Development of an „Attract & Kill“ strategy for the control of western corn rootworm larvae

Dissertation
zur Erlangung des Doktorgrades
der Fakultät für Agrarwissenschaften
der Georg-August-Universität Göttingen

vorgelegt von
Mario Schumann
geboren in Nürnberg

Göttingen, Februar 2012

_____

_____

D 7

1. Referent: Prof. Dr. Stefan Vidal

2. Korreferent: Prof. Dr. Stefan Schütz

Tag der mündlichen Prüfung: 10.02.2012

Table of contents

Summary

The western corn rootworm (WCR - *Diabrotica virgifera virgifera* Coleoptera: Chrysomelidae) is an important maize pest worldwide and the most damaging part of the beetle's life cycle is the below ground feeding of the larvae on the maize roots. The larvae use CO_2 to locate maize roots. This orientation cue can be incorporated into the chemical control of the larvae by attracting them to a soil insecticide (Tefluthrin) with CO_2 emitting capsules, implementing an "Attract & Kill" (A&K) strategy. This mechanism is eventually aiming at enhancing insecticide activity and reducing the application rate of Tefluthrin. Due to the cryptic feeding habit WCR larvae are difficult to observe, so non destructive methodologies were developed to evaluate larval behaviour, movement and spatial distribution. Furthermore the management of the larvae with the capsules alone and in an "Attract & Kill" combination were tested under semi field and field conditions.

1. Changes in the spatial distribution of WCR larvae were studied in an observation device at a fine scale (resolution 4.5 x 5 cm) and at a semi field scale with soil stratification (resolution 16 x 13 cm) to gain knowledge on basic WCR larval spatial ecology at a plant scale.
   - WCR larvae initially distribute in a major cluster close to their point of insertion and then actively disperse in the root system over time. The overall distribution in the root system remains aggregated
   - WCR larvae move to more developed root parts around the plant base and also exhibit an increased vertical movement over time.
   - Differences in root phenology have a minor influence on spatial distribution changes

2. The influence of CO_2 emitting capsules on the spatial and temporal distribution of WCR larvae was investigated in an observation device. Mortality rates of WCR larvae with an A&K strategy were analysed at different application rates of Tefluthrin and compared with a conventional treatment of Tefluthrin in the root system.
   - WCR larvae initially aggregate at and around the capsules but move away from the capsules over time

- With the addition of Tefluthrin to the capsules, WCR larvae were targeted in an A&K approach. The mortality of WCR larvae significantly increased compared to a conventional treatment of Tefluthrin in the root system at lower insecticide application rates

3. CO_2 emission by the capsules and the control of the larvae with the capsules alone and in an “Attract & Kill” strategy were tested. Under semi field conditions the reduction in larval densities in the greenhouse and under field conditions the reduction in root damage was measured.
   - CO_2 levels around the capsules increased in the soil for up to 20 days at greenhouse and 28 days under field conditions.
   - Application of the capsules between the maize rows alone reduced larval densities by up to 17 % under semi field conditions but could not reduce root damage under field conditions.
   - The combination of the capsules with Tefluthrin between the maize rows reduced larval densities by up to 27% under semi field and root damage up to 30% under field conditions. The insecticide activity could not be enhanced compared to a conventional application of Tefluthrin only in the maize rows.
   - The combination of the capsules with Tefluthrin in the maize rows under field conditions could increase the reduction in root damage by up to 15% at a quarter of the standard application of Tefluthrin compared to an application of Tefluthrin only.

„One challenge in agricultural entomology is to use our knowledge of arthropod behaviour in developing efficacious, environmentally benign, sustainable control tactics“ (Gould, F. (1991) Annu. Rev. Entomol, 36, 305 - 330)

Introduction

“What goes around comes around - The beetle that likes to travel “

Biological invasions are becoming a dominant concern of this time as the increasing modern travel and international trade of our society will favour the spread of invasive species. Besides having a negative effect on biodiversity (Wagner & Driesche 2010), invasive species cause threat to a country’s economy (Pimentel et al. 2005). Insects are good invaders as they exhibit *r*-selected life history characteristics such as a high fecundity or short generation time that contribute to invasion success (Sakai et al. 2001).

The western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) is a serious invasive root feeding pest of maize, *Zea mays* L. (Poacecae). After its successful spread in North America resulting in more than 1 billion dollars costs per year for its management and by crop losses (Spencer et al. 2009), it was first detected in Europe near Belgrade, Serbia in the early nineties (Kiss et al. 2005). Its first introduction was modelled to have occurred between 1979 and 1984 (Szalai et al. 2011). Additional subsequent independent introductions into other European regions followed (Ciosi et al. 2008) and the pest has now spread into 21 countries in Europe (EPPO 2011) (Fig. 1). Annual costs of up to 472 million € in a `no control` scenario are expected in Europe once it will have reached the full extent of its potential spread (Wesseler & Fall 2010). WCR is a univoltine species, the eggs overwinter in the soil and the larvae hatch in spring (Krysan 1986). The most damaging life stage is the larval below ground feeding on the maize roots (Meinke et al. 2009). The three larval instars feed upon the roots during a 3 week period, causing a disruption of water and nutrient uptake and plant lodging at higher larval densities (Levine & Oloumi-Sadeghi 1991).

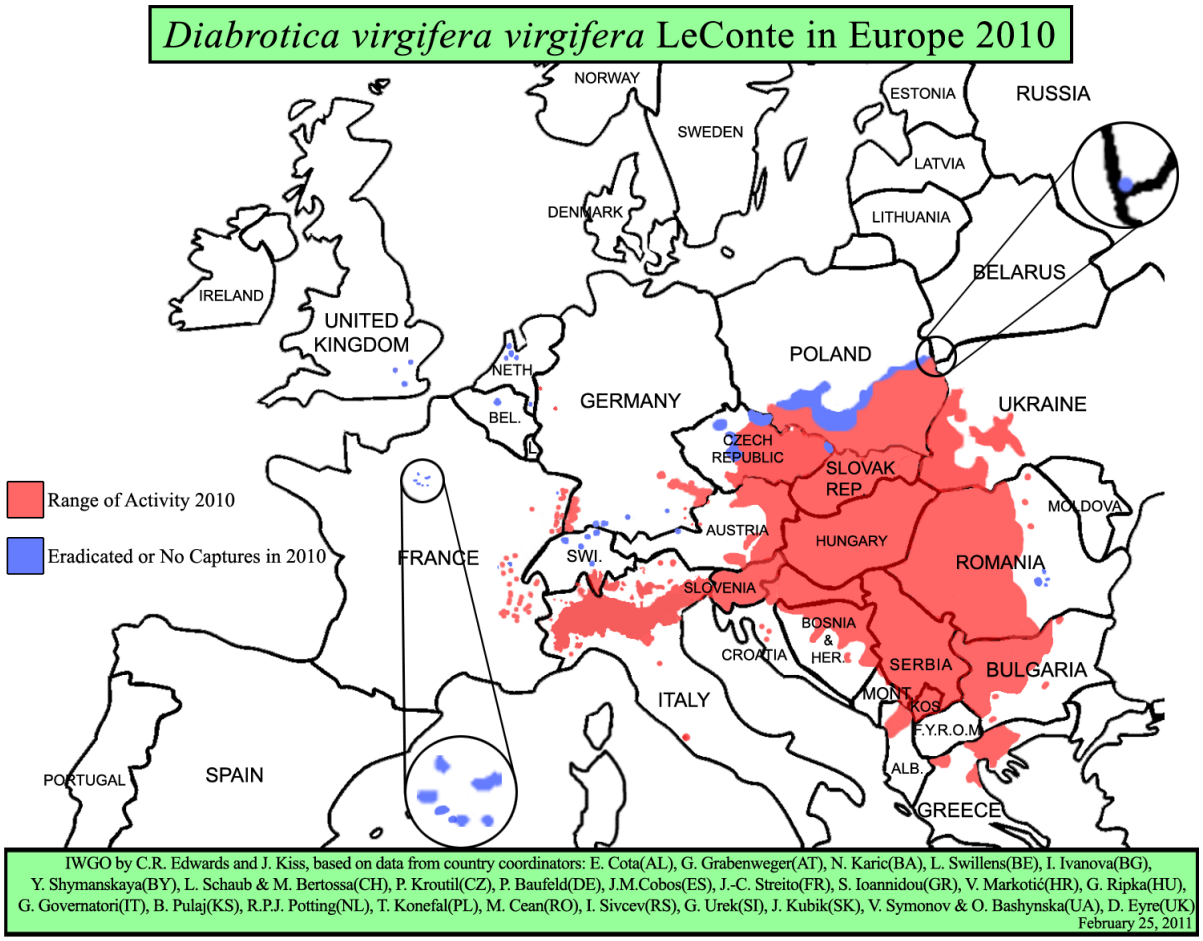


Figure 1 Extend of the distribution range of the western corn rootworm (WCR) in Europe in 2010

The main management options against the larvae vary according to their geographic spread. In North America for instance the use of transgenic cultivars is becoming increasingly adopted by farmers (Lefko et al. 2008; Chege et al. 2009; Hibbard et al. 2009). In Europe on the other hand chemical control with granular soil insecticides (Mayo 1980; Mayo & Peters 1978) or seed treatment (Furlan et al. 2006) is the most significant control option (Van Rozen & Ester 2010) when crop rotation (Gray et al. 2009) or biological control with entomopathogenic nematodes (Rasmann et al. 2005; Toepfer et al. 2010) are not viable. Chemical control of soil pests can cause enormous management problems as higher rates of pesticide need to be applied for their control than for above ground pests (Blackshaw & Kerry 2008) causing a bigger threat to the environment and human health (Ma et al. 2009). A recent key example is the application of insecticide coated seeds which has caused serious non-target effects on bees (Vincenzo et al. 2012). Thus on the basis of Regulation (EC) No 1107/2009 and Directive 2009/128/EC, the implementation of the principles of IPM to improve targeted use of all available pest control measures and reduce or even

eliminate pesticide use is obligatory (European Parliament and the Council of the European Union 2009).

The manipulation of insect behaviour makes it possible to utilize insecticides far more effectively (Harris 1972) by combining them with semiochemicals used in host finding as an attractant (Gould 1991). They can increase the chance of contact between the target and the toxic substance (Huang & Mack 2001). Such a combination is known as “Attract & Kill” (A&K) and has been shown to improve efficacy over other control methods (El-Sayed et al. 2009). For WCR larvae such a semiochemical is carbon dioxide (CO_2), a ubiquitous volatile released by respiring plant roots (Harris & Van Bavel 1957). It was first identified as an attractant for WCR larvae by Strnad et al. (1986) and further studies supported these findings (Hibbard & Bjostad 1988; Bernklau & Bjostad 1998a), corroborating CO_2 to be the only volatile attracting the larvae (Bernklau & Bjostad 1998b). This orientation behaviour is common across many orders of soil dwelling insects (see review by Johnson and Gregory 2006). It is a good cue for orientation as plants are unable to switch off its production (Johnson et al. 2006) and its low molecular weight allows a rapid diffusion over a long distance (Villani & Wright 1990; Pline & Dusenberry 1987). Bernklau et al. (2004) tested a wide range of CO_2 releasing compounds regarded to be attractive for western corn rootworm larvae aiming at disrupting their host location ability. They suggested that an encapsulation of these compounds would result in a more controlled and continuous release of CO_2 over a longer period of time. Such an encapsulated CO_2 source as an attractant for western corn rootworm larvae was first evaluated by Moeser et al. (unpublished). The capsules produced significantly more CO_2 than maize roots for seven days up to 20 cm from the source, attracting 2nd instar WCR larvae in longitudinal soil arenas. The integration of the capsules into chemical control with an A&K approach has not been evaluated so far.

The implementation of a behaviour based management strategy such as A&K requires a detailed study of the target insect’s biology (Loesel et al. 2000; Krupke et al. 2002). This is because the interaction between the semiochemical, the insecticide and the insect can be very complex (El sayed et al. 2009), especially due to any potential repellent effects of the A&K components. Despite the increasing impact of root feeding pests in agricultural production systems, studies on their biology are lacking compared to above ground counterparts (Hunter 2001). This lack of studies can mainly be related back to their cryptic feeding habit (Johnson et al. 2006). There

are certain aspects of a target organism's biology such as the spatial distribution that could help to evaluate success or failure of behavioural based management strategies. Overall the spatial distribution pattern of WCR larvae is determined by several factors across different spatial scales (Toepfer et al., 2007). Several studies dealt with a within field spatial distribution scale of the larvae (e.g. Ellsbury et al., 2005) but spatial distribution at a plant scale has only been intensively covered by Strnad & Bergman (1987). A more detailed knowledge on distribution changes at a plant scale could help to develop a more targeted management approach against the larvae.

It is difficult to assess the behaviour of soil organisms non destructively whilst maintaining thigmotactic cues and allowing lateral and vertical movement within the soil matrix (Bernklau & Bjostad 1998a; Johnson et al. 2006). Beside the fact that non invasive techniques like X – ray tomography (Harrison et al. 1993; Johnson et al. 2004) have had an increasing attention in the recent past (Mankin et al. 2008), the use of traditional non destructive methods to observe root herbivores (Reinecke et al. 2008; Wensler 1971) still remains an important tool to directly observe the behaviour of root herbivores (Dawson & Byers 2008).

Objectives

For this study an “Attract & Kill” strategy was developed that aims at reducing the application rate of a soil insecticide against western corn rootworm (WCR) larvae through the combination of an encapsulated CO_2 attractant (CO_2 emitting capsules). As part of the development process non destructive methodologies were used to identify potential mechanisms of the success or failure of the A&K strategy by examining WCR larval spatial ecology on a plant scale.

1. Quantification of the temporal and spatial distribution changes of western corn rootworm larvae in an observation device at a fine scale (resolution 4.5 x 5 cm) and with soil stratification at a semi field scale (resolution 16 x 13 cm) (Chapters 1 & 3)

    a. Do the larvae perform a certain sequence of distribution changes in the root system?

    b. Does dispersal and spatial distribution of WCR larvae in the maize root system change during their development?

    c. Does the availability of root material influence distributional changes of WCR larvae?

2. Evaluation of the length and rate of CO_2 production by CO_2 emitting capsules and their attractiveness for WCR larvae (Chapters 1, 2, 4 & 5)

    a. Do CO_2 emitting capsules affect the spatial and temporal distribution changes of WCR larvae?

    b. Do CO_2 emitting capsules build up CO_2 gradients in the soil outcompeting the ones built up by growing maize roots?

    c. Can the capsules be integrated into WCR management by disrupting larval host orientation?

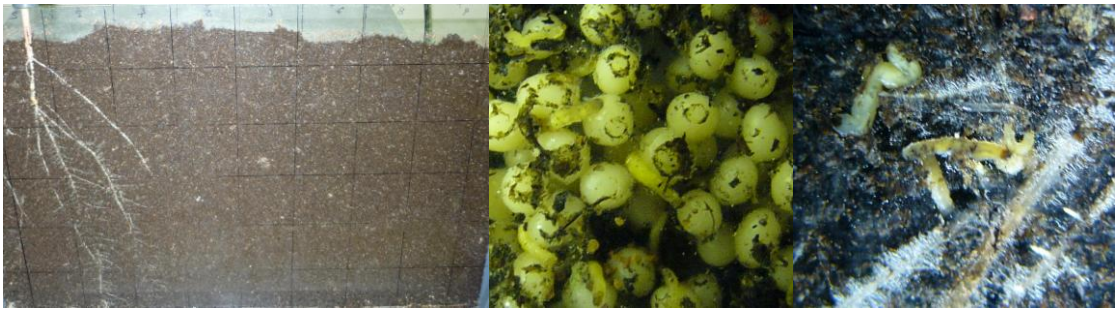
3. Evaluate the combination of a soil insecticide (Tefluthrin) and the CO_2 emitting capsules ("Attract & Kill" ("A&K")) (Chapters 1, 2, 4 & 5)

    a. Can Tefluthrin be combined with CO_2 emitting capsules to establish an A&K effect to target WCR larvae?

    b. Can A&K enhance the efficacy of Tefluthrin in terms of increasing larval mortality compared to its current conventional application?

Chapter 1

Evaluation of an attract and kill strategy for western corn rootworm larvae



Authors: Mario Schumann*, Anant Patel and Stefan Vidal

*Corresponding author

This chapter is published in:

Applied Soil Ecology (2013), 64, 178 – 189

Please access via: http://dx.doi.org/10.1016/j.apsoil.2012.12.007

© 2012 Elsevier

Evaluation of an attract and kill strategy for western corn rootworm larvae

M. Schumann[1], A. Patel[2] and S. Vidal[1]

[1] Georg–August University, Department of Crop Sciences, Agricultural Entomology, Grisebachstrasse 6, 37077 Göttingen, Germany
Phone: +49(0) 551–33733, Email: Mario.Schumann@agr.uni–goettingen.de

[2] University of Applied Sciences, Engineering and Alternative Fuels, Department of Engineering and Mathematics, Wilhelm – Bertelsmann-Strasse 10, 33602 Bielefeld

Abstract

Western corn rootworm larvae are serious soil dwelling maize pests, and use carbon dioxide (CO_2) to locate maize roots. The efficacy of insecticides can be enhanced by a combination with an attractant used in host finding, known as attract and kill. This study tested the use of CO_2 emitting capsules as an attractant in combination with the soil insecticide tefluthrin. An observation device was developed to study the temporal and spatial distribution changes of the larvae and to test whether these are influenced by the application of the capsules. Furthermore it was evaluated to what extent larvae are killed by the insecticide in combination with the capsules and whether this could be used for an attract and kill strategy to manage this pest.

The observation device enabled recovery of 20 – 40 % of the inserted larvae. The spatial analysis of distance indices revealed a sequence of spatial and temporal distribution patterns of the larvae in the root system. This sequence of spatial distribution was disrupted by an application of the capsules around which the larvae started to aggregate. Up to 40% mortality of the larvae with attract and kill was observed and thus could be increased over the conventional application (11% mortality) at lower application rates of tefluthrin. In conclusion an attract and kill strategy might be valuable to target this soil dwelling pest. Experiments under field conditions are needed to explore its potential as a management option for the western corn rootworm. Moreover, a further development of the capsules with host specific cues is needed to increase the attractiveness and subsequent mortality of the larvae.

Keywords: *Diabrotica virgifera virgifera*; below ground distribution; spatial analysis of distance indices; carbon dioxide; encapsulation; tefluthrin

1. Introduction

The western corn rootworm (WCR) *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) is a serious maize pest in the US Corn Belt with combined costs for management and damage exceeding 1 billion dollars per year (Spencer et al., 2009). Since its first introduction into Europe in the early eighties (Szalai et al., 2011), multiple introductions of the beetle from the US have been confirmed by genetic characterisations (Ciosi et al., 2008). This resulted in a rapid spread into 21 European countries by 2011 (EPPO, 2011). The most damaging life stage of this beetle are the three larval stages feeding on the maize roots (Meinke et al., 2009), causing a disruption of water uptake (Urias – Lopez et al., 2000) and, at high larval densities, plant lodging (Spike and Tollefson, 1991).

The proposed management options for the larvae vary according to their geographic location; in North America transgenic cultivars in combination with soil insecticides have increasingly been adopted by farmers since their commercialisation in 1996 (Huang et al., 2011). In Europe chemical control with soil insecticides only such as granulates or seed treatments is regarded the most promising option (Van Rozen and Ester, 2010) in case crop rotation (Gray et al., 2009) or biological control (Toepfer et al., 2010) are not viable.

In general, soil pests cause enormous management problems as higher concentrations of active ingredients of pesticides need to be applied for their control as compared to above ground pests (Blackshaw and Kerry, 2008) causing a threat to the environment and human health (Ma et al., 2009). Cryptic life stages, as it is the case for WCR larvae within the soil, create difficulties in targeting pests, making chemical control comparatively less effective (Hossain et al., 2007) as only a small fraction of the active ingredient reaches the target (Pimental, 1995). The manipulation of insect behaviour can allow utilization of insecticides in a more effective way (Harris, 1972). When combined with attractant semiochemicals used by the pest for host plant location (Gould, 1991), chances for a contact between the target and the toxic substance will markedly increase (Huang and Mack, 2001). Such a combination is known as attract and kill and has been shown to improve efficacy resulting in superior control levels as compared to other control methods (El–Sayed et al., 2009). Additionally, an attract and kill mechanism has the potential to target organisms from their cryptic habitats in complex environments that are normally difficult to reach with ordinary application techniques (El–Sayed et al., 2009).

WCR larvae use carbon dioxide (CO_2), a ubiquitous volatile emitted from respiring roots (Harris and Van Bavel, 1957), to locate maize roots. Such an orientation is common to soil dwelling larvae across numerous insect orders (Johnson and Gregory, 2006) with CO_2 acting as a general non specific semiochemical to locate roots by triggering a more directional response and intensifying the search for roots (Johnson et al., 2006). The importance of CO_2 for larval orientation was first identified for WCR larvae by Strnad et al. (1986). Further studies revealed that CO_2 was the most important attractant for the larvae (Hibbard and Bjostad, 1989; Bernklau and Bjostad, 1998b) and recent studies also identified additional cues (Bernklau and Bjostad, 2008; Hiltpold et al., 2012; Robert et al. 2012 a,b). Neonate larvae detect differences in CO_2 concentrations as small as 12% (Bernklau and Bjostad, 1998a) establishing the potential to integrate artificial CO_2 sources as an attractant for the control of the larvae. An extensive study by Bernklau et al. (2004) tested various CO_2 producing compounds that diverted the larvae away from the host; these authors proposed to encapsulate the CO_2 emitting products thus extending the time period of CO_2 production. Previous studies used an encapsulated CO_2 source (CO_2 emitting capsules) allowing the release of CO_2 at a distance up to 20 cm for 10 days; during this period 2nd instar larvae were attracted to these sources (Füser, 2006). However, a combination of these capsules in an attract and kill approach in terms of increasing the efficacy of larval control has never been tested before.

The implementation of such a behaviour based management strategy requires a detailed knowledge of the target insect's behaviour (Loesel et al., 2000; Krupke et al., 2002). Due to the potential repellent effects of any of the attract and kill components, the interactions between the semiochemical, the insecticide and the insect larvae may be very complex (El– Sayed et al., 2009). Furthermore, the cryptic feeding of below ground pests makes it difficult to study their behaviour in a non–destructive way, whilst maintaining thigmotactic cues and allowing lateral and vertical movement within the soil matrix (Bernklau and Bjostad, 1998a; Johnson et al., 2006). Beside the fact that non invasive techniques, such as X – ray microtomography (Harrison et al., 1993; Johnson et al., 2004) have received increasing attention recently (Mankin et al., 2008), the use of traditional non-destructive methods to directly observe root herbivores such as wireworms (Doane, 1975; Van Herk and Vernon, 2007) or scarab grubs (Reinecke et al., 2008; Wensler, 1971) still remain an important tool to study their behaviour (Dawson and Byers, 2008). This will be important when it comes to

assess behavioural based management tactics because potential behavioural resistance is more difficult to document than physiological resistance (Gould, 1991).
In this study we aimed at evaluating an attract and kill strategy for WCR larvae in a two step process using a non–destructive behavioural observation device: In step 1 we quantified WCR larval distribution to evaluate the attractiveness of CO_2 emitting capsules. In step 2 we assessed the attract and kill strategy by the combination of the CO_2 emitting capsules with an insecticide to evaluate the potential of this approach to enhance the control efficacy of WCR larvae over a conventional application.

2. Materials & Method

The distribution and state of the larvae were observed using a vertical observation device which consisted of a thin soil layer (45 cm x 30 cm x 6 mm) filled between two glass sheets. We used a distance of 6 mm between the glass sheets, because this is the minimal thickness for a maize seed to be inserted into the observation device undamaged. The observation device was divided into 60 grids with 10 vertical and 6 horizontal layers (Each grid 4.5 cm x 5 cm, Fig. 1) to quantify the dispersal, distribution and state of the larvae (described in detail below). The device was filled with 300 g of a peat soil mixture (Fruhstorfer Erde (Typ 25), Hawita Gruppe GmbH), as this type of soil allowed the larvae to move within the device without problems. The black colour of the soil also enabled to observe the white larvae more effectively. The side of the glass sheets were covered with opaque black cloths to avoid that light could interfere with the growth of the roots and larval movement.

2.1 Handling of WCR larvae and maize

Maize seeds (Cultivar: Prinz, KWS, Einbeck, Germany) were surface sterilised with sodium hydroxide for 5 minutes and soaked in sterile tap water for 12 hours. The seeds were transferred to a Petri dish covered with sterile paper towels, previously moistened with sterile tap water. The seeds were incubated for 24 hours at 25°C and 65% relative humidity. Seeds that had begun to germinate (radical root visible) were inserted between the glass sheets (Grid B2; Fig.1) at a depth of 7 cm. The plants were grown at 25°C and 65% RH until the required growth stage of maize used in the experiment was reached.

Late 2nd instar larvae were used in the experiment as they were large enough to be visualized in the observation device. The larvae were reared in feeding dishes (34 x 27 x 7 cm) that contained 30 maize plants (Cultivar: Prinz, KWS, Einbeck, Germany) in the same soil mixture as the soil used in the observation device. In each feeding dish 500 non diapausing WCR eggs, obtained from USDA–ARS, North Central Agricultural Research Laboratory, Brookings, were inoculated. Ten feeding dishes in total were prepared for each experiment. The eggs were stored at 8°C and incubated for 11 days at 25°C and 65% RH. Samples of the eggs were previously checked for the time of first hatch on day 13 of incubation. Soil was washed off from the eggs using a 250 µm sieve and then they were mixed in a 0.15% agar solution until they were evenly distributed. The number of eggs/ml of agar was calculated by taking 20

10 µl sub–samples and counting the number of eggs under a dissecting microscope (Leica, Wild, M3Z, Wetzlar, Germany). At five points in each feeding dish (one in each corner and one in the center; maize growth stage: BBCH 11 – 12) 100 eggs were applied with an Eppendorf pipette. 30 – 40 WCR eggs were prepared in a Petri dish to monitor the time of first hatch and the hatching pattern (N = 6; data not shown). The first larvae started to hatch 48 – 72 hours post inoculation and the majority had hatched after 7 days. After 10 days an additional 20 maize seeds, previously soaked in water for 12 hours, were inserted into each feeding dish to provide fresher root material for the larvae. The larvae were reared at 25°C and 65% humidity for 16 – 18 days. Following this period the majority of larvae were at the 2nd instar stage needed for the experiments. To extract the larvae from the soil each feeding dish was placed in a Kempson extraction chamber (Kempson et al., 1968) for 3 hours. In this system the soil of the feeding dishes was transferred to a box with netting at the bottom (mesh size 0.7 cm) and placed on a water container. A heat and moisture gradient produced by red light bulbs above the soil forced the larvae to move downwards and to fall into the water container. The larvae were skimmed off the water surface and placed in a Petri dish for 30 minutes to ensure that they are vital for use in the experiment.

2.2 Quantifying the distribution of root biomass

Once the plant had reached growth stage BBCH 13 (Lancashire et al., 1991), one glass sheet was carefully removed and soil cut with a scalpel according to the grid (Fig. 1). Each soil sample was washed using a 5 mm sieve, the cleaned roots were dried at 60°C for two weeks and weighed (Scale: H110, Sartorius, Göttingen, Germany). Six replicates were used to determine root biomass.

2.3 Assessment of larval distribution and behaviour

WCR larvae were inserted at a depth of 5 – 7 cm, 13 – 15 cm apart from the original sowing point of maize (Grid B5, Fig. 1). A plastic tube was placed between the glass sheets and the larvae were inserted into the soil through this tube to ensure that they were all placed at the required depth. After 4 hours and subsequently every 24 hours after the insertion of the larvae, the number and the state of the larvae was recorded for each grid (Fig. 1) looking at both sides of the observation device. For monitoring an observation device was transferred to a dark room, the black cloths were removed

and each grid was analysed with a white spotlight illuminating the grid to be analysed only avoiding disturbance of any neighbouring grids.

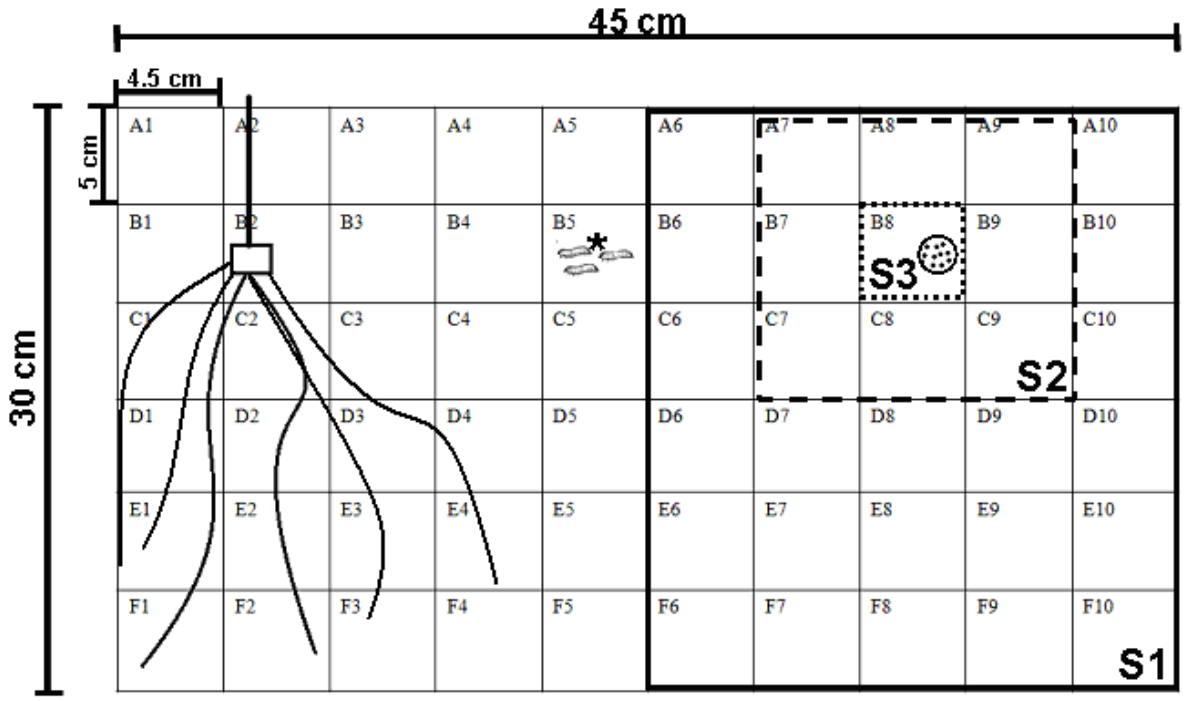


Figure 1 Side view of experimental set up to quantify western corn rootworm distribution and behaviour with a grid made up of 6 horizontal (A – F) and 10 vertical layers (1 – 10) (Total 60 grids: 4.5 x 5 cm each). Square, asterisk and circle represent the application area of the maize seed (Grid B2), western corn rootworm larvae (Grid B5) and CO_2 emitting capsules (Grid B8), respectively. For the quantitative analysis of the distribution of western corn rootworm larvae, the observation device is divided into section S1 (solid line ⁻), section S2 (dashed line **– –**) and section S3 (dotted line ····)

2.4 Attract & Kill components

For the attractant, an artificial CO_2 source (commercially available baker's yeast) was encapsulated in moist Ca-alginate capsules (CO_2 emitting capsules (CEC)) with a diameter of 2.3 mm and a moisture content of about 90%. These capsules were produced according to Patel and Vorlop (1994). In all experiments 5 g of the CEC were inserted 27 – 30 cm apart from the plant base at a depth of 5 – 10 cm (Grid B8; Fig. 1) as this distance is the equivalent of an application between two maize rows in the field. The CEC were previously weighed in small plastic boats (Scale: TE 1502s, Sartorius, Germany). For the application of the CEC the soil in grid A8 and B8 was removed with a spatula and the soil from grid B8 mixed with the CEC in the plastic boat. The soil and CEC mixture was placed between the glass sheets with the spatula and soil removed from grid A8 placed on top. The CEC were always applied

48 hours before the larvae were inserted, allowing the establishment of CO_2 gradients produced by the CEC.

The soil granulate Force 1.5 G (a.i.: tefluthrin 1g/100g of the active substance 2, 3, 5, 6 –Tetrafluoro– 4– methylbenzyl (Z)– (1RS, 3RS)– 3– (2–chloro–3, 3, 3–trifluoro–1–propenyl–2, 2–methylcyclopropanecarboxylate, Syngenta, Basel, Switzerland) was used as the insecticide (= kill) component. The granulates act through a gaseous phase upon contact with the target insect. Tefluthrin is classified as a pyrethroid which exhibits insecticidal activity by interfering with the sodium channels, disturbing the function of the nervous system (Soderlund, 2010). The granules were weighed in small glass vials before they were inserted into the observation device (Scale: H110, Sartorius, Germany). The application of tefluthrin is described in detail in step 2 of the evaluation process.

2.5 Evaluation of attract and kill components

2.5.1 Evaluation of the attractiveness of CO_2 emitting capsules (Step 1)

In the first step of the evaluation, one treatment with an application of the CO_2 emitting capsules (CEC) (applied in Grid B8 (Fig. 1) as described in section 2.4.), and one treatment without the capsules as the control were set up. In each observation device 50 WCR larvae were inserted into the observation device (Grid B5, Fig. 1) and the number of larvae at each grid of the observation was recorded after 4 hours and then every 24 hours for up to 3 days after the insertion of the larvae. This enabled us to determine the recovery rate, the dispersal and the distribution of the larvae in the observation device. Larvae that did not move off the point of insertion (Grid B5; Fig. 1), most likely due to stress or damage from transfer into the observation device, were regarded as “non dispersing” larvae and not included in data analysis. The evaluation was carried out in a series of 4 experiments (3 experiments with 6 replicates and 1 experiment with 5 replicates) for the controls and 3 experiments (6 replicates each) for the CEC. As no significant difference in the larval recovery was found between the experiments for the controls ($F_{3,19}$ = 3.04, P = 0.06) or for the capsule treatments ($F_{2,15}$ = 2.76, P = 0.10), the combined data from 23 replicates (= 23 observation devices) for the control and 18 replicates (= 18 observation devices) for the CEC were used for data analysis.

The dispersal of the larvae in the observation device was determined by counting the number of grids larvae were observed (= positive grids). Apart from the spatial analysis of distance indices (SADIE; see below), the distribution of the larvae was quantified by counting the total number of larvae observed i) directly at and up to 20 cm around the CEC (Section S1: All grids in column 6 – 10), ii) directly at and up to 5 cm around the CEC (Section S2: Grids A 7 – 9, B 7 – 9 and C 7 – 9) and iii) directly at the CEC (Section S3: Grid B8) (Fig. 1). Using these sections, the attractiveness of the capsules for the larvae could be quantified with regard to varying distances from the CEC. The number of larvae in each section was divided by the total number of larvae observed in the whole observation device (= percentage of recovered larvae).
For statistical analysis the recovery of larvae with the observation device, i.e. the number of larvae observed in the control or the CEC treatment, was tested with a repeated measure ANOVA with time and treatment as independent variables and number of larvae as the dependent variable. Quantification of CEC attractiveness with the percentage of recovered larvae observed in Section S1 and Section S2 were tested with the same tests after arcsine transformation. Additionally the percentage of recovered larvae between the control and CEC treatment was tested with a Student's t – test for each time of sampling (4 hours – 3 days). Data for section S3 were not statistically analysed as only a small proportion of larvae (< 1%) were recovered at this section in the control.

The spatial distribution of western corn rootworm larvae in the observation device was analyzed using spatial analysis by distance indices (SADIE). This program quantifies the spatial pattern in a sampled population and measures the degree of non–randomness in two dimensional spatial patterns (Perry, 1995). An index of aggregation (I_a) was calculated from the total number of larvae observed in all replicates at each time of sampling (4 hours – 3 days; total number ranged from 246 – 289 in the observation device for the control and 142 – 206 for the capsules). The index is calculated through the minimum distance that sampled individuals would need to move to achieve complete regularity (D). The observed counts are randomly allocated and D calculated again. In the analysis 26058 of these randomizations were carried out and the arithmetic mean D of all randomizations calculated (E_a). The aggregation index is an index of the observed value of distance to regularity with a mean randomized value ($I_a = D/E_a$), where $I_a = 1$, $I_a > 1$ and $I_a < 1$ indicates random,

aggregated and regular arrangements of counts, respectively (Perry and Dixon, 2002). The probability P_a tests for deviations from random dispersion, where $P_a > 0.975$ indicates regular dispersion; $P_a < 0.025$ spatial aggregation, and $0.025 < P < 0.975$, can not determine a spatial structure. A subsidiary index J_a indicates the presence of one major cluster ($J_a > 1$) or multiple clusters ($J_a \leq 1$ when $I_a > 1$) (Perry, 1998).

SADIE also calculates the contribution of each grid of the observation device to local clustering, expressed as unit–less sub – indices v_i and v_j, where v_i values > 1 contribute to patches and v_j values < -1 to gaps. These indices were used to develop contour maps of the spatial distribution of the larvae at each time of sampling in the observation device for the control and capsules (SigmaPlot, Version 11; Analytical Software, Tallahassee, FL, USA). We again used the total number of larvae from all replicates at each grid at each time of sampling to calculate local clustering.

Another feature of SADIE tests statistical association between the distributions of two groups of data. The extent to which local cluster indices of both distributions correlate at each point, provides a measure of spatial association, and produces an index of association (X). Positive values (association) were associated by a coincidence of two patches or gaps, whereas negative values (disassociation) result from a patch coinciding with a gap in both populations. The mean of local values of the two populations give the overall measure of association (X) (Perry & Dixon, 2002). The significance of X was tested against X_{rand} from a randomization test that included an adjustment procedure (Dutilleul, 1993). At the 5% significance level, the statistic $P < 0.025$ indicated significant association and $P > 0.975$ indicates significant disassociation. We tested the association of WCR larval distribution by comparing the spatial distribution in the observation device for the control and the CEC at each time of sampling.

2.5.2 Evaluation of "attract and kill" (Step 2)

Two treatments – "attract and kill" and "conventional" – were set up for this step in the evaluation process. For the attract and kill treatment, the CEC and tefluthrin were applied 27 – 30 cm apart from the plant base in 5 – 10 cm depth (Grid B8, Fig. 1). The application of the CEC was done the same way as it has already been described in section 2.4.. But in this step the first half of the CEC (~ 2.5 g) were applied between the glass sheets, the required amount of tefluthrin applied and the

remaining CEC were inserted on top ensuring that the granulates were embedded between the CEC. To guarantee that the insecticide was applied at the required depth, a thin glass rod (10 cm length and 3 mm diameter), connected to a plastic funnel, was placed between the glass sheet and the granulates were applied through this device. To compare the efficacy of an attract and kill treatment, a conventional treatment was set up where tefluthrin was directly applied at the original sowing point of the plant at a depth of 7 cm (Grid B2, Fig. 1). Half of the required amount was applied on each side of the seed. The attract and kill and the conventional treatment were tested at three application rates of tefluthrin: 150 mg (1.50 mg a.i. = HIGH) with 50 WCR larvae; 17 mg (0.17 mg a.i. = MEDIUM) with 100 WCR larvae and 9 mg (0.09 mg a.i. = LOW) with 100 WCR larvae.

Four hours and then every 24 hours for 4 days after the insertion of the larvae (Grid B5, Fig. 1) each grid of the observation device was specifically examined for larvae that showed “knock down” symptoms, expressed by writhing and curling of larvae (Michaelides and Wright, 1997; Bernklau et al., 2011) or which did not move (regarded as dead). With the grid the position of the larvae showing these symptoms was recorded. Furthermore the number of larvae in an observation device, knocked down and dead, was divided by the total number of larvae placed in the observation device for calculating the percentage mortality during the 4 days. Due to difficulties to decide whether a larva was dead, as larvae did not immediately start to curle and writhe when illuminated by the spotlight, the number of dead and knocked down larvae was combined for the analyses. The experiments were terminated after 4 days as most larvae had moved off their area of insertion (Grid B5, Fig. 1) and were expected to be targeted by the insecticide at that time, but also because larvae that were killed off shortly after insertion started to disintegrated making it difficult to count them.

For statistical analysis the percentage mortality of the larvae was arcsine transformed and tested with a repeated measure ANOVA with time and treatment as independent variables and mortality as the dependent variable. Additionally the mortality at each sampling date between the attract and kill and conventional treatment was tested with a Student’s t – test.

All statistical analyses were carried out with Statistica, Version 9 (StatSoft, Tulsa, OK, USA).

3. Results

3.1. Distribution of root biomass

The maize root system has spread across 22.50 ± 0.76 grids at growth stage BBCH 13 (Fig. 2) thus covering 506 cm^2 area of the 1350 cm^2 large the observation device (= 38% of total area). The dry root biomass recovered from all grids in the observation device was 0.069 ± 0.011 g per plant.

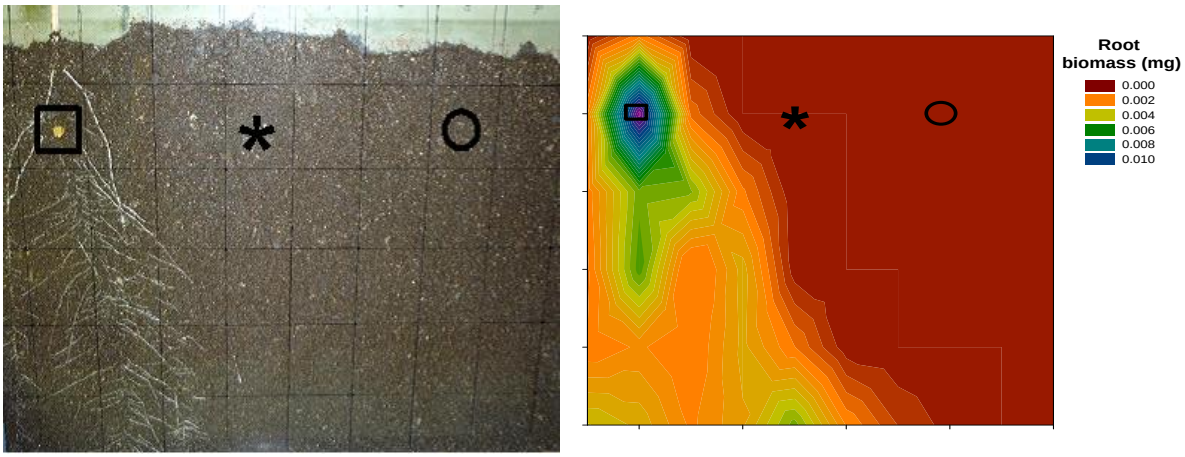


Figure 2 Extent of the maize root system (Left) and distribution of root biomass in the observation device at growth stage BBCH 13 (Right). Square, asterisk and circle represent the application area of the maize seed, western corn rootworm larvae and CO_2 capsules respectively.

3.2. Evaluation of the attractiveness of CO_2 emitting capsules (Step 1)

3.2.1. Recovery of non dispersing larvae in observation device

In the control 7.91 ± 0.84, 5.48 ± 0.91, 1.91 ± 0.46 and 0.65 ± 0.19 of non dispersing larvae were found at their original area of insertion (Grid B5, Fig. 1) after 4 hours, 1 day, 2 days and 3 days, respectively. The pattern of larvae slowly recovering from transfer could also be observed in all experiments with the CO_2 emitting capsules (CEC). Most larvae had recovered 2 days after their insertion.

3.2.2. Recovery of dispersing larvae in observation device

The recovery of dispersing larvae was influenced by time after insertion ($F_{3, 117}$ = 3.55; $P < 0.01$) but not by treatment (i.e. control vs. CEC) ($F_{1,39}$ = 0.72; P = 0.40) or an interaction of both ($F_{3,117}$ = 1.96; P = 0.12). The recovery of dispersing larvae (of the 50 larvae originally inserted in the observation device) was lowest after 4 hours (control: 10.70 ± 0.77 larvae; CEC: 9.89 ± 0.95 larvae). The recovery slightly increased on subsequent observations after 1 day (control: 12.57 ± 0.89 larvae; CEC: 9.50 ± 1.16 larvae), 2 days (control: 12.46 ± 1.05 larvae; CEC: 12.92 ± 1.32

larvae) and 3 days (control: 11.85 ± 0.95 larvae; CEC: 11.44 ± 1.17 larvae). Across all sampling dates, 23.78 ± 1.40 % and 21.88 ± 1.81 % of the inserted larvae were observed (= recovered for analysis) in the observation device for the control and the CEC treatment, respectively.

3.2.3. Dispersal of WCR larvae

WCR larval dispersal, expressed as the number of grids the larvae were observed at (=positive grid) (Fig. 3), was not affected by treatment (i.e. control vs. CEC) ($F_{1,39}$ = 0.27; P = 0.61), but by time after insertion ($F_{3,117}$ = 5.25; P < 0.01). An interaction between both parameters did not affect larval dispersal ($F_{3,117}$ = 1.19; P = 0.32). With 7.57 ± 0.60 positive grids, dispersal in the control was lowest after 4 hours and increased to 10.04 ± 0.60 positive grids after 1 day at which it remained during the next 2 days. A similar pattern was measured in the CEC treatment with the lowest dispersal after 4 hours (6.89 ± 0.71 positive grids) and 1 day (7.89 ± 1.32 positive grids) and then increasing to 10.89 ± 1.08 and 9.44 ± 1.18 after 2 and 3 days.

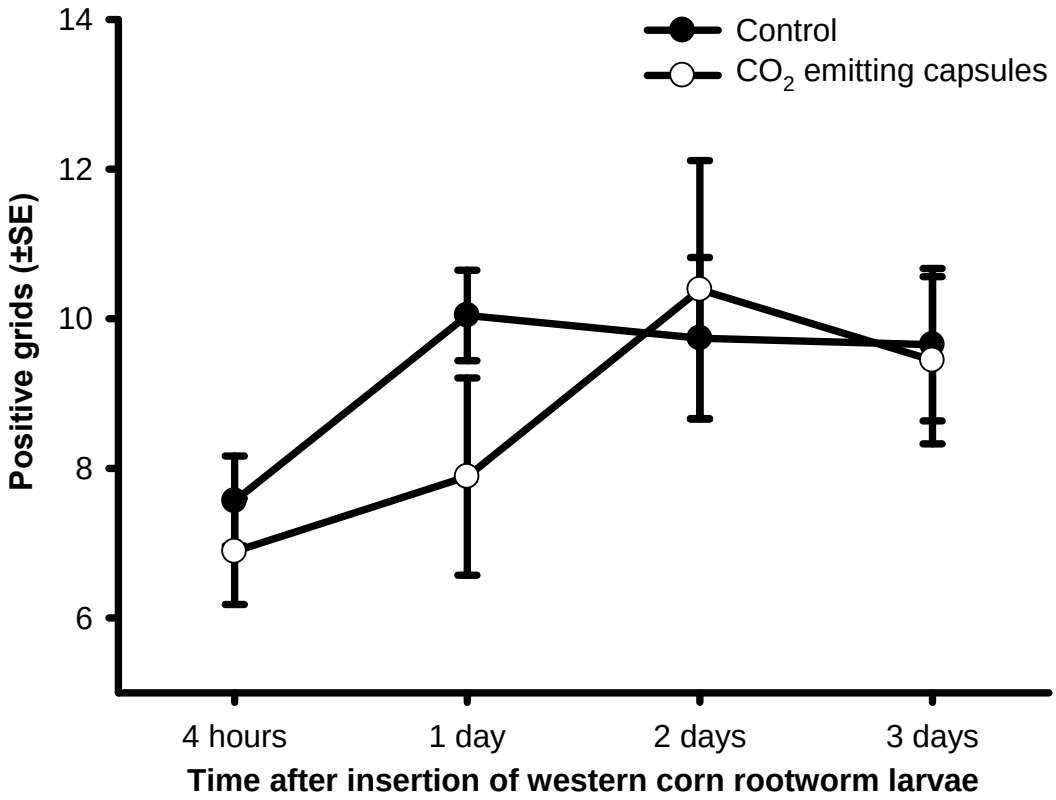


Figure 3 Dispersal of western corn rootworm larvae 4 hours, 1 day, 2 days and 3 days past the insertion into the observation device. Dispersal is determined by the number of grids western corn rootworm larvae were observed at (= “positive grid”)

3.2.4. Spatial analysis of WCR larval distribution

An aggregated distribution (I_a > 1) of the larvae could always be measured in both the control and CEC treatment at all sampling dates (Table 1). After 4 hours in the observation device for the control the larvae were significantly aggregated (I_a = 1.84; P < 0.01) in one major cluster (J_a = 1.29; P < 0.01) on the periphery of the root system near the point of their insertion (grids B3, C3 & D4) (Fig. 4a)). In the observation device with the CEC, strong clustering was also established at the same part in the root system (grid C4), but also at (Grid B8) and near (grid B7 and B9) the CEC (Fig. 5a). The additional cluster at the CEC reduced the I_a and J_a index to 1.38 (P = 0.05) and 1.08 (P = 0.11), respectively, so that the overall larval population was not significantly aggregated anymore. There was no association between the spatial distribution of the larvae in the observation device for the control and the CEC treatment after 4 hours (X = 0.15; P = 0.10) (Table 1).

On the subsequent observation days (day 1 – 3), the spatial analysis for the control showed strong clustering of the larvae around the original insertion area of maize (Grid B2) and at the bottom of the observation device (grids F1 – F3; Fig. 4 b) – d)). With the formation of more aggregations in different parts of the root system, the J_a index decreased to 1.07 (P = 0.15) after 3 days, indicating the formation of multiple clusters. In the observation device with the CEC, aggregation could still be measured at (Grid B8) and near (grid C6, C7 & C8) the CEC at any sampling day, but with a lower level of clustering (Fig. 5 b) – d)). As in the observation device for the control, the larvae started to aggregate around the original area of the insertion of maize (grid A1) and at the bottom of the observation device (grids F1 & F2) over subsequent sampling days. Through the clustering at the CEC the overall level of aggregation of the larval population was always lower than in the control (Table 1). The aggregation at the capsules also lowered the J_a index as more clusters were present on all sampling days. The larval distribution in the control and the CEC treatment started to become significantly associated after 1 day (X = 0.67; P < 0.01) and the level of association increased on subsequent sampling days (Table 1).

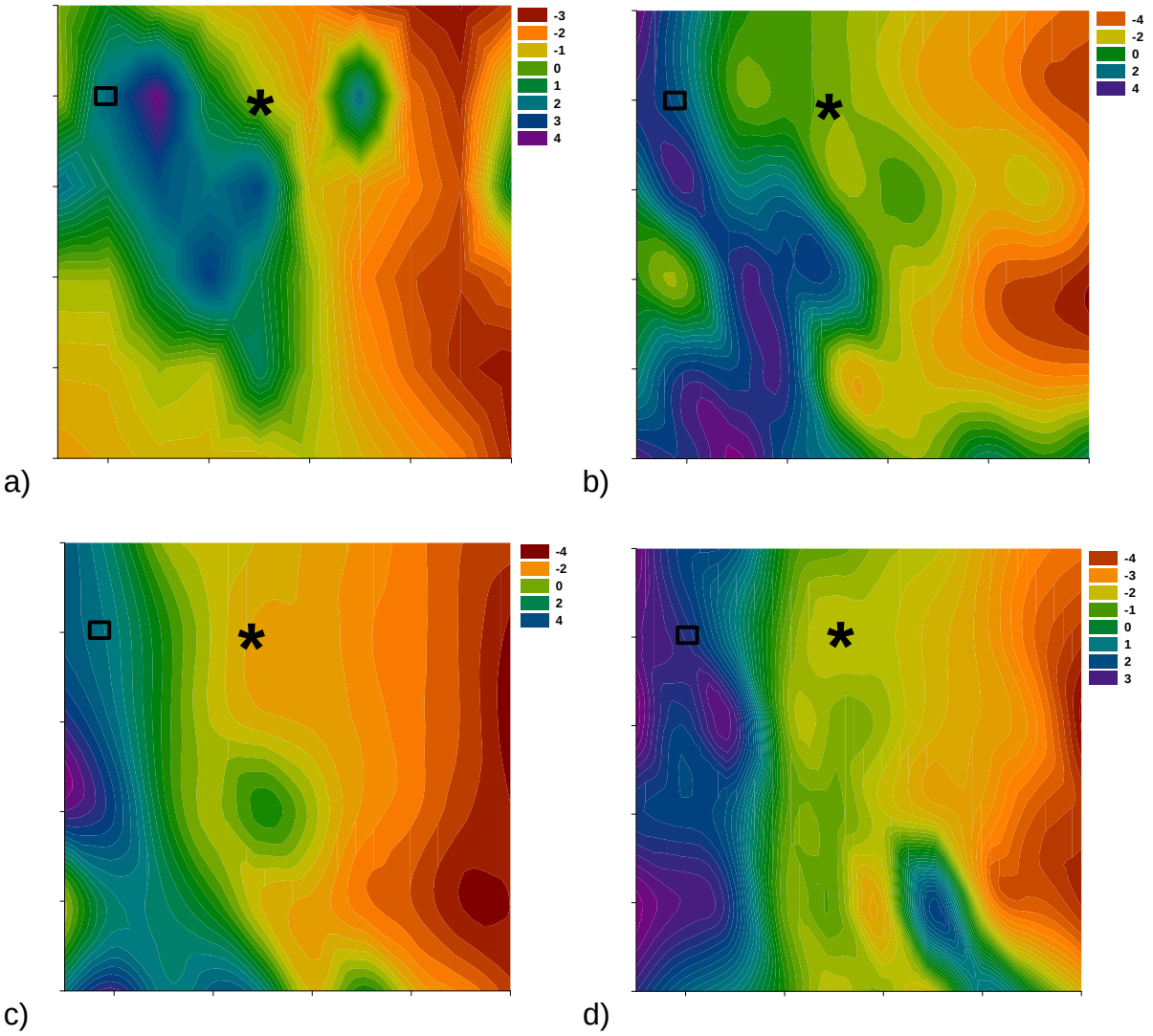


Figure 4 Time series of the distribution of western corn rootworm larvae in the control a) 4 hours b) 1 day c) 2 days and d) 3 days past the insertion into the observation device. Contour maps are based on local cluster indices calculated by spatial analysis of distance indices. A positive local cluster value > 1.5 indicates significant clustering of large counts (= number of larvae) close to one another (blue/purple), whereas a value of < –1.5 contributes significantly to a gap i.e. no or low counts to one another (brown). Square and asterisk represent the application area of the maize seed and western corn rootworm larvae respectively.

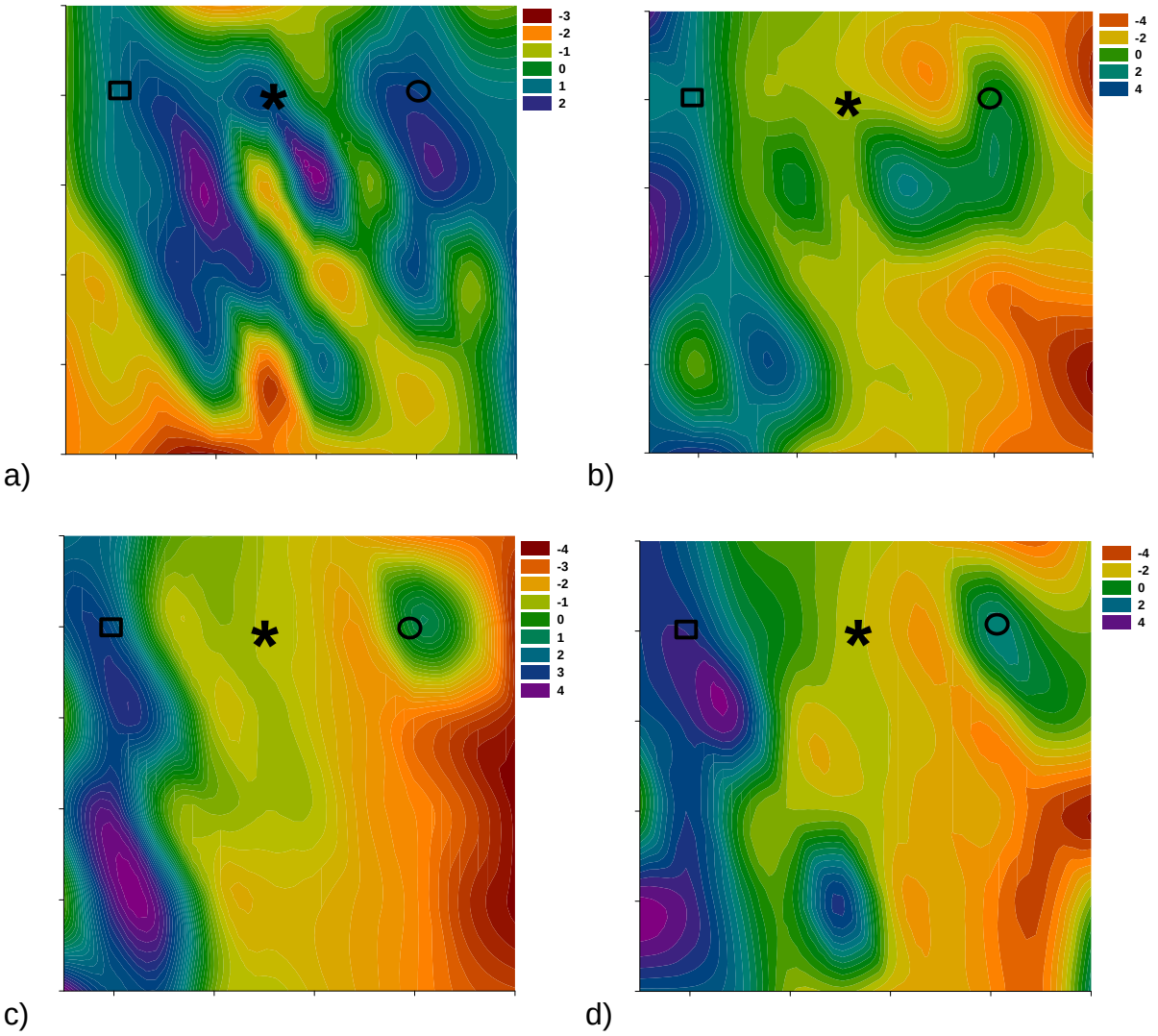


Figure 5 Time series of the distribution of western corn rootworm larvae a) 4 hours b) 1 day c) 2 days and d) 3 days past the insertion into the observation device with the application of CO_2 capsules. Contour maps are based on local cluster indices calculated by spatial analysis of distance indices. A positive local cluster value > 1.5 indicates significant clustering of large counts (= number of larvae) close to one another (blue/purple), whereas a value of < –1.5 contributes significantly to a gap i.e. no or low counts to one another (brown). Square, asterix and circle represent the application area of the maize seed, western corn rootworm larvae and CO_2 emitting capsules respectively

Table 1 Spatial parameters of western corn rootworm larval distribution 4 hours, 1 day, 2 days and 3 days past the insertion into the observation device for a control and with CO_2 emitting capsules using Spatial Analysis of Distance indices.

Treatment	**Control**				**CO_2-emitting capsules**				**Control vs. CO_2-emitting capsules**	
Time after insertion	**I_a**	**P**	**J_a**	**P**	**I_a**	**P**	**J_a**	**P**	**X**	**P**
4 hours	1.84	< 0.01	1.29	< 0.01	1.38	0.05	1.08	0.11	0.15	0.10
1 day	2.57	< 0.01	1.16	0.07	2.42	< 0.01	1.07	0.15	0.67	< 0.01
2 days	2.32	< 0.01	1.16	0.07	2.04	< 0.01	1.09	0.18	0.84	< 0.01
3 days	2.34	< 0.01	1.08	0.16	2.23	< 0.01	1.09	0.14	0.74	< 0.01

I_a and its associated P – value indicate the aggregation of an overall spatial pattern and associated significance test of the spatial pattern's departure from randomness. $I_a > 1$ indicates an aggregated distribution and a significant spatial aggregation is assumed at $P < 0.025$. $J_a \leq 1$ indicates the presence of multiple clusters when $I_a > 1$ and one major cluster when $J_a > 1$. X is the measure of spatial association between the distribution of western corn rootworm larvae. When $X > 0$ indicates that two populations are associated and are considered as significantly associated when $P < 0.025$

3.2.5. Quantitative analysis of WCR larval distribution

Recovery of WCR larvae in Section S1 of the observation device (= directly at and up to 20 cm around the CEC; Fig. 6) was affected by treatment (i.e. control vs. CEC) ($F_{1,39} = 7.03$; $P < 0.05$), by time after insertion ($F_{3,117} = 16.21$; $P < 0.001$) and an interaction between both parameters ($F_{3,117} = 8.37$; $P < 0.001$). 57.60 ± 6.08% of recovered larvae were observed in section S1 in the observation device with the CEC 4 hours after insertion (control: 21.42 ± 3.09%); this percentage decreased to 20.27 ± 4.41% after 1 day (control: 16.60 ± 2.64%), and to 15.93 ± 3.69% after 2 days (control: 10.98 ± 2.10%). After 3 days it increased to 18.38 ± 3.99% in the observation device with CEC and to 20.48 ± 3.15% in the control.

Recovery of WCR larvae in section S2 (= directly at and up to 5 cm around the CEC; Fig. 6) was significantly affected by treatment ($F_{1,39} = 34.78$; $P < 0.0001$), time after

insertion ($F_{3,117}$ = 13.56; P < 0.0001) and an interaction of both ($F_{3,117}$ = 6.45; P < 0.001). In the observation device with the CEC 35.52 ± 6.64% of recovered larvae were observed after 4 hours (control 6.05 ± 1.99%) but decreased to 10.86 ± 3.10% after 1 day (control: 1.07 ± 0.62%). It remained on this level on day 2 (control: 0.75 ± 0.42%; CEC: 8.15 ± 2.62 %) and increased to 5.32 ± 1.62% in the control after 3 days whereas it still remained on the same level with the CEC (8.77 ± 2.76%).
With the application of the CEC 13.74 ± 5.20 % of recovered larvae were found in section S3 (= directly at the CEC: Fig. 6) after 4 hours, but decreased to 3.95 ± 1.81% after 1 day. It changed to 6.59 ± 2.76% over the next two days. In the control < 1% of the recovered larvae could be observed at any time of sampling.

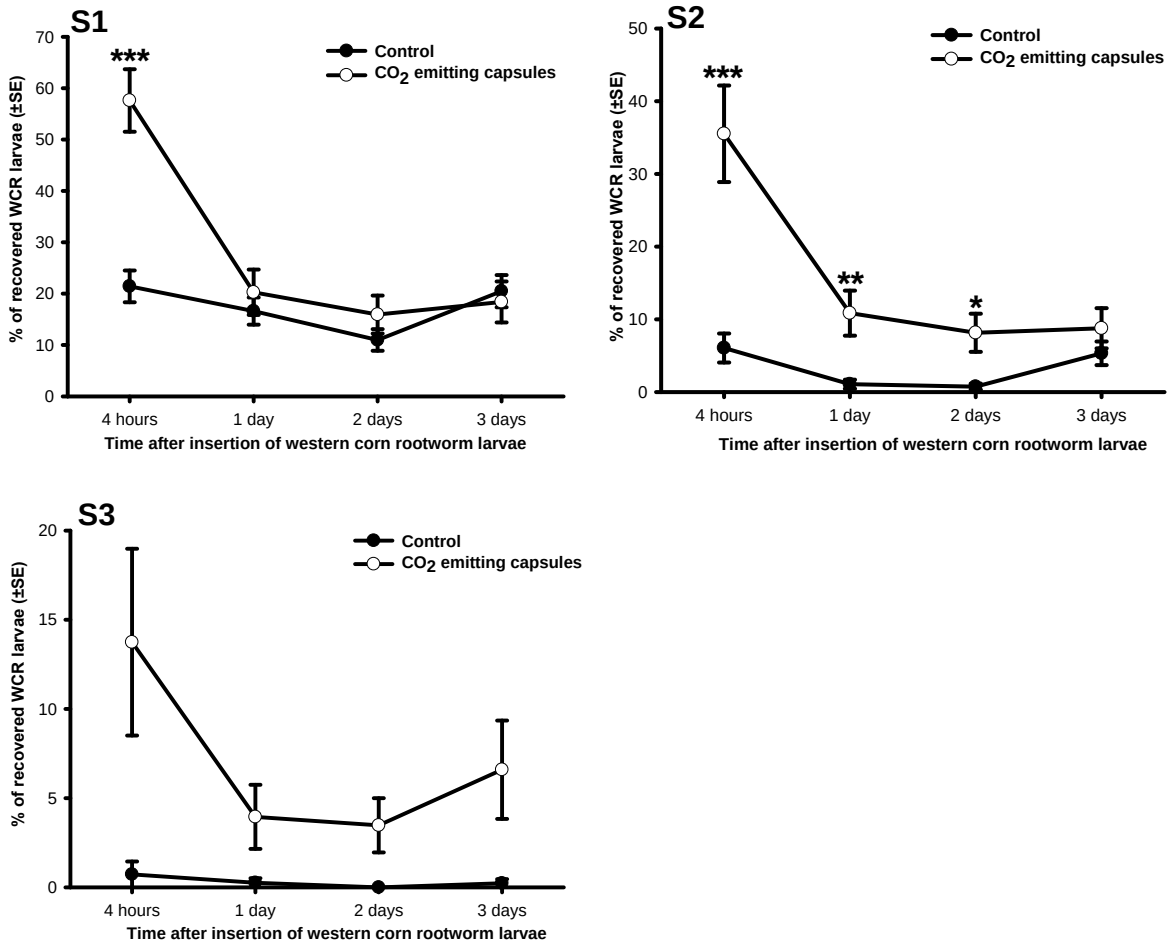


Figure 6 Percentage of recovered western corn rootworm larvae observed 4 hours, 1 day, 2 days and 3 days past the their insertion in section S1 (directly at and up to 20 cm around CO_2 emitting capsules), S2 (directly at and up to 5 cm around the CO_2 emitting capsules) and S3 (directly at CO_2 emitting capsules) of the observation device through the application of CO_2 emitting capsules. The asterix indicates a significant difference between the control and CO_2 emitting capsules on the time after insertion of WCR larvae (*** = P < 0.0001;** = P < 0.001; * = P < 0.01 according to Student's t – test). Please note the changing scale!

3.3. Evaluation of an attract and kill strategy (Step 2)

At a high application rate of tefluthrin, larvae with knock down symptoms and regarded as dead were observed in 6 grids in the conventional treatment (Grid A1 + B1; A2 – C2; B3) and in the attract and kill treatment (Grid B7; A8 – C8; B9 + C9). The percentage mortality was not significantly affected by the type of treatment ($F_{1,10}$ = 0.10; P = 0.76) but by time after insertion ($F_{4,40}$ = 28.96; P < 0.001) and an interaction of both parameters ($F_{4, 40}$ = 3.70; P < 0.01). In a conventional treatment mortality was lowest after 4 hours (0.33 ±0.33 %) and increased to 30.33 ± 2.15 % after 4 days. An increase in mortality could also be measured in the attract and kill treatment with a significantly higher mortality after 4 hours (8.17 ±2.34%) than in the conventional treatment. After 4 days a 22.00 ±2.09 % mortality was measured in the attract and kill treatment (Fig. 7; HIGH).

At a medium application rate of tefluthrin, larvae with knock down symptoms and regarded as dead were observed in 4 grids in the conventional treatment (Grid A1; A2 – C2) and 5 grids in the attract and kill treatment (Grid B7; A8 – C8; B9). There was a significant effect of treatment ($F_{1,10}$ = 5.69; P < 0.05) and time after insertion ($F_{4,40}$ = 57.95; P < 0.001) but not by an interaction of both parameters ($F_{4,40}$ = 1.83; P = 0.14) on percentage mortality. In the attract and kill treatment a 6.33 ± 1.15% mortality was measured after 4 hours which was significantly higher than with a conventional treatment. The mortality rate increased to 26.67 ± 3.48% after 4 days. In the conventional treatment mortality was lowest after 4 hours (1.67 ± 0.65%) and increased to 21.33 ± 2.20% after 3 days and then slightly dropped to 19.83 ± 2.77% after 4 days (Fig. 7; MEDIUM).

At a low application rate of tefluthrin, larvae with knock down symptoms and regarded as dead were observed in 4 grids in the conventional (Grid A1; A2 – C2) and 5 grids in the attract and kill (Grid B7; B8 + C8; B9 + C9) treatment. There was a significant effect of treatment ($F_{1,10}$ = 76.55; P < 0.0001) and time after insertion ($F_{4,40}$ = 53.12; P < 0.0001) on larval mortality, and also with the interaction of both parameters ($F_{4,40}$ = 8.26; P < 0.0001). Apart from day 2 a significantly higher mortality could be measured in the attract and kill treatment in which mortality increased from 9.17 ±0.60% after 4 hours to 39.67 ±3.81% after 3 days with a slight decrease to 37.50 ±4.93% after 4 days. Mortality in the conventional treatment was lowest after 4 hours (1.16 ±0.60%) and increased to 11.20 ±1.35% after 4 days (Fig. 7; LOW).

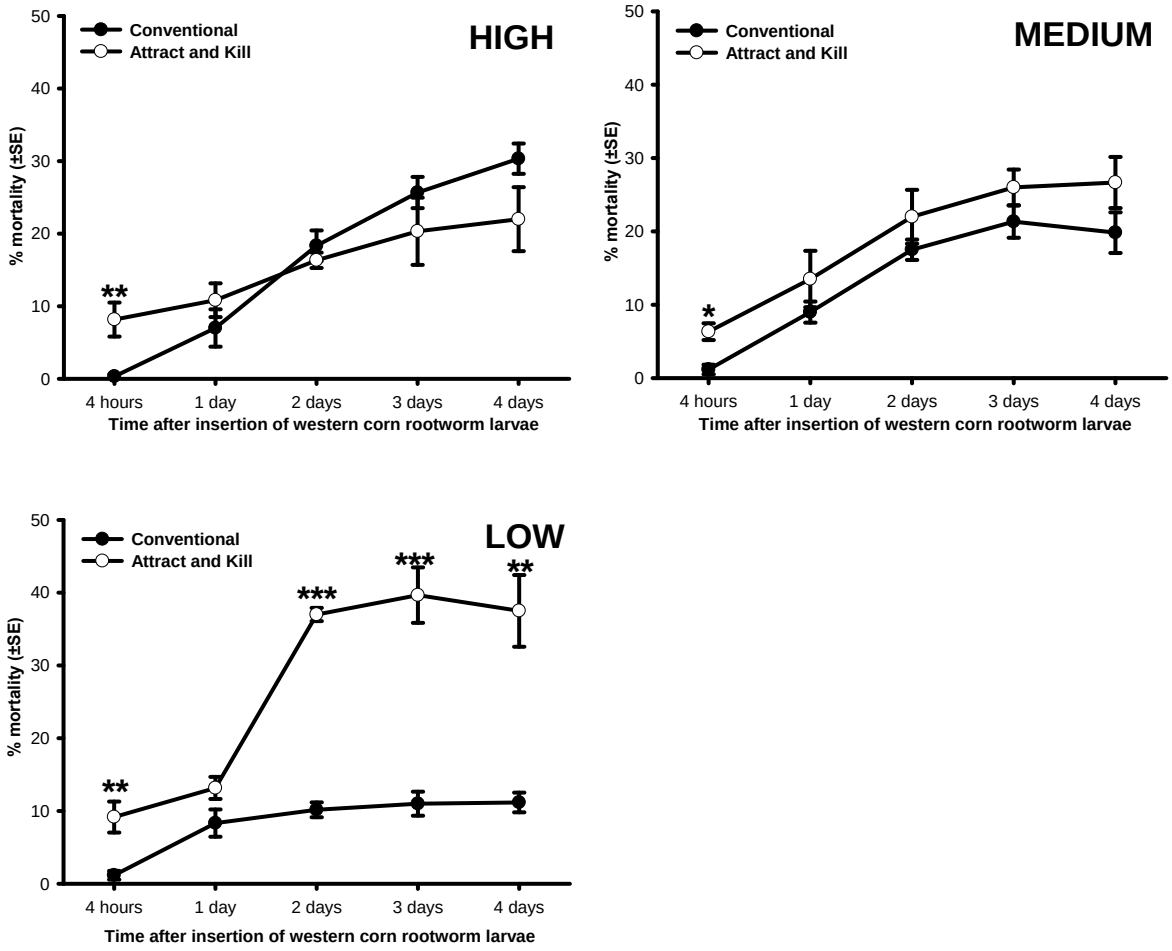


Figure 7 Effect of tefluthrin on western corn rootworm larvae shown as the percentage mortality (= knock down symptom or regarded as dead) of the inserted larvae in the different treatments at three different application rates of soil granulates per observation device (HIGH: 150 mg; MEDIUM: 17 mg; LOW: 9 mg) 4 hours, 1 day, 2 days, 3 days and 4 days past the insertion into the observation device. The asterisk indicates a significant difference between the conventional and attract and kill treatment on the time after insertion of the larvae (* = P < 0.01; ** = P < 0.001; *** = P < 0.0001 according to Student's t test).

4. Discussion

Our study showed that visualizing and quantifying movement with a non destructive observation device, adapted to the size of the study soil organism, enabled us to identify changes in distributional patterns. Furthermore it allowed evaluating a behavioural based pest management strategy. Step 1 of the evaluation showed that western corn rootworm (WCR) larvae exhibited a specific sequence of spatial and temporal distribution changes which can be disrupted with CO_2 emitting capsules (CEC). In step 2 of the evaluation, the CEC were combined with an insecticide, implementing an attract and kill strategy. With this strategy insecticide efficacy could significantly be enhanced over a conventional treatment at lower insecticide application rates used in this study.

4.1. Observation device

The use of the observation device simultaneously ensured visible root growth and larval movement. The observations were still constrained by the 6 mm thickness of the soil layer, which created an opaque medium, hampering observation of all inserted larvae to some extent. Moreover the 2nd instar larvae bore into roots, making it difficult to observe larval behaviour and thus reduced visualizing success. Consequently certain modification, like the use of larger 3rd instar larvae or a thinner soil layer (2 -4 mm), would need to be made in the future to improve visualization.

4.2. Evaluation of CO_2 emitting capsules (Step 1)

In the observation device for the control, the spatial distribution pattern of the larvae after 4 hours exhibited an establishment phase. We define this phase as larvae aggregating by feeding in one major cluster (highest J_a index, table 1) close to their point of insertion on the periphery of the root system (Fig. 4a)). Larval feeding established at this time on the root tips. This behaviour has also been reported by Clark et al. (2006) studying neonate larvae on maize seedlings in a transparent gel medium. Root tips are the point of new root cell formation and also a point of higher CO_2 emission (Strnad and Bergman, 1987b), explaining the preference of larvae when they start feeding in the root system. Following the initial establishment phase larvae started to actively disperse into the root system (Fig. 3), still however remaining in an aggregative distribution (Table 1). This aggregation behaviour is common in many orders of soil organisms (Brown and Gange, 1990), probably due to

aggregated suitable food resources, such as roots. WCR larvae fed on roots at the bottom (e.g. grids in layer F; Fig. 4) of the observation device and nearby the original point of insertion of the maize seed (Grids A1 – C1; Fig. 4). We are unable to rule out that the observation device did influence the distribution patterns of the larvae in a specific way: root biomass accumulated at the bottom of the observation device during the course of the experiments, causing larvae to exhibit an increased vertical movement to a depth of 30 cm. Moreover, we used 2nd instar larvae in the experiments that are known to feed on older and thicker roots at this stage of development which they typically find near the plant base (Chiang, 1973; Strnad and Bergman, 1987b). This might reflect the strong aggregation in this part of the root system (e.g. around grid B2; Fig. 4).

Our experiments demonstrated that an application of CEC changed the temporal and spatial distribution pattern, typically displayed by WCR larvae in the observation device, by luring them away from the plant roots. Both the quantitative and spatial analyses showed that the attraction of the CEC was highest 4 hours after larval insertion and decreased during subsequent measurements. As most larvae had already left their original area of insertion (grid B5) by the time of first monitoring, the highest attraction activity nearby the CEC was expected to have happened at this time interval. Few larvae initially remained at their insertion point, a problem also encountered in other studies investigating movement of 2nd instar larvae (e.g. Hiltpold et al., 2012). This is most likely due to stress experienced by the larvae during their insertion into the arena. These larvae, however, moved off during the next 48 hours and potentially reflect the low larval activity around the CEC during day 1 -3 (Fig. 5 B-D). Also larvae that had already been feeding in the root system might have been attracted to the CEC at the later sampling dates as a result of post establishment movement due to heavy root damage (Hibbard et al., 2003). In this movement the larvae could orientate towards a new CO_2 gradient to locate root material (Strnad and Dunn, 1990). Overall, however, larvae approaching the CEC were not rewarded food, resulting in continued search for roots thus moving away from the capsules so that larval activity at the CEC decreased.

The 2nd instar larvae used in our experiment had been feeding on maize roots < 6 hours before they were inserted in the observation device. They do not experience energetic constraints compared to unfed 1st instar larvae and can move in the soil matrix for a time sufficient to find maize roots. This could mislead the function of not

finding the host plant through the confusion by the CEC. Targeting 1st instar larvae with this luring technique could result in larval mortality as these larvae need to find roots within 24 – 48 hours after hatch, otherwise they die of starvation (Strnad and Bergman, 1987a; MacDonald and Ellis, 1990). Additionally ,as it has already been discussed for other studies (e.g. Hibbard and Bjostad, 1989), the use of 2nd instar larvae as the test organisms is one major drawback to evaluate larval orientation, because after the first contact with the root a localised searching behaviour of the larvae can be triggered (Bernklau et al., 2009). Also for a potential field application it is expected that 1st instar larvae will be targeted as they are the most important life stage for host plant selection (Bernklau and Bjostad, 1998b).

The effect of larval activity as a result of CO_2 attraction became more apparent with decreasing distance to the CEC (Fig. 5 & 6). Larvae were not only initially lured away from the plant, but also directly to the CO_2 source (Fig. 5 & 6 S1). This is an essential prerequisite for an attract and kill strategy as the larvae should move directly to the application point of the attractant so that they are affected by the killing agent.

4.3. Evaluation of "attract & kill" (Step 2)

Larvae recovered at and nearby the CEC showed curling and writhing, symptoms of intoxification due to tefluthrin. The addition of a pyrethroid such as tefluthrin with an attractant is regarded as an effective combination, resulting in a rapid knock down effects to the target organisms (Evenden and McLaughlin, 2004; Poullot et al., 2001). The addition of the insecticide did not result in an apparent repellent effect to the larvae. These observations support findings reported by Hibbard and Bjostad (1989) who characterised tefluthrin and other active ingredients as non repellent to WCR larvae in laboratory bioassays. However, some insect species are known to have evolved avoidance behavior of the plant toxin pyrethrum and related pyrethroids (Gould, 1991). Thus these compounds can also be repellent (Michaelides et al., 1997) which also been described for root herbivores such as the wireworm, *Agriotes lineatus/obscurus* (Van Herk and Vernon, 2007).

The proportion of repellent events in wireworms even increases with the concentration of tefluthrin (Van Herk and Vernon, 2008), which indicates that repellency might be more pronounced at higher application rates of the active ingredient. In our study this could explain lower mortality rates of WCR larvae in the attract and kill treatment at higher application rates of the insecticide (Fig. 7). We

speculate that the attractive effect of the CEC in this experimental set up was higher compared to the potential repellent effect of the garnulates. Thus any repellent effect would not be noticed by a target organism such as the WCR larvae until close contact when it is not possible for escape without death (Brockerhoff and Suckling, 1999). This potential masking effect should have become more pronounced at lower application rates of the killing agent, resulting in reduced repellent effects (Michaelides et al. 1997) and higher mortality rates. Assessment of this mechanism is regarded an important step in the evaluation for an attract and kill strategy, as an insecticide should not compromise the function of the attractant (El – Sayed et al., 2009).

Besides curling and writhing, regurgitation was also described as a symptom caused by a tefluthrin intoxification in southern corn rootworm larvae (*Diabrotica undecimpunctata howardi*), a mechanism helping in a detoxification process after which the larvae were able to recover (Michaelides and Wright, 1997). Regurgitated material was not observed in our experimental set up. At lower application rates in the attract and kill treatment (9 mg), however, we found a decrease in the number of larvae showing knock down symptoms from day 3 and 4 (Fig. 7, HIGH). This indicates that some larvae were affected by the insecticide but were able to recover and moved away from the capsules. A better understanding of such a sublethal effect is important for successfully implementing such a behaviour modifying pest management strategy (Krupke et al., 2002) and needs to be considered when reducing the active ingredient in attract and kill approaches.

The difference in mortality between the attract and kill and the conventional treatments became more evident by reducing the active ingredient. This effect was most probably related to a lack of contact of the larvae with the granulates. The efficacy of a conventional treatment depends on the behavioural preference of larvae feeding on roots in the zone of insecticidal activity (Boetel et al., 2003; Villani and Wright, 1990). Consequently, the spatial and temporal distribution patterns of the larvae in the root system of a plant determine their mortality in a conventional treatment. The spatial analysis of the WCR larval distribution in this study showed that larvae started to aggregate outside the zone of insecticidal activity after 4 hours (Fig. 4A). This resulted in only a minority of larvae (0.33 – 2%) to be affected by the insecticide (Fig. 7). In subsequent measurements larvae started to distribute into the root system with more larvae moving into the zone of insecticidal activity, reflected by

an increase in mortality over time. As previously noted, the set up of the observation device might have caused larvae to move outside the insecticidal zone through increased vertical movement. On the other hand, larvae were forced to move into the insecticidal zone, as 2nd instar larvae need to feed on the roots near the plant base. The size of the insecticidal zone was not defined or measured in this study; however, the distance from the granules to larvae with toxicity symptoms was up to 5 cm at the highest application rate (1.5 g a.i.). This distance was markedly reduced at the lower application rates (0.09 g a.i.) where as little as 6 mm between a larva that was feeding and a larva that showed knock down symptoms was measured (pers. observation). These observations imply that the probability of larvae moving to a zone of insecticidal activity decreases with reduced application rates, lowering the overall mortality of the larvae.

Given an attract and kill treatment the larval distribution patterns become less important as larvae are confined to the attractive source thus increasing the chance of contact with the insecticide. As attraction to the CEC has already been observed after 4 hours, the larvae were directly targeted with a rapid knock down response (up to 10% after 4 hours) (Fig. 7). This observation can have important implications for the implementation of this strategy in the field as larvae may be targeted quite early during their development, prior to their establishment in the root system.

4.4. Future development

For future field application of this attract and kill strategy a higher percentage of larvae need to be affected for sufficient WCR control. Due to the oligophagous feeding mode of WCR larvae (Moeser and Hibbard, 2005), CO_2 only acts as a general nonspecific semiochemical (Johnson et al., 2006). Once they have encountered a root, larvae switch their orientation behaviour to a more host specific semiochemical (Nicolas and Sillans, 1989; Villani and Wright, 1990; Johnson and Gregory, 2006). Various compounds apart from CO_2 have been identified in the orientation of WCR larvae (Bjostad and Hibbard, 1992; Hibbard et al., 1994; Hibbard et al., 1995; Hiltpold et al., 2012, Robert et a., 2012 a,b). Also host specific substances elicit localised search cues (Bernklau et al., 2009) and can act as feeding stimulants (Bernklau and Bjostad, 2008), which showed enhanced insecticide efficacy in laboratory studies (Bernklau and Bjostad, 2005; Bernklau et al., 2011). As their volatility compared to CO_2 is expected to be low (Bernklau et al., 2009) a

combination of these compounds could help to manipulate long and short range orientation of the larvae. This would enable to apply attract and kill components close to or even in the maize rows. For such an application, however, the role of CO_2 for orientation within the root system remains an essential issue for further investigation. Furthermore, more work on the CEC formulation needs to be conducted such as co–encapsulation of artificial CO_2 sources with feeding stimulants or additives for an enhanced and prolonged attraction.

Acknowledgements

We would like to thank Magdolna Weller, Biance Tappe and Isabella Rübig for their technical assistance, Syngenta, Inc (Basel, Switzerland) for providing of Tefluthrin, Dr. Desiree Jakobs–Schönwandt (FH Bielefeld, Germany) for producing the CO_2 emitting capsules. This research was funded by a fellowship for the first author by the German Environmental Foundation (DBU), Germany.

References

Bernklau, E.J., Bjostad, L.B., 1998a. Behavioral responses of first instar western corn rootworm to carbon dioxide in a glass bead bioassay. Journal of Economic Entomology 91, 444 – 456.

Bernklau, E.J., Bjostad, L.B., 1998b. Reinvestigation of host location by western corn rootworm larvae (Coleoptera: Chrysomelidae): CO_2 is the only volatile attractant. Journal of Economic Entomology 91, 1331–1340.

Bernklau, E.J., Bjostad, L.B., 2005. Insecticide enhancement with feeding stimulants in corn for western corn rootworm larvae (Coleoptera: Chrysomelidae). Journal of Economic Entomology 98, 1150 –1156.

Bernklau, E. J., Bjostad, L. B., 2008. Identification of feeding stimulants in corn roots for western corn rootworm (Coleoptera: Chrysomelidae) larvae. Journal of Economic Entomology 101, 2, 341 – 351.

Bernklau, E.J., Bjostad, L.B., Hibbard B.E., 2011. Synthetic feeding stimulants enhance insecticide activity against western corn rootworm larvae, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). Journal of Applied Entomology 135, 47 – 54.

Bernklau, E.J., Bjostad, L.B., Meihls, L.N., Coudron, T.A., Lim, E., Hibbard, B.E., 2009. Localized search cues in corn roots for western corn rootworm (Coleoptera: Chrysomelidae) larvae. Journal of Economic Entomology 102, 558 – 562.

Bernklau, E.J., Fromm, E.A., Bjostad, L.B., 2004. Disruption of host location of western corn rootworm larvae (Coleoptera: Chrysomelidae) with carbon dioxide. Journal of Economic Entomology 97, 330 – 339.

Bjostad, L.B., Hibbard, B.E., 1992. 6–Methoxy–2–benzoxazolinone: A semiochemical for host location by western corn rootworm larvae. Journal of Chemical Ecology 18, 931 – 944.

Blackshaw, R.P., Kerry, B.R., 2008. Root Herbivory in Agricultural Ecosystems, in: Johnson, S.J., Murray, P.J. (Eds.), Root Feeders – An Ecosystem Perspective CABI, Wallingford, UK, pp. 35 – 53.

Boetel, M.A., Fuller, B.W., Evenson, P.D., 2003. Emergence of adult northern and western corn rootworms (Coleoptera: Chrysomelidae) following reduced soil insecticide applications. Journal of Economic Entomology 96, 714 – 729.

Brockerhoff, E.G., Suckling, D.M.,1999. Development of an attracticide against light brown apple moth (Lepidoptera : Tortricidae). Journal of Economic Entomology 92, 853 – 859.

Brown, V.K., Gange, A.C., 1990. Insect herbivory below ground. Advances in Ecological Research 20, 1 – 58.

Chiang, H.C., 1973. Bionomics of the northern and western corn rootworms. Annual Review of Entomology 18, 47 – 72.

Ciosi M., Miller, N.J., Kim, K.S., Giordano, R., Estoup, A., Guillemaud, T., 2008. Invasion of Europe by the western corn rootworm, *Diabrotica virgifera virgifera*: multiple transatlantic introductions with various reductions of genetic diversity. Molecular Ecology 17, 3614 – 3627.

Clark, P.L., Vaughn, T.T., Meinke, L.J., Molina–Ochoa, J., Foster, J.E., 2006. *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) larval feeding behavior on transgenic maize (MON 863) and its isoline. Journal of Economic Entomology 99, 722 – 727.

Dawson, L.A., Byers, R.A., 2008. Methods for Studying Root Herbivory, in: Johnson, S.J., Murray Root, P.J. (Eds.), Root Feeders – An Ecosystem Perspective CABI, Wallingford, UK, pp. 3 – 19.

Doane, J. F., Lee, Y. W., Klingeler, J., Westcott, N. D., 1975. The orientation response of *Ctenicera destructor* and other wireworms (Coleoptera: Elateridae) to germinating grain and to carbon dioxide. Canadian Entomologist 107, 12, 1233 – 1252.

Dutilleul, P., 1993. Modifying the t – test for assessing the correlation between 2 spatial processes. Biometrics 49, 305 – 314.

El–Sayed, A.M., Suckling, D.M., Byers, J.A., Jang, E.B., Wearing, C.H., 2009. Potential of "lure and kill" in long–term pest management and eradication of invasive species. Journal of Economic Entomology 102, 815 – 835.

Evenden, M.L., McLaughlin, J.R., 2004. Factors influencing the effectiveness of an attracticide formulation against the Oriental fruit moth, *Grapholita molesta*. Entomologia Experimentalis Et Applicata 112, 89 – 97.

EPPO, 2011. www.eppo.org/QUARANTINE/Diabrotica_virgifera/diabrotica_virgifera

Füser,B., 2006. Using carbon dioxide to manipulate Western Corn Rootworm movement. Master Thesis, Georg August University, Göttingen.

Gould, F., 1991. Arthropod behaviour and the efficacy of plant protectants. Annual Review of Entomology 36, 305 – 330.

Gray, M.E., Sappington, T.W., Miller, N.J., Moeser, J., Bohn, M.O., 2009. Adaptation and invasiveness of western corn rootworm: Intensifying research on a worsening pest. Annual Review of Entomology 54, 303–321.

Harris, C.R., 1972. Factors influencing the effectiveness of soil insecticides. Annual Review of Entomology 17, 177 – 198.

Harris, D.G., Van Bavel, C.H.M, 1957. Root respiration of tobacco, corn, and cotton Plants. Agronomy Journal 49, 182 – 184.

Harrison, R.D., Gardner, W.A., Tollner, W.E., Kinard, D.J., 1993. X–Ray computed–tomography studies of the burrowing behavior of 4th–Instar pecan weevil (Coleoptera, Curculionidae). Journal of Economic Entomology 86, 1714 – 1719.

Hibbard, B.E., Bernklau, E.J., Bjostad, L.B., 1994. Long–chain free fatty acids: Semiochemicals for host location by western corn rootworm larvae. Journal of Chemical Ecology 20, 3335 – 3344.

Hibbard, B.E., Bjostad, L.B., 1989. Corn semiochemicals and their effects on insecticide efficacy and insecticide repellency toward western corn rootworm larvae (Coleoptera: Chrysomelidae). Journal of Economic Entomology 82, 773 – 781.

Hibbard, B.E., Peairs, F.B., Pilcher, S.D., Schroeder, M.E., Jewett, D.K., Bjostad, L.B., 1995. Germinating corn extracts and 6–Methoxy–2–Benzoxazolinone – western corn rootworm (Coleoptera: Chrysomelidae) larval attractants evaluated with soil insecticides. Journal of Economic Entomology 88, 716 – 724.

Hibbard, B.E., Duran, D.P., Ellersieck, M.R., Ellsbury, M.M., 2003. Post–establishment movement of western corn rootworm larvae (Coleoptera: Chrysomelidae) in Central Missouri corn. Journal of Economic Entomology 96, 599 – 608.

Hiltpold, I., Hibbard, B.E., French, B.W., Turlings, T.C.J., 2012. Capsules containing entomopathogenic nematodes as a Trojan horse approach to control the western corn rootworm. Plant and Soil 358, 1 – 2, 10 – 24.

Hossain, M.S., Williams, D.G., Hossain, M., Norng, S., 2007. Comparison of trap designs for use with aggregation pheromone and synthetic co–attractant in a user–friendly attract and kill system to control *Carpophilus* spp. (Coleoptera: Nitidulidae). Australian Journal of Entomology 46, 244 – 250.

Huang, X.P., Mack, T.P., 2001. Artificial carbon dioxide source to attract lesser cornstalk borer (Lepidoptera: Pyralidae) larvae. Journal of Economic Entomology 94, 860 – 867.

Huang, F. N., Andow, D. A., Buschman, L. L., 2011. Success of the high-dose/refuge resistance management strategy after 15 years of Bt crop use in North America. Entomologia Experimentalis Et Applicata 140, 1, 1 – 16.

Johnson, S.N., Gregory, P.J., 2006. Chemically mediated host plant location and selection by root feeding insects. Physiological Entomology 31, 1 – 13.

Johnson, S.N., Read, D.B., Gregory, P.J., 2004. Tracking larval insect movement within soil using high resolution X–ray microtomography. Ecological Entomology 29, 117 – 122.

Johnson, S.N., Zhang, X.X., Crawford, J.W., Gregory, P.J., Hix, N.J., Jarvis, S.C., Murray, P.J., Young, I.M., 2006. Effects of carbon dioxide on the searching behaviour of the root–feeding clover weevil *Sitona lepidus* (Coleoptera : Curculionidae). Bulletin of Entomological Research 96, 361 – 366.

Kempson, D., Lloyd, M., Ghelardy, R., 1968. A new extractor for woodland litter. Pedobiologia 3, 1 – 30.

Krupke, C.H., Roitberg, B.D., Judd, G.J.R., 2002. Field and laboratory responses of male codling moth (Lepidoptera: Tortricidae) to a pheromone–based attract–and–kill strategy. Environmental Entomology 31, 189 – 197.

Lancashire, P.D., Bleiholder, H., Vandenboom, T., Langeluddeke, P., Stauss, R., Weber, E., Witzenberger, A., 1991. A uniform decimal code for growth stages of crops and weeds. Annals of Applied Biology 119, 561 – 601.

Loesel, P.M., Penners, G., Potting, R.P.J., Ebbinghaus, D., Elbert, A., Scherkenbeck, J., 2000. Laboratory and field experiments towards the development of an attract and kill strategy for the control of the codling moth, *Cydia pomonella*. Entomologia Experimentalis Et Applicata 95, 39 – 46.

Ma, B.L., Meloche, F., Wie, L., 2009. Agronomic assessment of *Bt* trait and seed or soil–applied insecticides on the control of corn rootworm and yield. Field Crops Research 111, 189 – 196.

Macdonald, P.J., Ellis, C.R., 1990. Survival time of unfed, 1st–Instar western corn rootworm (Coleoptera: Chrysomelidae) and the effects of soil type, moisture, and compaction on their mobility in soil. Environmental Entomology 19, 666 – 671.

Mankin, R.W., Johnson, S.N., Grinev, D.V., Gregory, P.J., 2008. New experimental techniques for studying root herbivores, in: Johnson, S.J., Murray , P.J. (Eds.), Root Feeders – An Ecosystem Perspective CABI, Wallingford, UK. pp. 20 – 34.

Meinke, L.J., Sappington, T.W., Onstad, D.W., Guillemaud, T., Miller, N.J., Judith, K., Nora, L., Furlan, L., Jozsef, K., Ferenc, T., 2009. Western corn rootworm (*Diabrotica virgifera virgifera* LeConte) population dynamics. Agricultural and Forest Entomology 11, 29 – 46.

Michaelides, P.K., Cleverly, A.L., Wright, D.J., 1997. Sub–lethal effects of tefluthrin on *Diabrotica undecimpunctata howardi* (Barber) : Plant protection and larval development. Crop Protection 16, 423 – 429.

Michaelides, P.K., Wright, D.J., 1997. Insecticide penetration and symptomology studies on larvae of *Diabrotica undecimpunctata howardi* (Barber). Pesticide Science 49, 353 – 361.

Moeser, J., Hibbard, B.E., 2005. A Synopsis of the nutritional ecology of larvae and adults of *Diabrotica virgifera virgifera* (Le Conte) in the new and old world – Nouvelle cuisine for the invasive maize pest *Diabrotica virgifera virgifera* in Europe?, in Vidal, S., Kuhlmann, U., Edwards C.R. (Eds.), Western Corn Rootworm Ecology and Management, CABI, Wallington, UK, pp. 41 – 65.

Nicolas, G., Sillans, D., 1989. Immediate and latent effects of carbon dioxide on insects. Annual Review of Entomology 34, 97 – 116.

Patel, A.V., Vorlop, K.D., 1994. Entrapment of biological control agents applied to entomopathogenic nematodes. Biotechnology Techniques 8, 8, 569 – 574

Perry, J.N., 1995. Spatial analysis by distance indices. Journal of Animal Ecology 64, 303 – 314.

Perry, J.N., 1998. Measures of spatial pattern for counts. Ecology 79, 1008 – 1017.

Perry, J.N., Dixon, P.M., 2002. A new method to measure spatial association for ecological data count. Ecoscience 9, 133 – 141.

Pimentel, D., 1995. Amounts of pesticides reaching target pests – environmental impacts and ethics. Journal of Agricultural & Environmental Ethics 8, 17 – 29.

Poullot, D., Beslaym, D., Bouvier, C., Sauphanor B., 2001. Is attract–and–kill technology potent against insecticide–resistant Lepidoptera? Pest Management Science 57, 729 – 736.

Reinecke, A., Muller, F., Hilker, M., 2008. Attractiveness of CO_2 released by root respiration fades on the background of root exudates. Basic and Applied Ecology 9, 568 – 576.

Robert, C.A.M., Erb, M., Duployer, M., Zwahlen, C., Doyen, G.R., Turlings, T.C.J., 2012a. Herbivore-induced plant volatiles mediate host selection by a root herbivore. New Phytologist 194, 4, 1061 – 1069.

Robert, C.A.M., Erb, M., Hibbard, B.E., French, B.W., Zwahlen, C.,Turlings, T.C.J., 2012b. A specialist root herbivore reduces plant resistance and uses an induced plant volatile to aggregate in a density-dependent manner. Functional Ecology 26, 6, 1429 – 1440.

Soderlund, D.M., 2010. State-dependent modification of voltage-gated sodium channels by pyrethroids. Pesticide Biochemistry and Physiology 97, 2, 78 – 86.

Spencer, J.L., Hibbard, B.E., Moeser, J., Onstad, D.W., 2009. Behaviour and ecology of the western corn rootworm (*Diabrotica virgifera virgifera* LeConte). Agricultural and Forest Entomology 11, 9 – 27.

Spike, B.P., Tollefson, J.J., 1991. Yield response of corn subjected to western corn–rootworm (Coleoptera: Chrysomelidae) infestation and lodging. Journal of Economic Entomology 84, 1585 – 1590.

Strnad, S.P., Bergman M.K., 1987a. Movement of first–instar western corn rootworms (Coleoptera: Chrysomelidae) in soil. Environmental Entomology 16, 975 – 978.

Strnad, S.P., Bergman, M.K., 1987b. Distribution and orientation of western corn rootworm (Coleoptera: Chrysomelidae) larvae in corn roots. Environmental Entomology 16, 1193 – 1198.

Strnad, S.P., Bergman, M.K., Fulton W.C., 1986. 1st instar western corn rootworm (Coleoptera,Chrysomelidae) response to carbon dioxide. Environmental Entomology 15, 839 – 842.

Strnad, S.P., Dunn P.E., 1990. Host search behavior of neonate western corn rootworm (*Diabrotica virgifera virgifera*). Journal of Insect Physiology 36, 201–205.

Szalai, M., Komaromi, J.P., Bazok, R., Barcic, J.I., Kiss J., Toepfer S (2011) Generational growth rate estimates of Diabrotica virgifera virgifera populations (Coleoptera: Chrysomelidae). Journal of Pest Science 84, 133–142.

Toepfer, S., Burger, R., Ehlers, R.U., Peters, A., Kuhlmann U., 2010. Controlling western corn rootworm larvae with entomopathogenic nematodes: effect of

application techniques on plant–scale efficacy. Journal of Applied Entomology 134, 467–480.

Urias–Lopez M.A., Meinke L.J., Leon G.H., Haile F.J., 2000. Influence of western corn rootworm (Coleoptera:Chrysomelidae) larval injury on photosynthetic rate and vegetative growth of different types of maize. Environmental Entomology 29, 861 – 867.

Van Herk, W.G., Vernon, R.S., (2007). Soil bioassay for studying behavioral responses of wireworms (Coleoptera : Elateridae) to insecticide-treated wheat seed. Environmental Entomology 36, 6, 1441 – 1449.

Van Herk, W.G., Vernon, R.S., Roitberg, B.D., (2008). Repellency of a wireworm, *Agriotes obscurus* (Coleoptera : Elateridae), on exposure to synthetic insecticides in a soil-less bioassay. Environmental Entomology 37, 2, 534 – 545.

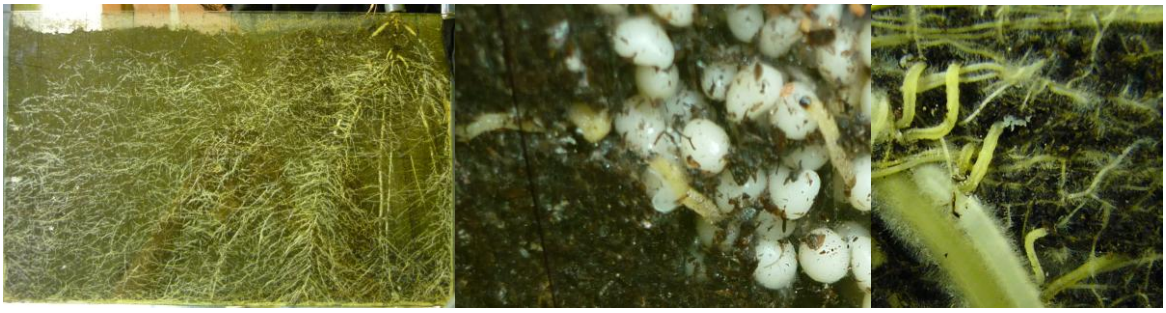
Van Rozen K., Ester, A., 2010. Chemical control of *Diabrotica virgifera virgifera* LeConte. Journal of Applied Entomology 134. 376 – 384.

Villani, M.G., Wright R.J., 1990. Environmetal influences on soil macroarthropod behavior in agricultural systems. Annual Review of Entomology 35, 249 – 269.

Wensler, R.J., 1971. Locomotor and feeding activity of larvae of scarabaeid *Sericesthis geminata* (Coleoptera). Entomologia Experimentalis Et Applicata 14, 270 – 282

Chapter 2

The role of carbon dioxide as an orientation cue for western corn rootworm larvae within the maize root system - implications for an attract and kill approach



Authors: Mario Schumann*, Marina Vemmer, Anant Patel and Stefan Vidal

*Corresponding author

This chapter is accepted for publication in:
Pest Management Science

Please access via: DOI: 10.1002/ps.3602

© 2013 Society of Chemical Industry

The role of carbon dioxide as an orientation cue for western corn rootworm larvae within the maize root system - implications for an attract and kill approach

Mario Schumann[*], Anant Patel[2], Marina Vemmer[2] and Stefan Vidal[1]

[1*]Georg–August University, Department of Crop Science, Agricultural Entomology, Grisebachstr.6, 37077 Göttingen, Germany, Phone: +49(0)551–39 33733, Email: Mario.Schumann@agr.uni–goettingen.de

[2]University of Applied Sciences, Department of Engineering and Mathematics, Engineering and Alternative Fuels, Wilhelm-Bertelsmann-Straße 10, 33602 Bielefeld, Germany

Abstract

BACKGROUND: Western corn rootworm larvae use CO_2 to locate maize roots. However, the importance of CO_2 as a specific orientation cue close to maize roots is not unequivocally investigated. This study aimed at elucidating the effect of CO_2 emitting capsules in combination with a soil insecticide (Tefluthrin = attract and kill) within the root system. We hypothesised that the capsules would result in an aggregation of the larvae at the soil insecticide, thus increasing its efficacy. A non-destructive observation device was used to study larval distribution and behaviour.

RESULTS: Spatial analysis of distance indices (SADIE) revealed an aggregation of the larvae around the capsules in an attract and kill treatment after 4 hours which was not found at the conventional treatment without the capsules. However, larval mortality did not differ in both treatments.

CONCLUSION: CO_2 is a weak attractant for western corn rootworm larvae within the root system. Consequently, an attract and kill strategy based on a CO_2 product will not contribute to a better control compared to conventional Tefluthrin applications. Host specific compounds, combined with a CO_2 source, should be used to target more larvae, making attract and kill feasible as a management option against this pest.

Keywords: *Diabrotica virgifera virgifera*; orientation cue; Tefluthrin; root herbivore

1 INTRODUCTION

The western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), is a serious maize pest in North America.[1] The costs for its management and yield loss exceed more than one billion USD per year.[2] Since the early 1990s multiple introductions[3-4] and stratified dispersal of the WCR[5] have resulted in a spread into more than 20 European countries.[6] A large economic impact on maize production systems is also expected in Europe in a "no control" scenario once the WCR has reached its full potential spread.[7-8] WCR is a univoltine species; the eggs overwinter in the soil and the larvae hatch in spring.[9] The larval stage is the beetle's most significant life stage contributing to economic loss,[10] as their feeding results in a disruption of water and nutrient uptake[11-12] and, at high larval densities, also plant lodging.[13] Chemical control of WCR larvae with soil insecticides, such as granules[14] or seed treatment,[15] is regarded as the main economic control option in regions of Europe with maize monocultures.[16] Targeting pests with a cryptic life stage, such as root herbivores in the soil, is difficult, making control measures ineffective[17] and causing higher insecticide application rates than for above ground pests.[18]

A combination of an attractant, mimicking host plant cues, and a toxic compound, known as an attract and kill, has been shown to improve the efficacy of insecticide applications compared to other control methods.[19] Carbon dioxide (CO_2), a product of root respiration,[20] is a common cue to locate host roots for numerous soil dwelling larvae.[21] Several studies have also identified this compound as an attractant for WCR larvae.[22-24] CO_2 emitting capsules have recently been evaluated as an attractant for WCR larvae and used in an attract and kill approach with Tefluthrin as a killing agent.[25] An attract and kill effect for western corn larvae was found when the compounds were placed 25 cm apart from the maize roots.[25]

We argue that a CO_2 source as part of an attract and kill approach with a soil insecticide needs to attract larvae closer to or even within maize root systems. This is because insecticidal treatments acting against WCR larvae also need to provide root protection.[15] WCR larvae are oligophagous[26] making CO_2 a general non-specific semiochemical to locate roots over a longer range by activating a more directional response and intensifying searching for roots.[27] Upon closer encounter or contact with a root, larvae change to a more localised searching behaviour,[28-29] triggered by

taste receptors.[30] This generally involves host specific semiochemicals,[31-33] and CO_2 is considered of minor importance.

To the best of our knowledge, the role of CO_2 attraction for larvae embedded in a root system has never been considered. In this study we aimed at analysing the attractiveness of CO_2 emitting capsules in an attract and kill strategy to enhance the efficacy of soil insecticides.

2 MATERIALS AND METHODS

A non-destructive observation device was used to examine the distribution and behavioural status of the larvae in the soil. This device consists of a thin soil layer (45 cm x 30 cm x 6 mm) between two glass sheets which was divided into 60 grids with 10 vertical and 6 horizontal layers (each grid: 4.5 cm x 5 cm, Fig. 1) to quantify larval parameters (described in detail below). The observation device was filled with 300 g of a peat soil mixture (FruhstorferErde (Typ 25), HawitaGruppe GmbH). The structure of the soil allowed the larvae to move in the observation device without problems (M. Schumann, personal observation) and the black colour of the soil also enabled observation of the white larvae more effectively. The whole observation device was covered with non-transparent black cloths so that light would not interfere with root growth and larval behaviour.

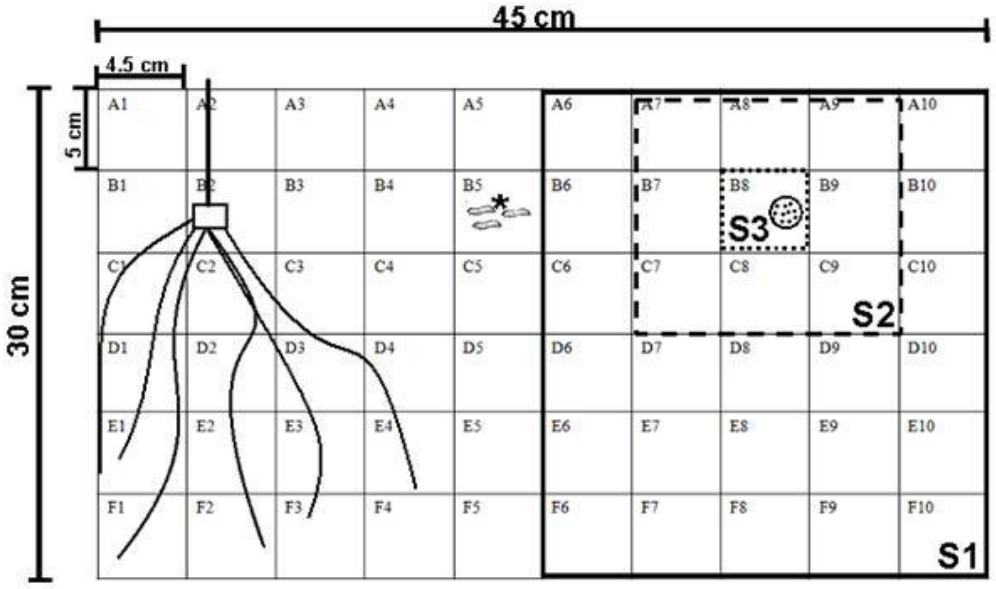


Figure 1 Side view of observation device to quantify WCR distribution and behaviour with a grid made up of 6 horizontal (A – F) and 10 vertical layers (1 – 10) (Total 60 grids: 4.5 x 5 cm each). Square, asterisk and circle represent the application point of the maize seed (Grid B2), WCR larvae (Grid B5) and CO_2 emitting capsules (Grid B8) respectively. The observation device is divided into section S1 (solid line –), section S2 (dashed line ‾ ‾) and section S3 (dotted line ····) for the quantitative analysis of larval distribution.

2.1 Handling of maize and WCR larvae

Maize seeds from the cultivar Prinz (KWS, Einbeck, Germany; not coated with insecticides or fungicides) were used in the experiment. The seeds were surface sterilised with sodium hydroxide for 5 minutes to eliminate bacteria and fungi and soaked in sterile tap water for 12 hours. The seeds were then placed in Petri dishes

on a sterile paper towel, which was previously moistened with sterile tap water, and incubated for 24 hours at 25°C and 65% relative humidity (RH). Most seeds had begun to germinate after this period and seeds with a visible radical root were inserted between the glass sheets (grid B2; Fig.1) at a depth of 7 cm. The plants were grown in the greenhouse (air temperature: 23 ± 2°C ; 65% RH) until they have reached maize growth stage BBCH 31 – 32[34-35] (growth stage V 8 – 9[36]). The plants were then transferred to a quarantine lab (air temperature: 25 ± 1 °C; 65% RH; 14:10 h light:dark) for use in the experiments.

Late 2nd instar larvae were used in the experiment as they were large enough to be observed in the device at this stage of larval development. Larvae from non-diapausing WCR eggs, obtained from USDA–ARS, North Central Agricultural Research Laboratory, Brookings, were reared and removed from the soil for use in the experiments as described in Schumann et al.[25]

2.2 Quantifying the distribution of root biomass

The distribution of root biomass was determined 2 days after the maize plants were transferred to the quarantine lab. The observation device was horizontally placed on a flat surface, the upper glass sheet carefully removed and the soil compartments were cut matching the grid contours. Each soil sample was washed using a 5 mm sieve and the cleaned roots were dried at 60°C for two weeks and then weighed in small plastic boats (scale: H110, Sartorius, Göttingen, Germany). The distribution of root biomass was analysed with four replicates (= four observation devices) in a separate experiment (see below).

2.3 Attract and Kill components

For the attractant, an artificial CO_2 source (commercially available baker's yeast) was encapsulated in Ca-alginate (diameter: 2.3 mm; moisture content: 90%) according to Patel and Vorlop.[37] The capsules will be referred to as CO_2 emitting capsules (CEC) in this study. 5 g of CEC were weighed in small plastic boats (scale: TE 1502s, Sartorius, Germany) before they were applied in the observation device. The granular soil insecticide Force 1.5 G (a.i.: Tefluthrin 1g/100g of the active substance 2, 3, 5, 6 – Tetrafluoro – 4 – methylbenzyl (Z) – (1RS, 3RS) – 3 – (2 – chloro – 3, 3, 3 –trifluoro – 1 – propenyl – 2, 2 – methylcyclopropanecarboxylate), Syngenta, Basel, Switzerland) was used as the kill component. The granules act through a gaseous

phase upon contact by disturbing the function of the peripheral and central nervous system through interference at the voltage gated sodium channels.[38-39] The insecticide blocks the channel closing causing the nerve cells to react in a state of abnormal hyperexcitability. This leads to an incapacitating (= knock down) effect in the insect followed by paralysis and eventually death.[40] 9 mg of the granules (= 0.09 mg a.i. Tefluthrin = 7 g a.i. Tefluthrin/ha) were applied in each observation device which is the equivalent of 4% of the recommended field application rate (= 200 g a.i./ha[41]). The granules were weighed in small glass vials before they were inserted into the observation device (Scale: H110, Sartorius, Germany).

Two treatments ("attract and kill" and "conventional") were set up 4 hours after the maize plants were transferred to the quarantine lab: In the attract and kill treatment the attract and kill components (CEC and Tefluthrin granules) were applied 30 cm from the plant base at 5 – 10 cm depth (grid B8, Fig. 1). Before the components were applied, the soil in grids A8+B8 (Fig. 1) was removed with a spatula. The soil removed from B8 was mixed with the CEC and half of the CEC (~2.5g) were inserted between the glass sheets; then the Tefluthrin granules were applied through a thin glass rod (10 cm length and 3 mm diameter). By placing the glass rod between the glass sheets, the granules could be applied at the required soil depth. A plastic funnel connected to the top of the glass rod ensured that granules would not be spilled during the application. The remaining CEC were inserted, so that the granules were surrounded by the CEC. The soil initially removed from grid A8 was placed on top of both components.

For the conventional treatment the same application rate of Tefluthrin granules were directly applied at the original sowing point of the plant at a depth of 7 cm (grid B2, Fig. 1). 4.5 mg of the granules were applied on each side of the seed by removing a small amount of surrounding soil. The application was also done with the thin glass rod/plastic funnel apparatus (previously described). Six replicates (= six observation devices) were set up for each treatment.

2.4 Assessment of larval parameters

2.4.1 Larval distribution and behaviour

2 days after the treatments were set up, 100 2nd instar larvae were inserted at a depth of 7 cm, 15 cm apart from the original sowing point of maize (grid B5, Fig.1).

The larvae were inserted into the soil through a plastic tube, previously inserted between the glass sheets, to ensure that they were all applied at the required depth. 4 hours later and subsequently every 24 hours after the application of the larvae, the number and the behavioural status of the larvae at each grid (Fig. 1) was recorded by visually inspecting both sides of the device. To reduce perturbing effects on the larvae during assessment, the observation devices were transferred to a dark room. The black cloths were removed and each grid was analysed with a white spotlight, illuminating the grid to be analysed only to avoid any disturbance of neighbouring grids.

2.4.2 Mortality of WCR larvae

To determine the mortality of larvae, each grid in an observation device was specifically examined for larvae that showed typical symptoms from Tefluthrin intoxication usually expressed by larvae as writhing and curling.[42-43] These larvae were recorded as “knocked down larvae”, or, when not moving, “dead larvae” during visual inspection. The position (i.e. the corresponding grid) and the number of knock down and dead larvae was recorded. The total number of knocked down and dead larvae in an observation device was divided by the number of larvae placed in the device (100 2nd instar larvae), giving the percentage mortality in each treatment during the 4 day period of examination. Due to difficulties to decide whether a larva was finally dead, as larvae did not immediately start to curle and writhe when illuminated by the spotlight, the number of knocked down and dead larvae was combined for the analyses. The experiments were terminated after 4 days as most larvae had moved off their point of insertion (grid B5, Fig. 1). Also larvae, that were killed off shortly after insertion, started to disintegrate, making it increasingly difficult to find them.

2.4.3 Dispersal and spatial distribution of WCR larvae

As a very low mortality rate was quantified after 4 hours (<2%) the effect of Tefluthrin was considered minimal, so we were able to determine the influence of CEC on larval distribution at this time point. For assessment of their distribution, the number of larvae at each grid was recorded 4 hours after the insertion of the larvae into the observation device. Some larvae started to curl up and did not move at the point of insertion (grid B5; Fig. 1), most likely due to stress or damage from transfer into the

device. These larvae were regarded as “non-dispersing larvae” and not included in the analysis. In addition to larval distribution, the dispersal of the larvae in the observation device was also recorded. We defined dispersal of WCR larvae as “number of positive grids” (= grids WCR larvae were observed at).

Apart from the spatial analysis of distance indices (SADIE; see below), the distribution of the larvae was quantified by summarising the total number of larvae observed: i) up to 25 cm from the CEC (section S1: All grids in columns 6-10), ii) up to 10 cm around the CEC (section S2: grids A 7-9, B 7-9 and C 7-9) and iii) directly at the CEC (section S3: grid B8) (Fig. 1). With these sections the attractiveness of the CEC for the larvae could be quantified at varying distances from CEC. The number of larvae in each section was divided by the total number of dispersing larvae counted in the observation device (= % of dispersing larvae).

2.5 Spatial and statistical analysis

2.5.1 Spatial analysis

The spatial distribution of WCR larvae was analyzed using spatial analysis by distance indices (SADIE). This program quantifies the spatial pattern in a sampled population and measures the degree of non-randomness in two dimensional spatial patterns.[44] An index of aggregation (I_a) was calculated from the total number of larvae observed in all replicates (128 in the conventional and 169 in the A&K treatment) by calculating the minimum distance that the observed counts individuals would need to move for complete regularity (D). These observed counts can be randomly allocated to calculate D again. This can be done for a number of randomizations (26058 were used in this study) to get the arithmetic mean D (= E_a). These indices then give the aggregation index ($I_a = D/E_a$), where $I_a = 1$, $I_a > 1$ and $I_a < 1$ indicates random, aggregated and regular arrangements (i.e. equal number of larvae in each grid) counts, respectively.[45] A probability (P_a) tests for deviations from random dispersion, where $P_a > 0.975$ indicates regular dispersion; $P_a < 0.025$ spatial aggregation, and $0.025 < P < 0.975$, randomness. A subsidiary index J_a indicates the presence of one major cluster ($J_a > 1$) or multiple clusters ($J_a \leq$ when $I_a > 1$) in the distribution of the larvae.[46]

SADIE also allows calculating the contribution of each grid to local clustering, expressed as unit - less sub - indices v_i and v_j, where v_i values > 1 contribute to

patches and v_j values < 1 to gaps. We used these indices to develop contour maps of the spatial distribution of the larvae after 4 hours in a conventional and an attract and kill treatment.[47] For local clustering we used the total number of dispersing larvae observed for each grid combined from all 6 replicates after 4 hours.

Another feature of SADIE tests statistical association between the distributions of two groups of data with an index of association (X). This index is calculated by the extent of the correlation of each local cluster index in both distributions. Positive values (= association) are generated by a coincidence of two patches or gaps (i.e. a positive or a negative local cluster index respectively), whereas negative values (= disassociation) result from a patch coinciding with a gap in both distributions. The mean of local association of the two distributions give the overall index of association (X).[45] A significance of X was tested against X_{rand} from a randomization test that included an adjustment procedure.[48] At the 5% significance level, the statistic P < 0.025 indicated significant association and P > 0.975 indicates significant disassociation. We tested the association of WCR larval distributions by comparing the conventional and the attract and kill treatment after 4 hours.

2.5.2 Statistical analysis

The dispersal (= number of positive grids) and recovery of the larvae after 4 hours was tested for significance with the Student`s t - test. The percentage of recovered WCR larvae in section S1 and S2 after 4 hours were arc sine transformed and also tested with a Student`s t - test. The percentage of recovered WCR larvae in section S3 was tested with a Mann Whitney U test. The mortality of larvae was arc sine transformed and tested with a repeated measures ANOVA with mortality as dependent and time and treatment as the independent variables. The Student`s t - test was applied when the normal distribution of the data (tested with Shapiro Wilks test and visual assessment of histogram) and the homogeneity of variances (tested with Leven`s test) were given.All statistical analyses were carried out with Statistica, Version 10.[49]

3 RESULTS

3.1 Distribution of root biomass

Root material was recovered from 42.50 ± 5.92 grids of the observation device (Fig. 2), thus covering 956.25 cm^2 of the 1350 cm^2 area given in the observation device (~ 71% of total area). The total dry root biomass recovered from all grids was 3.06 ± 0.91 g per plant.

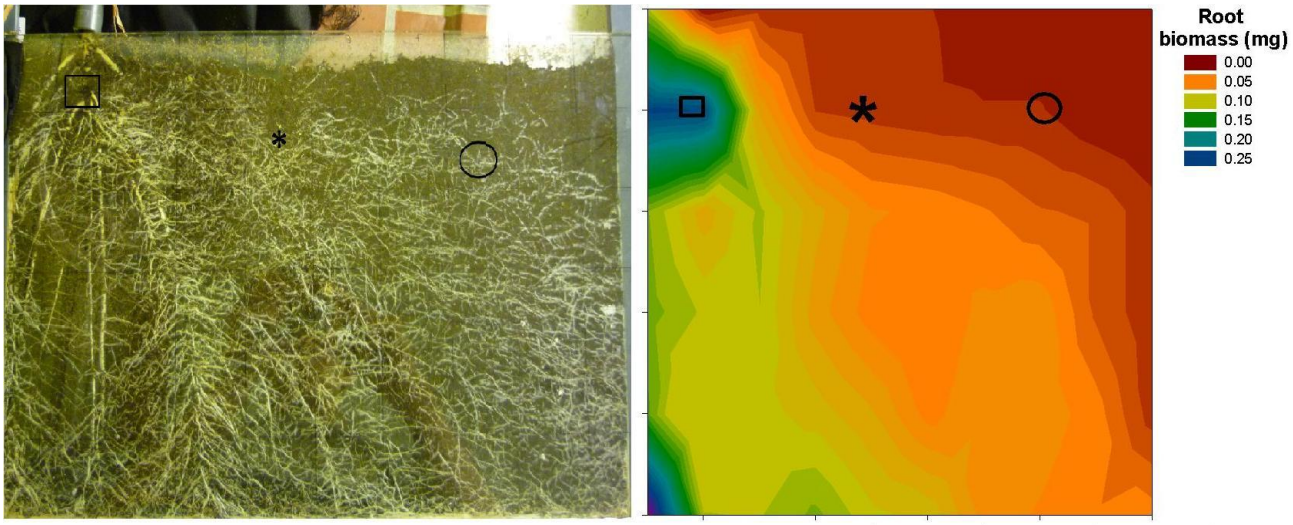


Figure 2 Spread of the maize root system (Left) and distribution of root biomass in the observation device at growth stage BBCH 32 (Right). Square, asterisk and circle represent the application point of the maize seed, western corn rootworm larvae and CO_2 emitting capsules respectively.

3.2 Larval parameters

3.2.1 Recovery of WCR larvae in observation device

After 4 hours 11.17 ±2.95 and 10.00 ±2.27 of non-dispersing larvae were counted in the conventional and attract and kill treatment, respectively. Thus, for the calculation of WCR larval dispersal and distribution, 20.67±2.30 and 28.50±4.60 dispersing larvae were observed in the conventional and the attract and kill treatment, respectively (t: -1.45; d.f. = 10; P = 0.18), giving a recovery of 21-28% across both treatments.

3.2.2 Dispersal of WCR larvae

After 4 hours a slightly higher, but non-significant, dispersal of the larvae was measured in the attract and kill treatment (16.50 ±2.74 positive grids) than in the conventional treatment (11.17 ±1.45 positive grids) (t: - 1.72; d.f. = 10; P = 0.12) (Fig. 3).

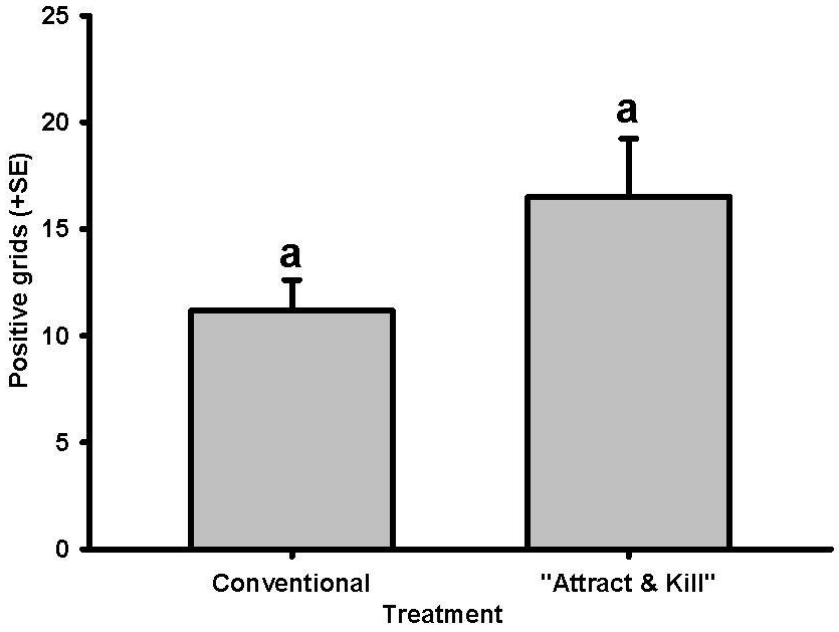


Figure 3 Dispersal of western corn rootworm larvae after 4 hours in a conventional and attract and kill treatment. Dispersal is determined by the number of grids western corn rootworm larvae were observed at (= “positive grid”)

3.2.3 Spatial analysis of WCR larval distribution

Larvae were significantly aggregated in both treatments (I_a> 1) and formed a major cluster (J_a> 1) 4 hours after their insertion. There was also a significant association between the distribution of larvae in an attract and kill and conventional treatment (X = 0.63) (Tab. 1).

Table 1 Spatial parameters of western corn rootworm (WCR) larval distribution using Spatial Analysis of Distance indices (SADIE) 4 hours past the insertion into the observation device with a conventional and an attract and kill treatment.

TREATMENT	I_a	P	J_a	P	X	P
Conventional	1.16	< 0.01	1.33	< 0.01	0.63	< 0.01
Attract & Kill	1.60	< 0.01	1.31	< 0.01		

I_a and its associated P – value indicate the aggregation of an overall spatial pattern and associated significance test of the spatial pattern’s departure from randomness. I_a >1 indicates an aggregated distribution and a significant spatial aggregation is assumed at P < 0.025. J_a ≤ 1 indicates the presence of multiple clusters when I_a > 1 and one major cluster when J_a > 1. X is the measure of spatial association between the distribution of WCR. X is the index of association and indicates when X > 0 that two populations are associated and are considered significantly associated when P < 0.025

In the conventional treatment the highest level of clustering of WCR larvae was measured around (grids B4 and B6) and directly at (grids A5 and B5) the grids WCR larvae were inserted at. In the attract and kill treatment strong clustering of WCR larvae was found around the grids of their insertion (grids B4 and B6) but also directly at and around the grids where CO_2 emitting capsules (CEC) were applied (grids B8 and B7 and C8 respectively; Fig. 4).

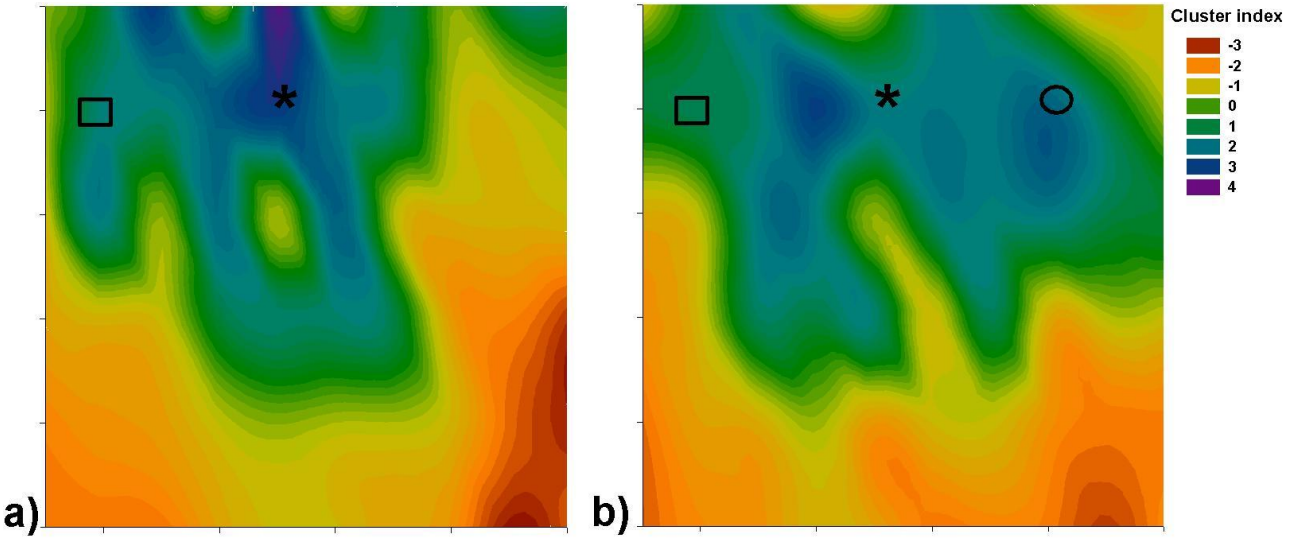


Figure 4 Distribution of WCR larvae 4 hours past insertion into the observation device in a) a conventional and b) an attract and kill treatment. Contour maps are based on local cluster indices calculated by SADIE. A positive local cluster index > 1.5 indicates significant clustering of large counts (= number of larvae) close to one another (blue/purple), whereas an index of < -1.5 contributes significantly to a gap i.e. no or low counts to one another (brown). Square, asterix and circle represent the application point of the maize seed, WCR larvae and CO_2 emitting capsules respectively.

3.2.4 Quantitative analysis of WCR larval distribution

52.72 ±4.74% of the dispersing larvae were observed in section S1 of the observation device after 4 hours in the attract and kill treatment compared to 42.93 ± 8.24% in the conventional treatment (t: - 1.05; d.f. = 10; P = 0.32) (Fig. 5, S1). In section S2 31.35 ±4.47% of the dispersing larvae were observed in the attract and kill treatment which was significantly higher than in the conventional treatment (13.98 ±1.36%) (t: - 3.95; d.f. = 10; P < 0.01) (Fig. 5, S2); in section S3 2.66 ±1.20% of the dispersing larvae were observed with an attract and kill treatment and 0.69 ± 0.69% with a conventional treatment (t: – 1.31; d.f. = 10; P = 0.22) (Fig. 5, S3).

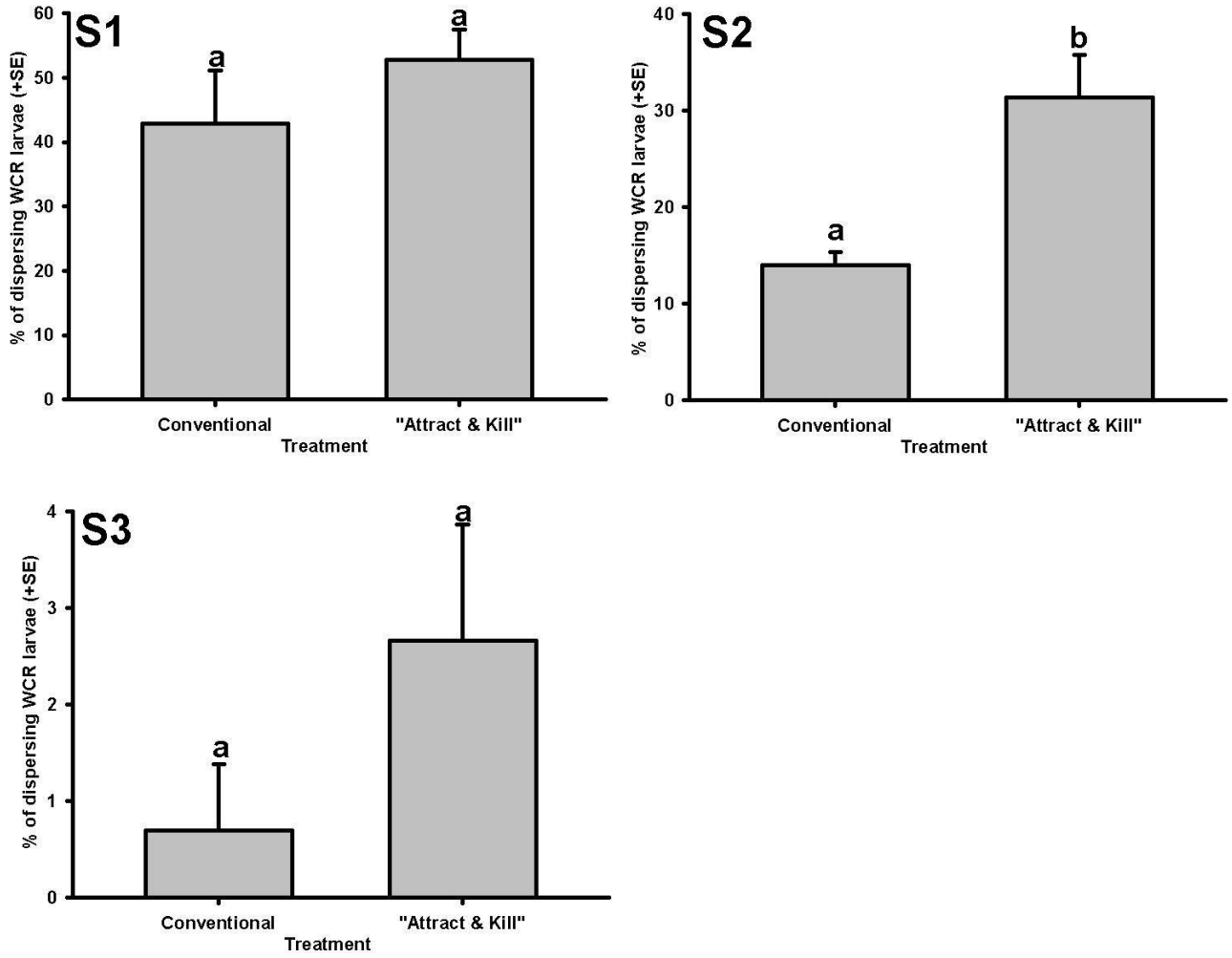


Figure 5 Percentage of dispersing WCR larvae observed 4 hours past their insertion in section S1, S2 and S3 of the observation device in a conventional and attract and kill treatment. Different lowercase letters indicate a significant difference between the treatments (Section S1 & S2: Student's t – test; Section S3: Mann Whitney U test). Please note the changing scale!

3.2.5 Mortality of WCR larvae

Larvae showing either knock down symptoms or being dead were observed in 4 grids in the conventional treatment (Grid A1; A2 & B2; A3) and 2 grids in the attract and kill treatment (Grid B8 & C9). There was a significant effect on mortality by time after insertion of WCR larvae ($F_{4,40} = 58.16$; $P < 0.001$) but not by treatment ($F_{1,10} = 0.05$; $P = 0.82$) or an interaction of both ($F_{4\ 40} = 2.06$; $P = 0.10$). Mortality rates of 0.67 ±0.42% in the attract and kill and 1.33 ±0.42% in the conventional treatment were measured after 4 hours. These increased to 16.50 ±1.61% and 15.17 ±1.42% after 4 days in the attract and kill and conventional treatment, respectively (Fig. 6).

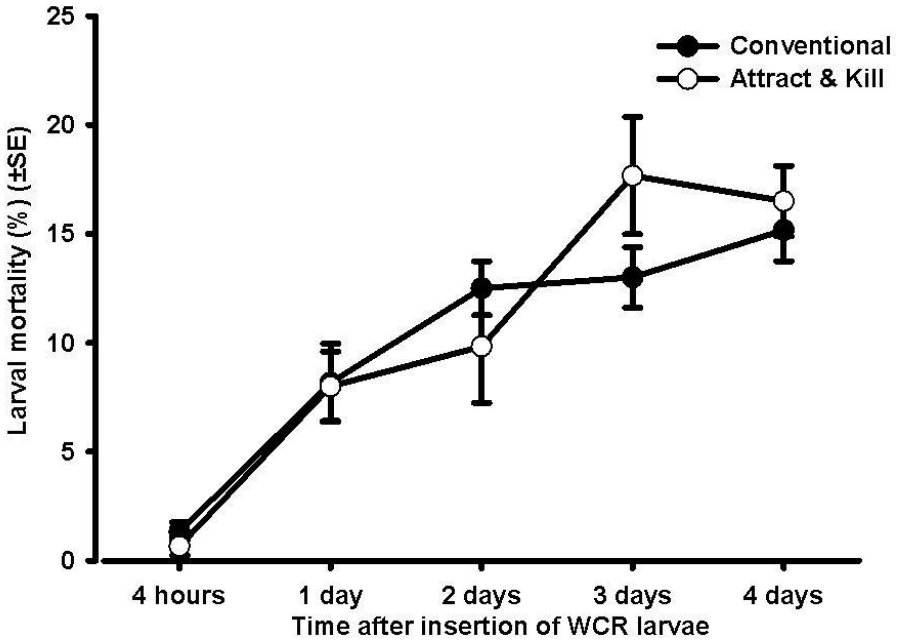


Figure 6 Percentage larval mortality (WCR larvae with knock down symptoms and regarded as dead) with an attract and kill and a conventional treatment of Tefluthrin 4 hours, 1 day, 2 days, 3 days and 4 days past the insertion into the observation device.

4 DISCUSSION

By visual inspection of western corn rootworm (WCR) larval movement and behaviour we were able to prove that CO_2 emitting capsules (CEC) are able to influence the spatial distribution of the larvae within the maize root system. The larval mortality in the attract and kill treatment, however, was low and did not significantly increase compared to the conventional treatment. This makes CO_2 a weak attractant for larval orientation within a root system and suggests that additional cues are also involved.

Despite the oligophagous nature of WCR larvae,[26] the spatial (Fig. 4) and quantitative (Fig. 5) analysis of the larval distribution indicate that CEC attract WCR larvae. This makes CO_2 an attractant for WCR larvae within the root system. Its function as an orientation cue could be explained by studying the movement of the larvae at a plant scale: WCR larvae change their distribution within the root system during their development[50-51] or move to a new root system as a result of heavy root damage [52]. When the larvae start feeding in a root system, they aggregate by feeding on root tips at the periphery of the root system.[25] This preference for root tips was also observed with neonate larvae feeding on maize seedlings in a transparent gel medium.[53] Root tips are the point of new root cell formation and higher CO_2 production.[54] Considering that WCR larvae are capable of detecting small changes (~12%) in CO_2 concentrations[55] and actively forage in the root system to exploit nutritious root tissue,[56] CO_2 may be an indicator for fresher and more suitable root material. Chemical defences are also expected to be low in new root tissue,[57] so that feeding at a root tip might also help the larvae to overcome the defence systems of the plant. Overall WCR actively seek new roots during their development and might follow a new CO_2 gradient during root switching.[29] Using 2nd instar larvae to study larval orientation is a slight drawback of this study as neonate larvae are the most important life stage in host finding.[58] However, neonate larvae are too small for behavioural observations whilst maintaining thigmotactic cues of the soil environment.[55]

In the attract and kill and conventional treatment WCR larvae showed signs of intoxication (= writhing and curling of WCR larvae) from Tefluthrin 24 hours after their insertion; however only a low mortality rate of 15 -16% could be measured in both treatments after 4 days. The low mortality in the conventional treatment (15.17%) can primarily be related to the low application rate of Tefluthrin (~4 % of the

recommended application rate in the field) used in this study. The majority of WCR larvae must have fed beyond the zone of insecticidal activity, most likely due to behavioural preferences of larvae for roots not reached by the gaseous phase of the insecticidal compound[59] (e.g. on the root tips as discussed above). The 4 day period used in this study to examine WCR larval mortality was probably also not long enough to measure high mortality rates. Consequently, the time period to study larval mortality needs to be extended in future experiments with higher insecticide application rates. The sensitivity to Tefluthrin may also change during WCR larval development as shown in southern corn rootworm (*Diabrotica undecimpunctata howardi*) larvae.[60] This implies that use of 2nd instar WCR larvae may have also contributed to a low mortality as the susceptibility is expected to be lower than with 1st instar WCR larvae.

Using the attract and kill approach to lure the larvae into the zone of insecticidal activity did not increase the efficacy of Tefluthrin and resulted in a similar mortality rate (16.50%) as in the conventional treatment. WCR larval mortality rate was also lower compared to an application of the attract and kill components at equivalent insecticide application rates 25 cm apart from the root system, where 38% of the larvae were targeted.[25] This adds evidence to the finding that CO_2 attraction is lower in the presence of plant roots,[61] but should not totally be discarded as an olfactory cue in the presence of maize roots.

The low mortality rate in the attract and kill treatment after 4 hours indicates that the CEC affected the distribution of the larvae but did not attract larvae within the zone of insecticidal activity (< 2% larval mortality; Fig. 6). The availability of roots around the CEC might have hindered the larvae to move within this zone. The addition of a more specific host volatile compound may result in an attraction directly to the Tefluthrin granules. Host specific compounds can trigger localised searching behaviour[62] or act as feeding stimulants.[63] The latter has already been used to enhance insecticide efficacy in laboratory studies[42] and also enabled to use active ingredient compounds that need to be taken up by ingestion, such as Thiamethoxam based products.[64] 6 – methoxy 2 – benzoxazolinone (MBOA)[65] or fatty acids,[66] are also involved in the orientation of WCR larvae and attract the larvae as a synthetic blend of fatty acids, sugars and MBOA coated on alginate capsules.[67] Furthermore ethylene, a gaseous phytohormone,[68] and (E)-b-caryophyllene (EBC) acted as attractants in addition to CO_2.[69-70] The latter compound is a sesquiterpene emitted by damaged maize roots

upon larval feeding[71] with a peak emission after 10 hours of larval feeding.[72] In our experimental set-up root damage during the insertion of the components Tefluthrin, CEC and WCR could not be avoided during handling, potentially interfering with the larval distribution in the observation devices. A mechanical damage of maize roots, however, only causes a short burst in EBC emission 3 hours after induction.[72] Thus we assume that EBC release by damaged maize roots can be regarded as negligible in our experimental set-up.

We argue that once a reliable orientation cue has been identified, a prolonged and steady release of the cue should be guaranteed to make a behaviour based strategy feasible in the field. This could be achieved through the formulation of products that continuously emit the orientation cue over a couple of weeks. A number of CO_2 emitting products have previously been tested by Bernklau et al.[23] under laboratory and field conditions. The unformulated baker's yeast tested in their study did not attract WCR larvae, which the authors explained by potential repellent effects from secondary metabolites produced by the test compound. The results of our study and Schumann et al.[25] demonstrated that a formulated baker's yeast does not appear to have a repellent effect on WCR larvae. Using an encapsulated baker's yeast might have changed or lowered the production of secondary metabolites, thus reducing the repellent effect. Bernklau et al.[23] also tested dried yeast granules mixed with maize based products which diverted larvae away from maize roots. Taking these and our results into account we hypothesize that the use of Baker's yeast as an attractant for WCR larvae will depend on its formulation.

5 CONCLUSIONS

The potential to use CO_2 for an attraction of WCR larvae within the root system could be an important step towards an attract and kill approach under field conditions. Future research on additional orientation cues of soil dwelling larvae within a root system needs to be given more attention to improve attract and kill strategies and subsequently increase its feasibility for a reliable control of the larvae in the field.

Acknowledgements

We would like to thank Bianca Tappe and Isabella Rübig for their technical assistance, Syngenta, Inc (Basel, Switzerland) for the provision of Force 1.5G. This research was funded by the German Environmental Foundation (DBU).

References

1. Meinke LJ, Sappington TW, Onstad DW, Guillemaud T, Miller NJ, Judith K, Nora L, Furlan L, Jozsef K and Ferenc T, Western corn rootworm (*Diabrotica virgifera virgifera* LeConte) population dynamics. *Agric For Entomol* **11**: 29-46 (2009).
2. Sappington TW, Siegfried BD and Guillemaud T, Coordinated *Diabrotica* genetics research: Accelerating progress on an urgent insect pest problem. *American Entomologist* **52**: 90-97 (2006).
3. Ciosi M, Miller NJ, Kim KS, Giordano R, Estoup A and Guillemaud T, Invasion of Europe by the western corn rootworm, *Diabrotica virgifera virgifera*: multiple transatlantic introductions with various reductions of genetic diversity. *Mol Ecol* **17**: 3614-3627 (2008).
4. Miller N, Estoup A, Toepfer S, Bourguet D, Lapchin L, Derridj S, Seok Kim K, Renaud P, Furlan L and Guillemaud T, Multiple transatlantic introductions of the western corn rootworm. *Science* **310**: 992 (2005).
5. Ciosi M, Miller NJ, Toepfer S, Estoup A and Guillemaud T, Stratified dispersal and increasing genetic variation during the invasion of Central Europe by the western corn rootworm, *Diabrotica virgifera virgifera*. *Evolutionary Applications* **4**: 54-70 (2011).
6. EPPO, *Diabrotica virgifera virgifera*. http://www.eppo.org/QUARANTINE/Diabrotica_virgifera/diabrotica_virgifera.htm [accessed 17.06. 2011].
7. Dillen K, Mitchell PD and Tollens E, On the competitiveness of *Diabrotica virgifera virgifera* damage abatement strategies in Hungary: a bio-economic approach. *J Appl Entomol* **134**: 395-408 (2010).
8. Wesseler J and Fall EH, Potential damage costs of *Diabrotica virgifera virgifera* infestation in Europe - the 'no control' scenario. *J Appl Entomol* **134**: 385-394 (2010).
9. Chiang HC, Bionomics of the Northern and Western Corn Rootworms. *Annual Review of Entomology* **18**: 47-72 (1973).
10. Spencer JL, Hibbard BE, Moeser J and Onstad DW, Behaviour and ecology of the western corn rootworm (*Diabrotica virgifera virgifera* LeConte). *Agric For Entomol* **11**: 9-27 (2009).

11. Kahler AL, Olness AE, Sutter GR, Dybing CD and Devine OJ, Root damage by western corn rootworm and nutrient content in maize. *Agronomy Journal* **77**: 769-774 (1985).
12. Gavloski JE, Whitfield GH and Ellis CR, Effect of larvae of western corn rootworm (Coleoptera: Chrysomelidae) and of mechanical root pruning on sap flow and growth of corn. *J Econ Entomol* **85**: 1434-1441 (1992).
13. Spike BP and Tollefson JJ, Yield response of corn subjected to western corn rootworm (Coleoptera: Chrysomelidae) infestation and lodging. *J Econ Entomol* **84**: 1585-1590 (1991).
14. Mayo ZB and Peters LL, Planting vs cultivation time applications of granular soil Insecticides to control larvae of corn rootworms (Coleoptera:Chrysomelidae) in Nebraska. *J Econ Entomol* **71**: 801-803 (1978).
15. Furlan L, Canzi S, Di Bernardo A and Edwards CR, The ineffectiveness of insecticide seed coatings and planting-time soil insecticides as *Diabrotica virgifera virgifera* LeConte population suppressors. *J Appl Entomol* **130**: 485-490 (2006).
16. van Rozen K and Ester A, Chemical control of *Diabrotica virgifera virgifera* LeConte. *J Appl Entomol* **134**: 376-384 (2010).
17. Hossain MS, Williams DG, Hossain M and Norng S, Comparison of trap designs for use with aggregation pheromone and synthetic co-attractant in a user-friendly attract and kill system to control *Carpophilus* spp. (Coleoptera : Nitidulidae). *Aust J Entomol* **46**: 244-250 (2007).
18. Blackshaw RP and Kerry BR. Root Herbivory in Agricultural Ecosystems. In *Introduction: Root Feeders – An Ecosystem Perspective*, ed. by Johnson SJ and Murray PJ. CABI: Wallingford,UK, pp. 35 - 53 (2008).
19. El-Sayed AM, Suckling DM, Byers JA, Jang EB and Wearing CH, Potential of "Lure and Kill" in long-term pest management and eradication of invasive species. *J Econ Entomol* **102**: 815-835 (2009).
20. Kuzyakov Y and Larionova AA, Root and rhizomicrobial respiration: A review of approaches to estimate respiration by autotrophic and heterotrophic organisms in soil. *J Plant Nutr Soil Sci* **168**: 503-520 (2005).
21. Johnson SN and Nielsen UN, Foraging in the dark - Chemically mediated host plant location by belowground insect herbivores. *J Chem Ecol* **38**: 604-614 (2012).

22. Bernklau EJ and Bjostad LB, Behavioral responses of first instar western corn rootworm to carbon dioxide in a glass bead bioassay. *J Econ Entomol* **91**: 444-456 (1998).
23. Bernklau EJ, Fromm EA and Bjostad LB, Disruption of host location of western corn rootworm larvae (Coleoptera: Chrysomelidae) with carbon dioxide *J Econ Entomol* **97**: 330-339 (2004).
24. Strnad SP, Bergman MK and Fulton WC, 1st instar western corn rootworm (Coleoptera, Chrysomelidae) response to carbon dioxide. *Environ Entomol* **15**: 839-842 (1986).
25. Schumann M, Patel A and Vidal S, Evaluation of an attract and kill strategy for western corn rootworm larvae. *Applied Soil Ecology* **64**: 178-189 (2013).
26. Moeser J and Hibbard BE. A synopsis of the nutritional ecology of larvae and adults of *Diabrotica virgifera virgifera* (Le Conte) in the New and Old World – Nouvelle cuisine for the invasive maize pest *Diabrotica virgifera virgifera* in Europe? In *Western Corn Rootworm- Ecology and Management*, ed. by Vidal S, Kuhlmann U and Edwards CR. CABI: Wallington,UK, pp. 41-65 (2005).
27. Johnson SN, Zhang XX, Crawford JW, Gregory PJ, Hix NJ, Jarvis SC, Murray PJ and Young IM, Effects of carbon dioxide on the searching behaviour of the root-feeding clover weevil *Sitona lepidus* (Coleoptera : Curculionidae). *Bulletin of Entomological Research* **96**: 361-366 (2006).
28. Bernklau EJ, Bjostad LB, Meiiils LN, Coudron TA, Lim E and Hibbard BE, Localized search cues in corn roots for western corn rootworm (Coleoptera: Chrysomelidae) larvae. *J Econ Entomol* **102**: 558-562 (2009).
29. Strnad SP and Dunn PE, Host search behavior of neonate western corn rootworm (*Diabrotica virgifera virgifera*). *Journal of Insect Physiology* **36**: 201-205 (1990).
30. Branson TF, Olfactory response of larvae of *Diabrotica virgifera virgifera* to plant roots. *Entomology Experimental and Application* **31**: 303-307 (1982).
31. Johnson SN and Gregory PJ, Chemically mediated host plant location and selection by root feeding insects. *Physiological Entomology* **31**: 1-13 (2006).
32. Nicolas G and Sillans D, Immediate and latent effects of carbon dioxide on insects. *Annual Review of Entomology* **34**: 97-116 (1989).

33. Villani MG and Wright RJ, Environmetal influences on soil macroarthropod behavior in agricultural systems. *Annual Review of Entomology* **35**: 249-269 (1990).
34. Lancashire PD, Bleiholder H, Vandenboom T, Langeluddeke P, Stauss R, Weber E and Witzenberger A, A Uniform Decimal Code for Growth-Stages of Crops and Weeds. *Annals of Applied Biology* **119**: 561-601 (1991).
35. Julius Kühn-Institut, *The extended BBCH-scale*. http://pub.jki.bund.de/index.php/BBCH/article/view/465/415 [accessed 30.05.2013].
36. Nafziger E. Corn. In *Illinois Agronomy Handbook: 24th Edition*, ed. by Service UoIaU-CCE. University of Illinois at Urbana-Champaign, College of Agriculture, Cooperative Extension Service (2012).
37. Patel AV and Vorlop KD, Entrapement of biological control agents applied to entomopathogenic nematodes. *Biotechnol Tech* **8**: 569-574 (1994).
38. Soderlund DM, State-dependent modification of voltage-gated sodium channels by pyrethroids. *Pesticide Biochemistry and Physiology* **97**: 78-86 (2010).
39. Insecticide Resistance Action Committee, IRAC MoA Classification Scheme. www.irac-online.org/content/uploads/MoA-classification.pdf [accessed 29.05.2013].
40. Davies TGE, Field LM, Usherwood PNR and Williamson MS, DDT, pyrethrins, pyrethroids and insect sodium channels. *IUBMB Life* **59**: 151-162 (2007).
41. Austrian Federal Office of Food Safety, Plant Protection Product Register-Force 1,5 G. http://pmg.ages.at/export/PMG/PMG/web/reg/3293_0_e.html [accessed 30.05.2013].
42. Bernklau EJ, Bjostad LB and Hibbard BE, Synthetic feeding stimulants enhance insecticide activity against western corn rootworm larvae, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). *J Appl Entomol* **135**: 47-54 (2011).
43. Michaelides PK and Wright DJ, Insecticide penetration and symptomology studies on larvae of *Diabrotica undecimpunctata howardi* (Barber). *Pesticide Science* **49**: 353-361 (1997).
44. Perry JN, Spatial analysis by distance indices. *Journal of Animal Ecology* **64**: 303-314 (1995).

45. Perry JN and Dixon PM, A new method to measure spatial association for ecological data count. *Ecoscience* **9**: 133-141 (2002).
46. Perry JN, Measures of spatial pattern for counts. *Ecology* **79**: 1008-1017 (1998).
47. Systat Software I. SigmaPlot for Windows Version 11.0 (2008).
48. Dutilleul P, Modifying the T-Test for Assessing the Correlation between 2 Spatial Processes. *Biometrics* **49**: 305-314 (1993).
49. StatSoft I, STATISTICA for Windows (data analysis software), Version 10.0. www.statsoft.com. [accessed 01.02] 2013.
50. Schumann M and Vidal S, Dispersal and spatial distribution of western corn rootworm larvae in relation to root phenology. *Agric For Entomol* **14**: 331-339 (2012).
51. Strnad SP and Bergman MK, Distribution and orientation of western corn rootworm (Coleoptera: Chrysomelidae) larvae in corn roots. *Environ Entomol* **16**: 1193-1198 (1987).
52. Hibbard BE, Duran DP, Ellersieck MR and Ellsbury MM, Post-establishment movement of western corn rootworm larvae (Coleoptera : Chrysomelidae) in Central Missouri corn. *J Econ Entomol* **96**: 599-608 (2003).
53. Clark PL, Vaughn TT, Meinke LJ, Molina-Ochoa J and Foster JE, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) larval feeding behavior on transgenic maize (MON 863) and its isoline. *J Econ Entomol* **99**: 722-727 (2006).
54. Bidel LPR, Renault P, Pages L and Riviere LM, An improved method to measure spatial variation in root respiration: application to the taproot of a young peach tree *Prunus persica*. *Agronomie* **21**: 179-192 (2001).
55. Bernklau EJ and Bjostad LB, Behavioral responses of first-instar western corn rootworm to carbon dioxide in a glass bead bioassay. *J Econ Entomol* **91**: 444-456 (1998).
56. Robert CAM, Veyrat N, Glauser G, Marti G, Doyen GR, Villard N, Gaillard MDP, Kollner TG, Giron D, Body M, Babst BA, Ferrieri RA, Turlings TCJ and Erb M, A specialist root herbivore exploits defensive metabolites to locate nutritious tissues. *Ecology letters* **15**: 55-64 (2012).
57. van Dam NM, Belowground herbivory and plant defenses. *Annu Rev Ecol Evol Syst* **40**: 373-391 (2009).

58. Bernklau EJ and Bjostad LB, Reinvestigation of host location by western corn rootworm larvae (Coleoptera: Chrysomelidae): CO_2 is the only volatile attractant. *J Econ Entomol* **91**: 1331-1340 (1998).
59. Boetel MA, Fuller BW and Evenson PD, Emergence of adult northern and western corn rootworms (Coleoptera : Chrysomelidae) following reduced soil insecticide applications. *J Econ Entomol* **96**: 714-729 (2003).
60. Michaelides PK, Cleverly AL and Wright DJ, Sub-lethal effects of tefluthrin on *Diabrotica undecimpunctata howardi*, Barber: Plant protection and larval development. *Crop Protection* **16**: 423-429 (1997).
61. Reinecke A, Muller F and Hilker M, Attractiveness of CO_2 released by root respiration fades on the background of root exudates. *Basic and Applied Ecology* **9**: 568-576 (2008).
62. Bernklau EJ, Bjostad LB, Meihls LN, Coudron TA, Lim E and Hibbard BE, Localized search cues in corn roots for western corn rootworm (Coleoptera: Chrysomelidae) larvae. *J Econ Entomol* **102**: 558-562 (2009).
63. Bernklau EJ and Bjostad LB, Identification of feeding stimulants in corn roots for western corn rootworm (Coleoptera : Chrysomelidae) larvae. *J Econ Entomol* **101**: 341-351 (2008).
64. Bernklau EJ and Bjostad LB, Insecticide Enhancement with Feeding Stimulants in Corn for Western Corn Rootworm Larvae (Coleoptera: Chrysomelidae). *J Econ Entomol* **98**: 1150-1156 (2005).
65. Bjostad LB and Hibbard BE, 6-Methoxy-2-benzoxazolinone: A semiochemical for host location by western corn rootworm larvae *J Chem Ecol* **18**: 931-944 (1992).
66. Hibbard BE, Bernklau EJ and Bjostad LB, Long-chain free fatty acids: Semiochemicals for host location by western corn rootworm larvae *J Chem Ecol* **20**: 3335-3344 (1994).
67. Hiltpold I, Hibbard BE, French BW and Turlings TCJ, Capsules containing entomopathogenic nematodes as a Trojan horse approach to control the western corn rootworm. *Plant Soil* **358**: 10-24 (2012).
68. Lin ZF, Zhong SL and Grierson D, Recent advances in ethylene research. *J Exp Bot* **60**: 3311-3336 (2009).

69. Robert CAM, Erb M, Duployer M, Zwahlen C, Doyen GR and Turlings TCJ, Herbivore-induced plant volatiles mediate host selection by a root herbivore. *New Phytol* **194**: 1061-1069 (2012).
70. Robert CAM, Erb M, Hibbard BE, French BW, Zwahlen C and Turlings TCJ, A specialist root herbivore reduces plant resistance and uses an induced plant volatile to aggregate in a density-dependent manner. *Functional Ecology* **26**: 1429-1440 (2012).
71. Rasmann S, Kollner TG, Degenhardt J, Hiltpold I, Toepfer S, Kuhlmann U, Gershenzon J and Turlings TCJ, Recruitment of entomopathogenic nematodes by insect-damaged maize roots. *Nature* **434**: 732-737 (2005).
72. Erb M, Glauser G and Robert CAM, Induced Immunity Against Belowground Insect Herbivores- Activation of Defenses in the Absence of a Jasmonate Burst. *J Chem Ecol* **38**: 629-640 (2012).

Chapter 3

Dispersal and spatial distribution of western corn rootworm larvae in relation to root phenology

Authors: Mario Schumann* and Stefan Vidal

*Corresponding author

This chapter is published in:

Agricultural and Forest Entomology (2012), 14, 331–339

Please access via doi: 10.1111/j.1461-9563.2012.00573.x

© 2012 The Royal Entomological Society

Dispersal and spatial distribution of western corn rootworm larvae in relation to root phenology

M. Schumann and S. Vidal

Georg–August University, Department of Crop Sciences, Agricultural Entomology, Grisebachstr.6, 37077 Göttingen, Germany; Phone: +49 (0) 551 – 39 33733, Fax: +49 (0)551 39 12105, Email: Mario.Schumann@agr.uni–goettingen.de

Abstract

1. Despite the increasing economic importance of root feeding pests such as the western corn rootworm (WCR – *Diabrotica virgifera virgifera*) basic parameters about their below ground biology are only partly understood. This study investigated the dispersal and distribution of western corn rootworm larvae in the maize root system during their development at two growth stages of maize (BBCH 13 – 14 and BBCH 17 – 18).
2. Dispersal of the WCR larvae increased as they developed; the larvae moved off their original place of hatch and into deeper soil layers. Overall, changes in the horizontal distribution of the larvae were more extensive than changes in the vertical distribution.
3. The spatial analysis of distance indices (SADIE) revealed that the larvae have an aggregative distribution throughout their development. The feeding site of larvae in the root system was determined by the stage of larval development. Initially WCR larvae started feeding in close proximity of their hatching location and moved to more developed root tissue towards the end of their development.
4. Differences in root phenology mainly influenced the distribution of the larvae at the end of their development wherein larvae exhibited increased vertical movement at a later growth stage of maize.
5. Mechanisms of these distributional changes and the implications for the management of WCR larvae are discussed especially with regard to chemical control as fewer larvae are expected to be targeted at a later growth stage of maize.

Keywords: *Diabrotica virgifera virgifera; Zea mays*; root herbivore; below ground distribution; soil stratification; SADIE

Introduction

Despite the increasing impact of root feeding pests in agricultural production systems studies on their biology are limited compared to their above ground counterparts (Hunter, 2001), mainly because their cryptic feeding habit makes it difficult to assess their behaviour (Johnson *et al*., 2006). A better understanding of the behavioural ecology of insect pests, however, can help to improve sustainable strategies for their management (Coyle *et al.*, 2010).

The western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), is a serious root feeding pest of maize, *Zea mays* L. (Poacecae). In the U.S. Corn Belt, costs for management and crop losses exceed 1 billion US dollars per year (Spencer *et al.*, 2009). In the early 1990s, the WCR was first detected in Europe near Belgrade, Serbia, and by calculating the generational growth rates a first introduction into Europe is estimated between 1979 and 1984 (Szalai *et al.*, 2011). Subsequent independent introductions into other European regions were also identified (Miller *et al.*, 2005) and the pest has now spread into 21 countries (EPPO, 2011). Annual costs of up to 472 million € in a `no control` scenario are expected in Europe once it will have reached the full extent of its potential spread (Wesseler & Fall, 2010). WCR is a univoltine species. The eggs overwinter in the soil and the larvae hatch in spring (Krysan, 1986). The three larval instars feed upon the roots during a 3 week period, causing a disruption of water and nutrient uptake and plant lodging at higher larval densities (Levine & Oloumi – Sadeghi, 1991).

The root feeding of the larvae usually coincides with the early to mid vegetative growth stages of maize (Musick *et al.*, 1980). Different growth stages of maize differ in their root architecture, which affects the amount of suitable root tissue available to larvae when establishing in the root system (Branson *et al.*, 1982). Hence the planting dates of maize can affect the western corn rootworm population dynamics (Stavisky & Davis, 1997) mainly due to a lack of adequate food supply when larvae hatch (Bergman & Turpin, 1984). Hibbard et al. (2008) have shown that larval recovery, plant damage and adult emergence can be affected by maize phenology. The spatial distribution of WCR larvae in the root system at different growth stages of maize has not been investigated, but may provide important information regarding the understanding of host – herbivore interactions.

The spatial distribution pattern of the larvae is determined by several factors across different spatial scales (Toepfer *et al.*, 2007). At a within field scale the spatial

distribution of the larvae is mainly determined by soil moisture and texture (Ellsbury *et al.*, 2005), female oviposition behaviour (Foster *et al.*, 1979) and the limited movement of the larvae (Bergman *et al.*, 1983). WCR larvae are able to move up to 100 cm from the location of egg hatch to where adults emerge (Short & Luedtke, 1970) and between as many as three plants within a row (Hibbard *et al.*, 2003). Overall, these factors result in an aggregated distribution of soil dwelling larvae (Brown & Gange, 1990).

At a plant scale, the WCR larvae are associated with the maize root system (Strnad & Bergman, 1987b). After hatch they start feeding on the root hairs and outer cortical tissue (Chiang, 1973) specifically on roots of 2 mm diameter or less (Strnad & Bergman, 1987b). As the larvae develop they burrow into the cortical parenchyma and move towards newly grown root whorls (Chiang, 1973), which are larger in diameter. As feeding preference of larvae seems to change with time, they repeatedly redistribute themselves in the root system (Strnad & Bergman, 1987b).

We assessed dispersal and spatial distribution of WCR larvae during their development in a maize root system and monitored how the availability of root biomass influenced changes in the spatial distribution of feeding larvae. Insights from this study of host – herbivore interaction in the root system may ultimately improve the control of WCR larvae as the knowledge of the feeding location can help to target the larvae more effectively.

Materials and Method

The experiment was set up in a microhabitat container (120cm x 80cm x 60cm), simulating 1 m^2 portion of a maize field where soil parameters can be kept homogenous. *Haplic luvi* soil was taken from an arable land near Göttingen (51°29`52.88 N, 9°55`38.26 E) and homogenized using a soil shredder (Unifix 300, Moeschle, Ortenberg, Germany). The soil was passed through a 1 cm mesh sieve to create an even soil structure for all samples. The soil in each container was filled to a depth of 39 cm, horizontal sheets of wire mesh with ~ 5 cm openings, so that roots can grow through, were added every 13 cm to define three soil layers. Once a container was filled with one soil layer, the soil was slightly compacted with a wooden panel to mimic a more realistic soil profile as found in the field.

Handling of maize and WCR eggs

Maize (Cultivar: Prinz, KWS, Einbeck, Germany) was grown in plastic trays at 25°C and transplanted to the containers 7 days after sowing. Two maize rows were set up with a 60 cm row spacing with each row consisting of 9 plants each 13 cm apart. The plants were watered with 2 – 3 litres per container per day and fertilised once a week with a 2% Hakaphos Blau solution (Compo, Münster, Germany). To test the distribution of the larvae at an early and a mid vegetative growth stage, two experiments were set up: In the first experiment, the plants were allowed to grow for 4 weeks (Growth stage BBCH 13 – 14) until WCR eggs were applied. In the second experiment the plants were allowed to grow for 7 weeks (Growth stage BBCH 17 – 18) (Lancashire et al., 1991) until WCR eggs were applied. Further references to ‘BBCH 13 – 14’ and ‘BBCH 17 – 18’ always relate to the growth stage of maize when the eggs were added. Eight containers (= replicates) were set up for each experiment.

WCR eggs from a non – diapausing strain were obtained from the USDA – ARS, North Central Agricultural Research Laboratory, Brookings, North Dakota, USA. This laboratory strain does not show a significant performance difference compared with the wild type strains (Hibbard *et al.*, 1999). The eggs were stored in Petri dishes at 8°C. Hatch tests with egg samples were carried out at 25°C and 65% relative humidity (RH) and showed that the first eggs hatched after 13 days. About two days prior hatching (day 11 of incubation) eggs were washed from the soil matrix in which they were held with a 250 µm sieve and mixed in a 0.15% agar solution until they

were evenly distributed. The egg concentration was determined by counting the number of eggs in 10 µl subsamples. Agar-water-solution was added until a concentration of 100 eggs in a 200 µl agar solution was reached. 100 eggs were applied 15 cm from each plant base at a depth of 7 cm (Fig. 1) with an Eppendorf pipette. At this egg density intraspecific competition can be minimised (Weiss *et al.*, 1985). Hatching time and rate were measured by applying 30 eggs on wet filter paper in Petri dishes and placing them in pots with soil near the containers. The larvae started to hatch 48 hours post inoculation in both experiments. In the experiment with BBCH 13 –14 the larvae completed their hatch after 9 days with a peak hatch (18 – 20% of larvae hatch in one day) on day 4. In the experiment with BBCH 17 – 18, the larvae completed hatch after 8 days with a peak hatch on day 3.

Sampling

Due to quarantine regulations in Germany, experiments had to be terminated 21 days after the first larval hatch to avoid adult emergence. Thus sampling took place on the following days of larval development; on day 7, 14 and 21 after the first larval hatch. On day 7 the majority of larvae were expected to be at the 1st instar stage (L1), on day 14 at the 2nd instar stage (L2) and on day 21 at the 3rd instar stage (L3). Each container was sub – sampled on these days during larval development. For each sub-sampling the soil was stratified into 15 soil cubes (16 cm x 13 cm x 13 cm; Fig. 1) between the first three maize plants of the maize rows, giving a total of 45 soil cubes. Using this stratification, the horizontal distribution was measured at the maize plant (Soil cubes A1 & A5), the point of egg inoculation (Soil cubes A2 & A4) and between the maize rows (Soil cube A3). The vertical distribution was determined in soil layers A (0 – 13 cm depth), B (13 – 26 cm depth) and C (26 – 39 cm depth). As the soil stratification was carried out in association with three maize plants per row three samples of each soil cube could be used for further analysis. One soil cube was used to extract larvae, the second one to quantify root biomass and the third one was discarded (= 8 replicates of a soil cube available for an analysis of larval numbers and 8 replicates for the recovery of root biomass on each sampling date). Soil cubes were selected at random to minimise potential edge effects of the container on larval behaviour or root growth. Each soil cube was cut out with an ordinary kitchen knife and once the stratification was complete, a 5 mm plastic sheet fitted to container dimensions (PVC CAW, Simona, Germany) was fixed in front of

the remaining soil in the container to fully enclose the experimental setup and avoid desiccation. Due to the compaction at the beginning of the experiment the soil was solid and no soil cube could fall onto the others during soil stratification.

The larvae were extracted from the separately sampled soil cubes with a high gradient Kempson extraction system (Kempson *et al.*, 1968). In this system the soil cubes were transferred to a box with netting at the bottom (mesh size 0.7 cm) and placed above water – filled containers. Red light bulbs placed over the soil created a heat and moisture gradient that forced the larvae to move downwards and to fall into the water. When larvae were recovered from a soil cube, that cube was recorded as 'positive'. The number of larvae in a positive soil cube was recorded and the larvae were placed in 70% ethanol for later analysis. The head capsule width of the larvae was measured under a dissecting microscope (Leica, Wild, M3Z, Wetzlar, Germany) fitted with an ocular micrometer and was used to determine the larval instar stage (Hammack *et al.*, 2003).

The amount of root biomass available for larval feeding in a soil cube was determined by washing the soil from the root material in the second cube using a 5 mm sieve. The cleaned roots were dried at 60°C for two weeks and weighed (H110 balance, Sartorius, Göttingen, Germany). These data were affected by larval feeding but were used as a proxi for the distribution of root biomass.

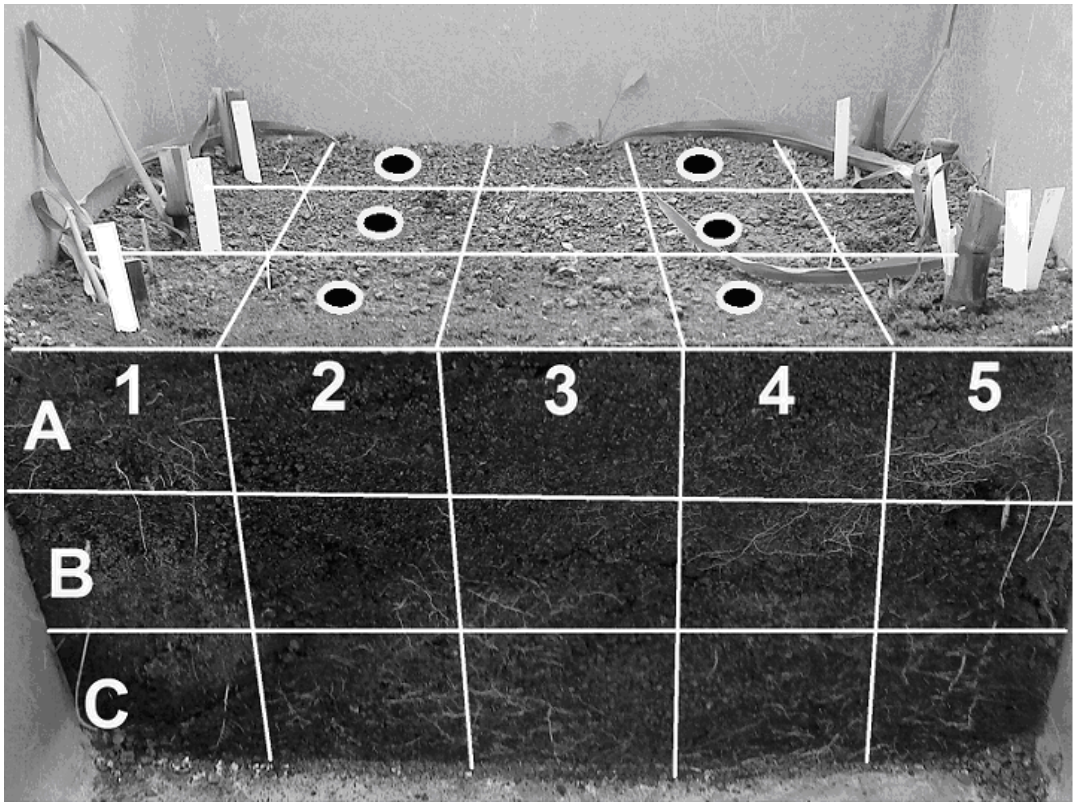


Figure 1 Experimental set up of soil stratification (45 soil cubes; Size of each cube 16 cm x 13 cm x 13 cm) at the last sub-sampling date (day 21 of larval development). The soil at and between three plants per maize row was sampled so that three samples of each soil cube were available for analysis: one soil cube was used to extract larvae, the second to extract roots and one was discarded. Vertical distribution of the larvae was measured with soil layers A, B and C; horizontal distribution was measured directly at the plant (A1 & A5), the point of egg inoculation (A2 & A4; marked by the black/white symbols) and between the maize rows (A3).

Statistical analysis

WCR density, larval development and availability of root biomass

The total number of larvae recovered from all soil cubes was analyzed with a repeated measures ANOVA with day of larval development and growth stage of maize as independent variables and number of larvae as the dependent variable using a general linear model (GLM). A Tukey`s post hoc was performed to test for between and within group effects. The analysis of the total root biomass in all soil cubes was done with the same tests.

Linear regression models were used to describe the relationship between the distribution of root biomass and the number of larvae. Only root biomass of positive soil cubes was correlated with larval density. Differences in larval development between the growth stages of maize were tested as larval ratios which were calculated with the formulae (number of L2 larvae – number of L1 larvae) + K on day

14 and (number of L3 larvae – number of L2 larvae) + K on day 21 of larval development, where K is a constant to generate positive values (Kurtz *et al.*, 2010). The larval development was tested between the growth stages of maize with a Student's t – test for each day of larval development. No statistical test was performed for day 7 of larval development as only L1 larvae were found.

Dispersal and spatial distribution of WCR larvae

The number of positive soil cubes is a measure of larval dispersal in the root system. Dispersal was analysed with a repeated measures ANOVA derived from a GLM with time and growth stage as independent variables and the number of positive soil samples as the dependent variable. A Tukey's post hoc test was performed to test for between and within group effects.

Changes in the vertical distribution of western corn rootworm larvae were calculated by combining the total number of larvae in the 5 cubes of layers A, B and C separately. Larvae in layer C were not statistically analysed as only a small proportion of larvae were recovered, not sufficient for analysis. As the majority of larvae were found in layer A the horizontal distribution changes of the larvae were analysed by combining soil cubes A1 & A5 (larvae at the plant), A2 & A4 (larvae at the point of inoculation) and A3 (larvae in the middle of the rows). The vertical and horizontal distribution changes of the larvae are expressed as proportional data and were arcsine transformed prior to each analysis. Horizontal and vertical distribution changes were analysed individually in a GLM with a repeated measures ANOVA with time and growth stage as independent variables and the transformed proportion of larvae as the dependent variable. A Tukey's post hoc test was performed to test for between and within group effects.

All statistical analyses were carried out with Statistica, Version 9 (StatSoft, Tulsa, OK, USA).

Spatial analysis

The spatial distribution of western corn rootworm larvae was analyzed with Spatial Analysis by Distance IndicEs (SADIE, Rothamsted Experimental Station, Harpenden, Herts, UK). SADIE quantifies the spatial pattern in a sampled population and measures the degree of non – randomness in two dimensional spatial patterns (Perry, 1995). An index of aggregation (I_a) is calculated through the minimum

distance that sampled individuals would need to move to achieve complete regularity (D). The observed counts are randomly allocated and calculated again. 26058 of these randomizations were carried out and the arithmetic mean (D) of all randomizations calculated (E_a). The aggregation index is an index of the mean observed value of distance to regularity with the means randomized value ($I_a = D/E_a$). $I_a = 1$, $I_a > 1$ and $I_a < 1$ indicates random, aggregated and regular arrangements of counts respectively. The probability P_a tests for deviations from random dispersion, where $P_a > 0.975$ indicates regular dispersion, $P_a < 0.025$ spatial aggregation, and $0.025 < P < 0.975$, randomness. A subsidiary index J_a indicates the presence of one major cluster ($J_a > 1$) or multiple clusters ($J_a \leq 1$ when $I_a > 1$) (Perry, 1998). For these analyses we used the total number of larvae extracted from all replicates in each experiment when the containers were subsampled (= on day 7, 14 and 21 of larval development).

SADIE can also test for a statistical association between the distributions of two groups of data by calculating cluster indices for each distribution, representing the local spatial pattern as patches or gaps. The extent to which cluster indices of both distributions correlate at each point provides a measure of spatial association and produces an association index (X). Positive values (association) result from a coincidence of two patches or gaps, whereas negative values (disassociation) result from a patch coinciding with a gap in both populations. The mean of local values of the two populations gives the overall measure of association (X) (Perry & Dixon, 2002). The significance of X was tested against X_{rand} from a randomization test that included an adjustment procedure (Dutilleul, 1993). At the 5% significance level, the statistic $P < 0.025$ indicated significant association and $P > 0.975$ indicates significant disassociation. We used the index to test the similarities of larval distributions at the two tested growth stages of maize each time the container was subsampled (= on day 7, 14 and 21 of larval development).

Results

WCR density, development and availability of root biomass

The total number of larvae extracted from the containers was neither influenced by the growth stage of maize ($F_{1,14}$ = 0.63, P = 0.44) or the day of larval development ($F_{1,14}$ = 1.94, P = 0.16). On day 7 of larval development only L1 larvae were extracted at both growth stages of maize. On day 14 L1 and L2 larvae were extracted with a significant higher proportion of L2 larvae at BBCH 13 – 14 (t = 3.13, d.f. = 14, P < 0.01). On day 21 L2 and L3 larvae were extracted with no differences in larval development between the growth stages of maize (t = -0.46, d.f. = 14, P = 0.65) (Table 1).

Table 1 Total number of larvae extracted from all soil cubes in a soil stratification and their larval instar composition on day 7, 14 and 21 of development at growth stages BBCH 13 – 14 and BBCH 17 – 18 of maize. Lower case letters in each column indicate significant difference between growth stage BBCH 13 – 14 and BBCH 17 – 18 (N = 8) for total number of larvae and the different instar stages (±SE).

Day of larval development	Total number of larvae		1st instar larvae		2nd instar larvae		3rd instar larvae	
	BBCH 13–14	BBCH 17–18	BBCH 13–14	BBCH 17–18	BBCH 13–14	BBCH 17–18	BBCH 13–14	BBCH 17–18
7	54.28 ± 6.20 a	51.50 ± 5.37 a	54.28 ± 6.20 a	51.50 ± 5.37 a	–	–	–	–
14	56.75 ± 5.72 a	49.13 ± 4.27 a	15.50 ± 1.80 a	24.75 ± 4.27 b	40.50 ± 3.79 a	24.00 ± 3.61 b	0.75 ± 0.48 a	0.38 ± 0.18 a
21	48.25 ± 6.01 a	42.63 ± 6.25 a	0.88 ± 0.23 a	0.38 ± 0.26 a	15.00 ± 1.68 a	10.13 ± 1.60 a	32.38 ± 4.87 a	32.13 ± 6.42 a

Availability of root biomass was significantly affected by the growth stage of maize ($F_{1,14}$ = 22.92, P < 0.001) and the day of larval development ($F_{2,28}$ = 10.39, P < 0.001) but not by an interaction of both ($F_{2,28}$ = 0.63, P = 0.54). At growth stage BBCH 13 – 14 0.94 g ± 0.10, 2.22 g ± 0.12 and 2.58 g ± 0.16 (± SE) of dry root biomass was recovered on day 7, 14 and 21 of larval development, respectively. Comparatively, at

BBCH 17 – 18 3.02 g ± 0.41, 3.88 g ± 0.43 and 5.16 g ± 0.93 (± SE) of dry root biomass was recovered, respectively.

The relationship between the distribution of root biomass and the distribution of WCR larvae was negatively correlated on day 7 of larval development at both growth stages of maize, and became positively correlated on the subsequent days of development. Correlation between the distribution of WCR larvae and the distribution of root biomass at growth stage BBCH 13 – 14 became significant on day 14 and on day 21 at BBCH 17 – 18 ($P < 0.01$) (Table 2).

Table 2 Relationship between the spatial distribution of root biomass and spatial distribution of WCR larvae in the soil on day 7, 14 and 21 of larval development at growth stages BBCH 13 – 14 and BBCH 17 – 18 of maize.

Day of larval development	Growth stage	Linear regression equation	R^2	*P* value
7	BBCH 13 – 14	y = 8.86 – 5.74x	0.0072	0.54
	BBCH 17 – 18	y = 10.61 – 2.64x	0.02	0.36
14	BBCH 13 – 14	y = 4.06 + 15.10x	0.35	<0.001
	BBCH 17 – 18	y = 8.24 + 0.29x	0.0008	0.84
21	BBCH 13 – 14	y = 1.14 + 15.37x	0.54	<0.001
	BBCH 17 – 18	y = 2.29 + 3.37x	0.35	<0.001

Dispersal and spatial distribution of WCR larvae

Dispersal of WCR larvae was significantly influenced by the day of larval development ($F_{2,28} = 21.04$, $P < 0.001$) but not by the growth stage of maize ($F_{1,14} = 0.0038$, $P = 0.95$). On plants inoculated with eggs at BBCH 13 – 14, WCR larvae were recovered from 6.12 ± 0.44, 6.75 ± 0.45 and 8.38 ± 0.96 soil cubes on day 7, 14 and 21 of larval development. Dispersal on day 21 is significantly higher than on day 7 ($P < 0.05$). On plants inoculated with eggs at BBCH 17 – 18, WCR larvae were recovered from 5.38 ± 0.56, 6.75 ± 0.47 and 9.00 ± 0.65 soil cubes on day 7, 14 and 21 of larval development. Dispersal increased significantly from day 7 to day 21 of larval development ($P < 0.05$) (Fig. 2).

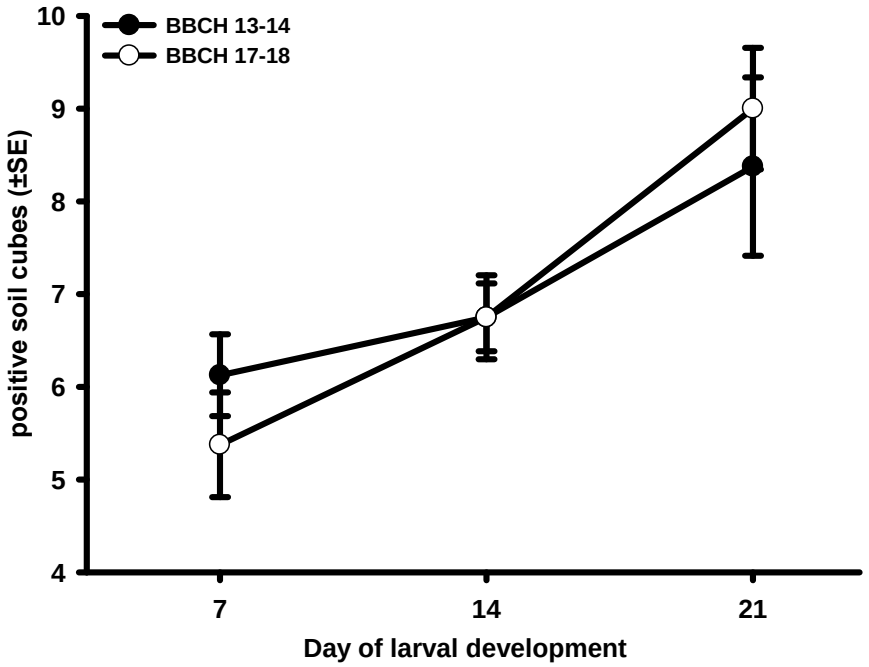


Figure 2 Dispersal of WCR larvae on day 7, 14 and 21 of larval development at growth stages BBCH 13 – 14 and BBCH 17 – 18 of maize. Dispersal is determined by the number of soil cubes WCR larvae could be extracted from (= "positive soil cubes")

Distribution of WCR larvae in layers A (0 – 13 cm depth) & B (13 – 26 cm depth) was not affected by the growth stage of maize (Layer A: $F_{1,14}$ = 0.009, P = 0.76; layer B: $F_{1,14}$ = 0.06, P = 0.81), but by the day of larval development (Layer A: $F_{2,28}$ = 13.07, P < 0.01; layer B: $F_{2,28}$ = 8.08, P < 0.01) and by the interaction of both (Layer A: $F_{2,28}$ = 10.31, P < 0.01; layer B: $F_{2,28}$ = 9.54, P < 0.01). 89 – 96% of the larvae were recovered in layer A and 4 – 10% in layer B at both growth stages of maize throughout the whole larval development except for day 21 at BBCH 17 – 18 when the proportion of larvae significantly dropped to 75% in layer A and increased to 18% in layer B. 0.26% and 2% of the larvae at BBCH 13 – 14 and 17 – 18 were recovered in layer C (26 – 39 cm depth) on day 14 and increased to 2% and 7% on day 21 of larval development (Fig. 3).

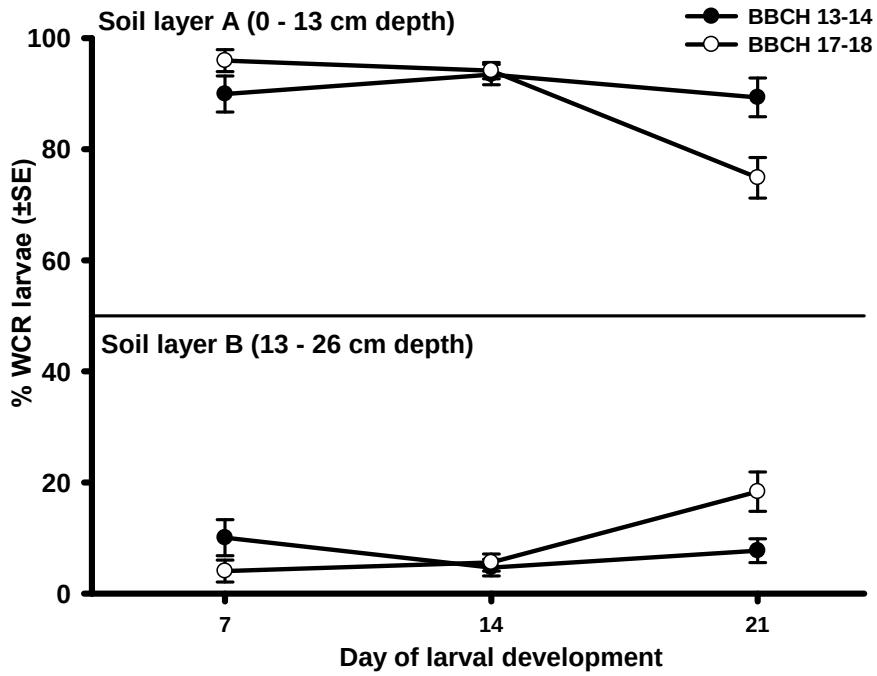


Figure 3 WCR larvae extracted in soil layers A and B on day 7, 14 and 21 of larval development at growth stages BBCH 13 – 14 and BBCH 17 – 18 of maize. Larvae recovered from layer C was very low and is therefore not included.

The proportion of larvae extracted directly at the plant (soil cubes A1 & A5) was affected by the growth stage of maize ($F_{1,14}$ = 5.26, P < 0.05) and the day of larval development ($F_{2,28}$ = 41.20, P < 0.01). At BBCH 13 – 14 the proportion of larvae directly at the plant significantly increased from 14% on day 7 to 57% on day 14 of larval development (P < 0.001), and slightly increased to 69% on day 21. At BBCH 17 – 18 the same pattern was observed with 23% of larvae recovered on day 7, increasing to 32% and 51% on day 14 and 21 of larval development, respectively. Significant differences in larvae extracted were measured between day 7 and 21 of larval development (P < 0.001) (Fig. 4). The growth stage of maize at the time eggs were added affected the proportion of larvae on day 14 with a significantly higher proportion recovered at growth stage BBCH 13 – 14 than at BBCH 17 – 18 (P < 0.05).

The proportion of larvae recovered in the cubes at point of inoculation i.e. 15 cm from the plant base (soil cubes A2 & A4) was only influenced by the day of larval development ($F_{2,28}$ = 46.05, P < 0.01) and not by the growth stage of maize ($F_{1,14}$ = 1.49, P = 0.24). The proportion of larvae recovered significantly decreased from 65 – 66% on day 7 to 16 – 19% on day 21 of larval development at both growth stages (P

< 0.001). The proportion of larvae from day 7 to day 14, however, decreased to 26% at BBCH 13 – 14 ($P < 0.01$) and 46% at BBCH 17 – 18 ($P = 0.47$) (Fig. 4).

Movement to the middle of the maize rows (soil cube A3) was influenced by the day of larval development ($F_{2,28} = 4.28$, $P < 0.05$) but not by the growth stage of maize ($F_{1,14} = 0.006$, $P = 0.98$). At both growth stages of maize the same pattern was measured with an increase from 8 – 10% on day 7 to 11% at BBCH 13 – 14 and 19% at BBCH 17 – 18 on day 14 and a decrease to 4% at both growth stages of maize on day 21 of larval development.

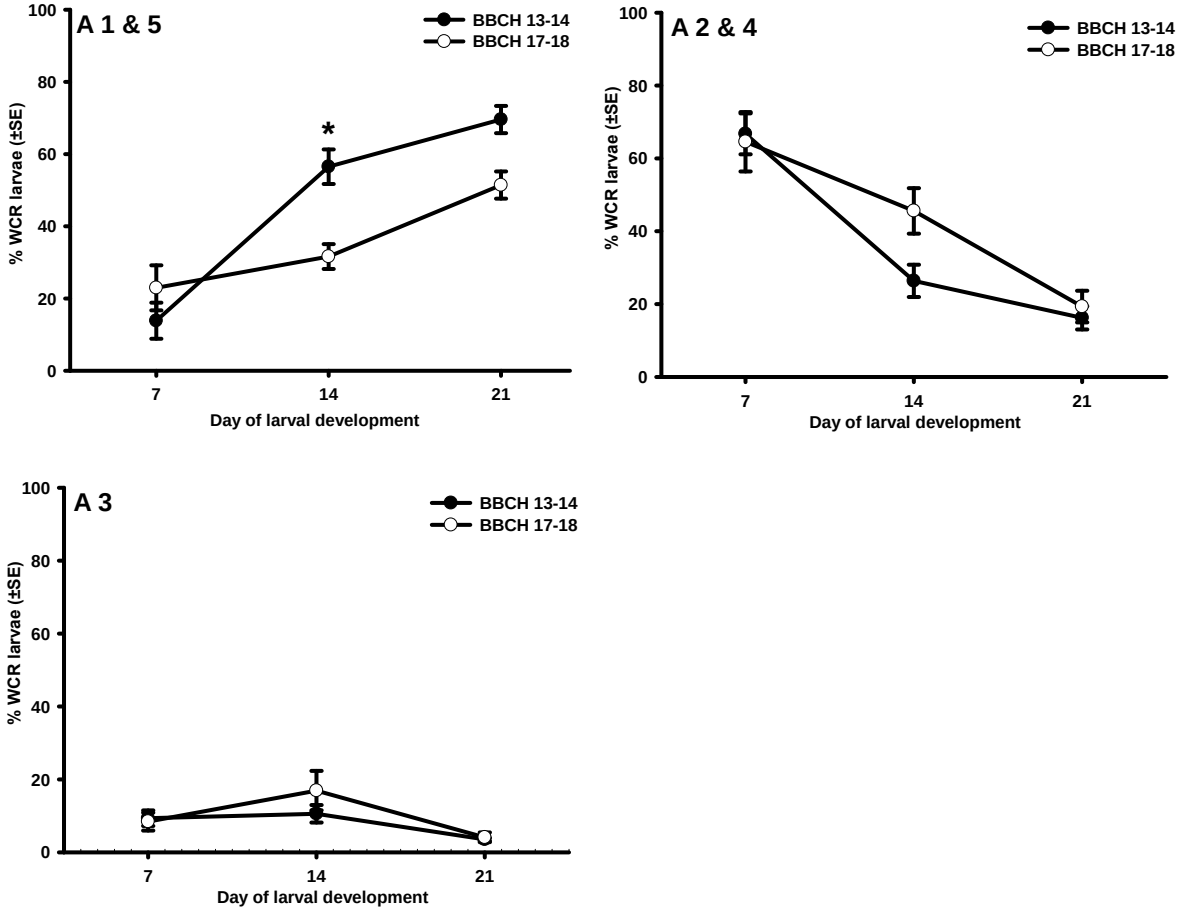


Figure 4 WCR larvae extracted directly at the plant (soil cubes A 1 & 5), point of inoculation (soil cubes A 2 & 4) and between the plant rows (soil cube A3) on day 7, 14 and 21 of larval development at growth stages BBCH 13 – 14 and BBCH 17 – 18 of maize. The asterix (*) indicates a significant difference between the growth stages of maize on the day of larval development (Tukey test $P < 0.05$).

Spatial analysis

The SADIE index indicated aggregation of WCR larvae on all days of larval development ($I_a > 1$) at both growth stages of maize. On day 7 of larval development, SADIE identified one major cluster ($J_a > 1$) at both growth stages of maize. On day 14 multiple clusters were identified at growth stage BBCH 13 – 14 and the presence of one major cluster at BBCH 17 – 18. On day 21 multiple clusters were identified at both growth stages ($J_a \leq 1$). The degree of association was always significant between the distributions of WCR larvae at both tested growth stages of maize ($X > 0$) (Table 3).

Table 3 Spatial analysis of the total number of WCR larvae on day 7, 14 and 21 of larval development at growth stages BBCH 13 – 14 and BBCH 17 – 18 of maize with Spatial Analysis of Distance indices (SADIE).

Day of larval development	Growth stage	I_a	*P*	J_a	*P*	X	*P*
7	BBCH 13 – 14	1.04	0.32	1.13	0.33	0.91	<0.01
	BBCH 17 – 18	1.04	0.33	1.11	0.37		
14	BBCH 13 – 14	1.18	0.16	0.89	0.75	0.98	<0.01
	BBCH 17 – 18	1.18	0.14	1.08	0.34		
21	BBCH 13 – 14	1.12	0.23	0.73	0.91	0.92	<0.01
	BBCH 17 – 18	1.26	0.10	0.87	0.82		

I_a and its associated *P* – value indicate the aggregation of an overall spatial pattern and associated significance test of the spatial pattern's departure from randomness. $I_a > 1$ indicates an aggregated distribution and a significant spatial aggregation is assumed at $P < 0.025$. $J_a \leq 1$ indicates the presence of multiple clusters when $I_a > 1$ and one major cluster when $J_a > 1$. X is the measure of spatial association between the distribution of WCR larvae at the two growth stages of maize on day 7, 14 and 21 of larval development and indicates when $X > 0$ that two populations are associated and are considered significantly associated when $P < 0.025$.

Discussion

The use of soil stratification at different development stages of western corn rootworm larvae (WCR) allowed a detailed examination of WCR larval spatial distribution in the root system at a plant scale. Larval dispersal increased during the course of their development, but distribution remained aggregated throughout. The location where the majority of larvae were recovered in the root system was dependent on the composition of larval instars. Root phenology had a minor influence on the distributional changes and dispersal of the WCR larvae.

Age dependent dispersal of soil dwelling larvae is described “like the ripples from a stone dropped into water” (Salt & Hollick, 1946). As older larvae tend to have better dispersal abilities (Salt & Hollick, 1946; Doane, 1977) the more developed WCR larvae have the potential to occupy more parts of a root system, moving away from their original point of hatch and into deeper soil layers. The lower J_a index we found in our experiments supported this behavioural pattern as the larvae formed a major cluster at the beginning of their development ($J_a > 1$) and formed multiple clusters as they dispersed ($J_a < 1$).

The proportion of WCR larvae exhibiting vertical movement in our experimental set up was minimal (up to 25% were deeper than 13 cm and up to 7% were deeper than 27 cm) and the depth where most WCR larvae were found at was 0 – 13 cm (Layer A). Vertical movement increased at the later growth stage of maize (BBCH 17 – 18) and also increased as the larvae developed. Such larval development dependent vertical movement has also been demonstrated for the scarab beetle *Pyllophaga cuyabana* (Oliveira *et al.*, 2009). Strnad & Bergman (1987a) reported that 20% of L1 WCR larvae exhibited vertical movement to depths between 10 cm and 30 cm and suggested that negative geotaxis and increased soil weight might be limiting factors for vertical movement. As abiotic factors are known to influence below ground distribution of soil dwelling insects (Curry, 1987; Villani & Wright, 1990) differences in soil moisture might have also played a key role in the vertical movement patterns in our experimental set up. This is because the soil of the containers in our experiments became drier with depth and only the first 15 cm contained in excess (~20%) moisture (Pers. observation, M. Schumann) as the plots were watered on the soil surface and no ground water layer was simulated. First instar larvae require adequate levels of soil moisture for survival especially after hatch (Gaylor & Frankie, 1979). Based on this finding, our results also indicate that susceptible early instar

larvae may have simply avoided drier soil layers, whereas older larvae were able to move into portions of the root system with lower soil moisture. Patterns of vertical movement should be considered for the biological control of the larvae with entomopathogenic nematodes (Toepfer *et al.*, 2010; Rasmann *et al.*, 2005). Studies by Duncan & McCoy (1996) and Hanula (1993) for example have shown that the degree of infection of soil dwelling weevil larvae at different depths varied according to the entomopathogenic nematode species used.

Although the WCR larvae were actively dispersing, SADIE identified an aggregated distribution throughout their development ($I_a > 1$). Aggregation of soil dwelling larvae during their development is common across many insect orders (Brown & Gange, 1990) and can be caused by various factors: The distribution of the 1st instar larvae in the soil cubes A2 & A4 (compare Fig. 1) reflected the initial clumped application of the eggs in these locations. Most larvae must have started feeding close to their point of hatch as they have a limited mobility at this stage (Bergman *et al.*, 1983) and need to find roots within 36 – 48 hours for nutrition as well as to ensure that they can bore into the root successfully (Strnad & Bergman, 1987a). We hypothesise that aggregated feeding may facilitate first successful colonization of a root by neonate larvae reducing establishment mortality compared to that experienced by an isolated larva. When the larvae start feeding they also orientate towards softer developing roots that are easy to infest (Strnad & Bergman, 1987b; Clark *et al.*, 2006) and move to the middle of the maize rows (= soil cube A3; compare Fig.1) where fine root material is present (Strnad & Bergman, 1987b; personal observation, M. Schumann). This movement was not common in our study but underlines the importance of inter – maize row movement, an essential prerequisite for *Bt* resistance management (Hibbard *et al.*, 2003) as larvae may switch to nontransgenic plants.

As the larvae develop, their feeding site preferences change along with their nutritional requirements; a mechanism also described for *Helicoverpa zea* in soybeans (Eckel *et al.*, 1992). This causes the WCR larvae to re – distribute as they develop and to move from their original point of hatch to locate younger growing but also more developed, thicker roots. By day 14 of larval development more larvae had moved from their point of inoculation (= soil cubes A2 & A4 compare Fig.1) at growth stage BBCH 13 – 14 than at growth stage BBCH 17 – 18 (Fig. 4), either due to the faster development of the larvae (Table 1) or an earlier depletion of suitable food sources (Stavisky & Davis, 1997). Following a period of larval feeding, declining

amounts of suitable root tissue at a feeding site may trigger a shift in the distribution of larvae as intraspecific competition for suitable resources becomes more acute. This forces the larvae to move away from the original point of hatch into other parts of the root system. Compared to BBCH 13 – 14, the higher initial root biomass available to larvae whose eggs were inoculated onto plants at growth stage BBCH 17 – 18 are able to sustain damage by the larvae for a longer time. Consequently the motivation by the larvae to search for suitable food sources (Hibbard *et al.*, 2004) is lower and changes in their distribution are delayed. This is supported by the relationship of the distribution of root biomass and WCR larvae (Table 2). They became more correlated earlier during larval development at growth stage BBCH 13 – 14 and also had a higher correlation at the end of larval development.

Most larvae moved to the more suitable thicker roots around the plants base (= soil cubes A1 & A5 compare Fig.1) as they developed (Fig. 4); however at BBCH 17 – 18 fewer larvae moved into this part of the root system. This could be because the thicker roots are longer (Strnad & Bergman, 1987b) so that the larvae can find suitable root material outside this part of the root system. The roots in older plants also have a higher lignin content which is unsuitable for the larvae (Hibbard *et al.*, 2008). Thus the roots provide fewer suitable resources at this growth stage of maize and the larvae need to move to other parts of the root system, in this case to deeper soil layers (Fig. 3). This phenological host effect has important implications for the chemical control of the larvae because the soil granules that are applied in furrow during sowing will most probably target larvae in soil cubes A1 & A5 only. Thus i) larvae will only be targeted at the end of their development and ii) control is less effective at a higher growth stage of maize as a higher proportion of larvae that feed beyond the zone of insecticidal activity will survive (Gray et al.,1992). Consequently larvae should be targeted at the beginning of their development before they start to distribute in the root system.

A more detailed knowledge of WCR larval dispersal and distribution can also contribute to improved larval sampling procedures. For sampling the majority of larvae are assumed to be found within the major portion of the root system (10 cm around the plant base by 10 cm deep; Bergman *et al.*, 1983). As this study showed the larval distribution depends both on the day of larval development and growth stage of maize. This needs to be considered for sampling protocols of WCR larvae in the future.

Conclusions

Understanding the effect of plant phenology on host – herbivore interactions can help to improve and develop more sustainable pest management strategies (Leather, 2010). The use of soil stratification to study the movement of the WCR larvae in the maize root system showed that distributional changes were found to be linked with the feeding preferences of the three larval instars for different root types and newly developed roots. In contrast changes in root phenology had a minor influence on the distribution pattern, but indicated that distributional changes can also be driven through availability of suitable food resources and root quality. The efficacy and sustainability of WCR management approaches may benefit by incorporating more specific information about WCR larval dispersal and distribution patterns at different growth stages of maize.

Acknowledgements

We like to thank Magdolna Weller, Jaqueline Platzek, Katja Fischer and Thorsten Ogon for their assistance during the trials and KWS, Einbeck, Germany for providing the seeds. We thank Joseph Spencer (Illinois Natural History Survey) for comments on an earlier version of this manuscript. This research was funded by the German Environmental Foundation (DBU).

References

Bergman, M.K., Tollefson, J.J. & Hinz, P.N. (1983) Spatial dispersion of corn rootworm larvae (Coleoptera: Chrysomelidae) in Iowa cornfields. *Environmental Entomology*, **12**, 1443–1446.

Bergman, M.K. & Turpin, F.T. (1984) Impact of corn planting date on the population dynamics of corn rootworms (Coleoptera: Chrysomelidae). *Environmental Entomology*, **13**, 898–901.

Branson, T.F., Sutter, G.R. & Fisher, J.R. (1982) Comparison of a tolerant and a susceptible maize inbred under artificial infestations of *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae): Yield and adult emergence. *Environmental Entomology*, **11**, 371–372.

Brown, V.K. & Gange, A.C. (1990) Insect herbivory below ground. *Advances in Ecological Research*, **20**, 1–58.

Chiang, H.C. (1973) Bionomics of the northern and western corn rootworms. *Annual Review of Entomology*, **18**, 47–72.

Clark, P.L., Vaughn, T.T., Meinke, L.J., Molina – Ochoa, J. & Foster, J.E. (2006) *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) larval feeding behavior on transgenic maize (MON 863) and its isoline. *Journal of Economic Entomology*, **99**, 722–727.

Coyle, D.R., Jordan, M.S. & Raffa, K.F. (2010) Host plant phenology affects performance of an invasive weevil, *Phyllobius oblongus* (Coleoptera: Curculionidae), in a northern hardwood forest. *Environmental Entomology*, **39**, 1539–1544.

Curry, J.P. (1987) The Invertebrate fauna of grassland and its influence on productivity 2. Factors affecting the abundance and composition of the fauna. *Grass and Forage Science*, **42**, 197–212.

Doane, J.F. (1977) Spatial pattern and density of *Ctenicera destructor* and *Hypolithus bicolor* (Coleoptera: Elateridae) in soil in spring wheat. *Canadian Entomologist*, **109**, 807–822.

Duncan, L.W. & McCoy, C.W. (1996) Vertical distribution in soil, persistence, and efficacy against citrus root weevil (Coleoptera: Curculionidae) of two species of entomogenous nematodes (Rhabditida: Steinernematidae; Heterorhabditidae). *Environmental Entomology*, **25**, 174–178.

Dutilleul, P. (1993) Modifying the t Test for assessing the correlation between two spatial processes. *Biometrics*, **49**, 305–314.

Eckel, C.S., Terry, L.I., Bradley, J.R. & Van Duyn, J.W. (1992) Changes in within–plant distribution of *Helicoverpa zea* (Boddie) (Lepidoptera, Noctuidae) on soybeans. *Environmental Entomology*, **21**, 287–293.

Ellsbury, M.M., Clay, A.C., Clay, E.D. & Malo, D.D. (2005). Within - field spatial variation of northern corn rootworm distributions. *Western Corn Rootworm-Ecology and Management* (ed. by S. Vidal, U. Kuhlmann and C.R. Edwards), pp. 145–153. CABI publishing, Wallington, UK.

EPPO (2011) *Diabrotica virgifera virgifera* [WWW document] http://www.eppo.fr/QUARANTINE/Diabrotica_virgifera/diabrotica_virgifera.htm#map-dia [Last accessed 10.07.2011]

Foster, R. E., Ruesink, W. G. & Luckmann, W. H. (1979) Northern corn rootworm (Coleoptera: Chrysomelidae) egg sampling. *Journal of Economic Entomology*, **72**, 659–663.

Gaylor, M.J. & Frankie, G.W. (1979) Relationship of rainfall to adult flight activity and of soil moisture to oviposition behavior and egg and 1st instar survival in *Phyllophaga crinita* (Scarabaeidae: Coleoptera). *Environmental Entomology*, **8**, 591–594.

Gray,M.E., Felsot, A.S.,Steffey, K.L. & Levine,E. (1992) Planting time application of soil insecticides and western corn rootworm (Coleoptera: Chrysomelidae) emergence: Implications for long – term management programs. *Journal of Economic Entomology*, **85**, 544–553.

Hammack, L., Ellsbury, M.M., Roehrdanz, R.L. & Pikul, J.L. (2003) Larval sampling and instar determination in field populations of northern and western corn rootworm (Coleoptera: Chrysomelidae). *Journal of Economic Entomology*, **96**, 1153–1159.

Hanula, J.L. (1993) Vertical distribution of black vine weevil (Coleoptera: Curculionidae) immatures and infection by entomogenous nematodes in soil columns and field soil. *Journal of Economic Entomology*, **86**, 340–347.

Hibbard, B.E., Barry, B.D., Darrah, L.L., Jackson, J.J., Chandler, L.D., French, L.K. & Mihm, J.A. (1999) Controlled field infestations with western corn rootworm (Coleoptera: Chrysomelidae) eggs in Missouri: Effects of egg strains, infestation

dates, and infestation levels on corn root damage. *Journal of the Kansas Entomological Society*, **72**, 214–221.

Hibbard, B.E., Duran, D.P., Ellersieck, M.R. & Ellsbury, M.M. (2003) Post–establishment movement of western corn rootworm larvae (Coleoptera: Chrysomelidae) in Central Missouri corn. *Journal of Economic Entomology*, **96**, 599–608.

Hibbard, B.E., Higdon, M.L., Duran, D.P., Schweikert, Y.M. & Ellersieck, M.R. (2004) Role of egg density on establishment and plant–to–plant movement by western corn rootworm larvae (Coleoptera: Chrysomelidae). *Journal of Economic Entomology*, **97**, 871–882.

Hibbard, B.E., Schweikert, Y.M., Higdon, M.L. & Ellersieck, M.R. (2008) Maize phenology affects establishment, damage, and development of the western corn rootworm (Coleoptera: Chrysomelidae). *Environmental Entomology*, **37**, 1558–1564.

Hunter, M.D. (2001) Out of sight, out of mind: the impacts of root–feeding insects in natural and managed systems. *Agricultural and Forest Entomology*, **3**, 3–9.

Johnson, S. N., Zhang, X. X., Crawford, J. W., Gregory, P. J., Hix, N. J., Jarvis, S. C., Murray, P. J. & Young, I. M. (2006) Effects of carbon dioxide on the searching behaviour of the root–feeding clover weevil *Sitona lepidus* (Coleoptera: Curculionidae). *Bulletin of Entomological Research*, **96**, 361–366.

Kempson D., Lloyd, M. & Ghelardy, R. (1968) A new extractor for woodland litter. *Pedobiologia*, **3**, 1–30.

Krysan, J.L. (1986) Introduction: Biology, distribution and identification of pest Diabrotica. In: *Methods for the Study of Pest Diabrotica* (ed. by J.L. Krysan and A.M. Miller), pp. 1–23. Springer Verlag, New York, New York.

Kurtz, B., Karlovsky, P. & Vidal, S. (2010) Interaction between western corn rootworm (Coleoptera: Chrysomelidae) larvae and root–infecting *Fusarium verticillioides*. *Environmental Entomology*, **39**, 1532–1538.

Lancashire, P. D., Bleiholder, H., Vandenboom, T., Langeluddeke, P., Stauss, R., Weber, E. & Witzenberger, A. (1991) A uniform decimal code for growth stages of crops and weeds. *Annals of Applied Biology*, **119**, 561–601.

Leather, S.R. (2010) Precise knowledge of plant growth stages enhances applied and pure research. *Annals of Applied Biology*, **157**, 159–161.

Levine, E. & Oloumi–Sadeghi, H. (1991) Management of Diabroticite rootworms in corn. *Annual Review of Entomology*, **36**, 229–255.

Miller, N., Estoup, A., Toepfer, S., Bourguet, D., Lapchin, L., Derridj, S., Seok Kim, K., Renaud, P., Furlan, L. & Guillemaud, T. (2005) Multiple transatlantic introductions of the western corn rootworm. *Science*, **310**, 992.

Musick, G.J., Chiang, H.C., Luckmann, W.H., Mayo, Z.B. & Turpin, F.T. (1980) Impact of planting dates of field corn on beetle emergence and damage by the western and the northern corn rootworms (Coleoptera, Chrysomelidae) in the corn belt. *Annals of the Entomological Society of America*, **73**, 207–215.

Oliveira, L.J., Farias, J.R.B., Hoffmann–Campo, C.B., Do Amarali, M.L.B. & Garcia, M.A. (2009) Seasonal and vertical distribution of *Phyllophaga cuyabana* (Moser) (Coleoptera: Melolonthidae) in the soil profile. *Neotropical Entomology*, **38**, 582–588.

Perry, J.N. (1995) Spatial analysis by distance indices. *Journal of Animal Ecology*, **64**, 303–314.

Perry, J.N. (1998) Measures of spatial pattern for counts. *Ecology*, **79**, 1008–1017.

Perry, J.N. & Dixon, P.M. (2002) A new method to measure spatial association for ecological count data. *Ecoscience*, **9**, 133–141.

Rasmann, S., Koellner, T.G., Degenhardt, J., Hiltpold, I., Toepfer, S., Kuhlmann, U., Gershenzon, J. & Turlings, T.C.J. (2005) Recruitment of entomopathogenic nematodes by insect–damaged maize roots. *Nature*, **434**, 732–737.

Salt, G. & Hollick, F.S.J. (1946) Studies of wireworm populations. 2. Spatial distribution. *Journal of Experimental Biology*, **23**, 1–46.

Short, D.E. & Luedtke, R.J. (1970) Larval migration of the western corn rootworm. *Journal of Economic Entomology*, **63**, 325–326

Spencer, J. L., Hibbard, B. E., Moeser, J. and Onstad, D. W. (2009) Behaviour and ecology of the western corn rootworm (*Diabrotica virgifera virgifera* LeConte) *Agricultural and Forest Entomology*, **11**, 9–27.

Stavisky, J. & Davis, P.M. (1997) The effects of corn maturity class on western corn rootworm (Coleoptera: Chrysomelidae) phenology. *Journal of the Kansas Entomological Society*, **70**, 261–271.

Strnad, S.P. & Bergman, M.K. (1987a) Movement of first–instar western corn rootworms (Coleoptera: Chrysomelidae) in soil. *Environmental Entomology*, **16**, 975–978.

Strnad, S.P. & Bergman, M.K. (1987b) Distribution and orientation of western corn rootworm (Coleoptera: Chrysomelidae) larvae in corn roots. *Environmental Entomology*, **16**, 1193–1198.

Szalai, M., Komaromi, J.P., Bazok, R., Barcic, J.I., Kiss, J. & Toepfer, S. (2011) Generational growth rate estimates of *Diabrotica virgifera virgifera* populations (Coleoptera: Chrysomelidae). *Journal of Pest Science*, **84**, 133–142.

Toepfer, S., Ellsbury, M.M., Eschen, R. & Kuhlmann, U. (2007) Spatial clustering of *Diabrotica virgifera virgifera* and *Agriotes ustulatus* in small–scale maize fields without topographic relief drift. *Entomologia Experimentalis et Applicata*, **124**, 61–75.

Toepfer, S., Burger, R., Ehlers, R. – U., Peters, A. & Kuhlmann, U. (2010) Controlling western corn rootworm larvae with entomopathogenic nematodes: effect of application techniques on plant–scale efficacy. *Journal of Applied Entomology*, **134**, 467–480.

Villani, M.G. & Wright, R.J. (1990) Environmental influences on soil macroarthropod behavior in agricultural systems. *Annual Review of Entomology*, **35**, 249–269.

Weiss, M.J., Seevers, K.P., & Mayo, Z.B. (1985) Influence of western corn rootworm larval densities and damage on corn rootworm survival, developmental time, size and sex ratio (Coleoptera: Chrysomelidae). *Journal of the Kansas Entomological Society*, **58**, 397–402.

Wesseler, J. & Fall, E.H. (2010) Potential damage costs of *Diabrotica virgifera virgifera* infestation in Europe – the 'no control' scenario. *Journal of Applied Entomology*,**134**,385–394.

Chapter 4

Soil application of an encapsulated CO_2 source and its potential for management of western corn rootworm larvae

Authors: Mario Schumann*, Anant Patel and Stefan Vidal

*Corresponding author

Soil application of an encapsulated CO_2 source and its potential for management of western corn rootworm larvae

M. Schumann[1], A.Patel[2] and S. Vidal[1]

[1]Georg-August University, Department of Crop Sciences, Agricultural Entomology, Grisebachstr. 6, 37077 Göttingen, Germany,

Phone: +49 (0) 551–39 33733, E – mail: Mario.Schumann@agr.uni–goettingen.de

[2]University of Applied Sciences, Department of Engineering and Mathematics, Engineering and Alternative Fuels, Wilhelm-Bertelsmann-Strasse 10, 33602 Bielefeld, Germany

Abstract

Western corn rootworm (*Diabrotica virgifera virgifera*LeConte; WCR) larvae use CO_2 to locate the roots of their hosts. This study investigated whether an encapsulated CO_2 source (CO_2-emitting capsules) are able to outcompete CO_2 gradients established by corn root respiration in the soil. Furthermore, two management options with the capsules were tested in semi-field experiments (0.5- to 1-m^2 greenhouse plots): the disruption of host location and an “attract-and-kill” strategy in which larvae were lured to a soil insecticide (Tefluthrin) between the corn rows. The attract-and-kill strategy was compared to an application of Tefluthrin in the corn rows (conventional treatment) at 33% and 18% of the standard field application rate.

Application of the CO_2-emitting capsules 30 cm from the plant base increased CO_2 levels near the application point for up to 20 days with a peak at day 10. Both the disruption of host location and an attract-and-kill strategy caused a slight but non-significant reduction in larval densities. The disruption of host location caused a 17% reduction in larval densities, whereas an attract-and-kill strategy with Tefluthrin added at 33% and 18% of the standard application rate caused a 24 and 27% reduction in larval densities, respectively. As presently formulated, the CO_2-emitting capsules, either with or without insecticide, do not provide adequate control of WCR.

Keywords: *Diabrotica virgifera virgifiera*; carbon dioxide gradients; encapsulated carbon dioxide source; Tefluthrin; attract-and-kill

Introduction

For decades, insecticides have been used to control subterranean herbivorous insect pests (Lilly 1956). One of these insect herbivores is the western corn rootworm (*Diabrotica virgifera virgifera* LeConte Coleoptera: Chrysomelidae; WCR), an invasive pest of corn in North America that reduces grower profits by up to 1 billion dollars as a result of yield loss and management costs (Gray et al. 2009). Since its first detection in Europe (Belgrade, Serbia) at the beginning of the early 1990s (Kiss et al. 2005), WCR has been introduced multiple times across Europe (Ciosi et al. 2008) and has currently spread to more than 20 European countries (EPPO 2011).

Most of the damage caused by WCR results from the belowground feeding of the larvae (Urias-Lopez and Meinke 2001). Larval feeding on corn roots disrupts water uptake (Riedell and Reese 1999, Urias-Lopez et al. 2000) and causes plant lodging when larval densities are high (Levine and Oloumi-Sadeghi 1991, Godfrey et al. 1993). Several management options are used for controlling the larvae, including crop rotation (Gray et al. 2009), *Bt*-toxin expressing transgenic cultivars (Moellenbeck et al. 2001, Vaughn et al. 2005), insecticide seed treatments (Furlan et al. 2006), biological control with entomopathogenic nematodes (Rasmann et al. 2005, Toepfer et al. 2010a, Toepfer et al. 2010b), and the application of granular soil insecticides (Mayo and Peters 1978, Mayo 1980).

In continuous corn, chemical control of WCR larvae will continue to be regarded as the most important management option in the EU for the near future as long as case crop rotation remains impractical (Dillen et al. 2010, Van Rozen and Ester 2010). A dependency on chemical control, however, will eventually pose a threat to the environment and human health (Ma et al. 2009, Van Rozen and Ester 2010) and will potentially result in conflicts with regulation (EC) No. 1107/2009 and directive 2009/128/EC. This regulation and directive require the implementation of IPM principles to improve targeted use of all available pest control measures and therefore require the reduction in pesticide use (European Parliament and the Council of the European Union 2009).

For control of WCR larvae, granular soil insecticides are applied at planting time as a band in the corn row (Mayo and Peters 1978). The effectiveness of this approach is limited, however, because only a small fraction of the active ingredient reaches the target (Pimentel 1995). This may be due to the highly variable interaction of the compound with various soil parameters like moisture, soil type, and temperature

(Gray et al. 1992, Wright et al. 2000, Van Rozen and Ester 2010) but may also be due to the behaviour of the target insect (Harris 1972).

Manipulating insect behaviour has the potential to enhance insecticide efficacy (Harris 1972, Gould 1991) by increasing the probability that the insect will contact toxic substances with formulations that combine an attractant and a killing agent; this is referred to as an “attract-and-kill” strategy (El-Sayed et al. 2006). Attract-and-kill strategies have been widely employed against numerous aboveground pests (El-Sayed et al. 2009) but only for a limited number of belowground herbivores. For WCR larvae, an attract-and-kill strategy under field conditions was first evaluated by Hibbard et al. 1995. They used 6 -methoxy 2 -benzoxazolinone (MBOA), which was previously identified as a host location semiochemical (Bjostad and Hibbard 1992), in combination with the insecticidal compound chlorethoxyphos (Hibbard et al. 1995). These authors also suggested that attractiveness of WCR larvae towards this compound could be increased in combination with carbon dioxide (CO_2).

CO_2 is an ubiquitous volatile released by respiring plant roots (Kuzyakov and Larionova 2005) and is commonly used by many soil dwelling insects in many orders as a cue for finding host plants (Johnson and Gregory 2006). It is regarded as a reliable cue for orientation because plants are unable to switch off CO_2 production (Johnson et al. 2006) and because CO_2 diffuses well in the soil (> 10 cm) (Hinsinger et al. 2005). CO_2 was first identified as an attractant for WCR larvae by Strnad et al. (1986). Further studies supported these findings (Hibbard and Bjostad 1988, Bernklau and Bjostad 1998b, Bernklau and Bjostad 1998a) and also showed that CO_2-releasing products can lure larvae away from plants and reduce root damage in the field (Bernklau et al. 2004). CO_2-emitting capsules have recently been identified as an attractant for WCR larvae in laboratory studies and have been integrated in an attract-and-kill approach with the insecticide Tefluthrin (Schumann et al. 2013).

In this study, we investigated whether CO_2-emitting capsules will increase CO_2 levels in the soil and interfere with the ability of WCR to locate corn roots. We also evaluated an attract-and-kill strategy, i.e. the use CO_2-emitting capsules with added insecticide, for the control of WCR. Both management options are aimed at developing a more sustainable strategy for control of WCR larvae.

Materials & Methods

Handling of corn and WCR eggs

The corn cultivar Prinz (KWS, Einbeck, Germany) was used in all experiments. Plants were watered daily and fertilised once each week with a 2% HakaphosBlau solution (Compo, Münster, Germany).

Non-diapausing WCR eggs from the USDA-ARS, North Central Agricultural Research Laboratory, Brookings, North Dakota, USA were used in all experiments. Larvae hatching from this laboratory strain of eggs do not show significant differences in damage levels compared to larvae hatching from wild-type eggs (Hibbard et al. 1999). The eggs were stored at 8°C and were previously determined to begin hatching after 13–14 days at 25 °C and 65% relative humidity. Two days before the expected hatch, the eggs were placed on a 250-µm sieve, and the attached soil was washed off. The eggs were then mixed in a 0.15% agar solution until they were evenly distributed and the desired concentration was obtained. Hatching time and rate were monitored in Petri dishes placed near the experiments. The first larvae began to hatch in 48–72 h after addition to soil in the experiments and continued to hatch over a period of 7–9 days.

Attract-and-kill components

CO_2-emitting capsules (referred to as “capsules”) were prepared by encapsulating commercially available baker’s yeast (as an artificial source of CO_2) in moist calcium alginate. The capsules, which represented the “attract” component, had a diameter of 2.3 mm and a moisture content of about 90% (Patel and Vorlop 1994).

These capsules were used with and without a “kill” component, which consisted of granules of the insecticide Force 1.5 G (Syngenta, Basel, Switzerland). The active ingredient in Force 1.5 G is Tefluthrin (2, 3, 5, 6-tetrafluoro-4-methylbenzyl (Z)-(1RS, 3RS)-3-(2-chloro-3, 3, 3-trifluoro-1-propenyl-2, 2-methylcyclopropanecarboxylate).

In all experiments, the capsules were applied at 7–10 cm soil depth by digging up the soil, inserting the capsules, and then covering them with soil. When the capsules were applied with Tefluthrin granules in an attract-and-kill strategy, half of the capsules were applied, the granules were sprinkled on the capsules, and the remaining capsules were added. The capsules and the granules were always applied at the same time as the WCR eggs so that CO_2 gradients could establish during the

48 h before the first larvae began to hatch. The capsules and granules were weighed before they were applied in the experiments. The capsules were weighed in small plastic boats (Scale: TE 1502s, Sartorius, Germany) and the granules in small glass vials (Scale: H110, Sartorius, Germany).

CO_2 measurements

The concentration of CO_2 in soil was measured with a hand-held CO_2 meter (CARBCOCAP GM 70, Vaisala, Finland). The meter was attached to a flexible tube which was in turn attached to a thin metal pipe (3 mm diameter) with three holes at the tip. The tip of the metal tube was inserted into the soil so that the holes in the tip collected air from 7–10 cm depth. Air was pumped from the soil into the meter's measuring chamber. The CO_2 in the chamber absorbs light emitted by an infrared source, and the meter quantifies light absorbance with a non-dispersive infrared (NDIR) sensor. A Fabry-Perot Interferometer (FPI) interference filter coincides its pass band with the absorption wavelength of CO_2. Finally, the IR detector measures the strength of the signal that passes through (Vaisala 2012). Each air collection lasted for 15 minutes. CO_2 was measured as a mixing ratio of parts per million (ppm). Soil moisture was measured with an absolute humidity reader (PCE–SMM 1, PCE, Germany), and soil moisture was maintained between 20–25%.

CO_2 emission by CO_2-emitting capsules (experiment 1)

The length and rate of CO_2 emission by the capsules was measured in soil arenas (longitudinal plastic boxes: 80 cm long x 14 cm wide x 17 cm high). *Haplic luvi* soil was collected from a field near Göttingen, Germany (51°29`52.88 N, 9°55`38.26 E; Field B: 51°31`16.21 N, 9°57`49.30 E) and was homogenized using a soil shredder (Unifix 300, Moeschle, Ortenberg, Germany). It was then passed through a 1-cm-mesh sieve, so that soil structure was similar in all arenas, and each soil arena was filled with the soil to a depth of 11 cm. One corn seed was planted 25 cm from one end of the arena, and arenas were kept at 23 ± 2°C, 65% air humidity and ambient light. When the corn plants had reached growth stage V3–V4, 50 g of CO_2-emitting capsules were inserted at one point 30 cm from the plant base.

CO_2 was measured along a line at 0, 15, and 30 cm from the plant base so that the 30-cm measurement was at the application point of the capsules. The sampling locations were sampled in a random sequence to ensure that any previous

measurement in the arena would not influence the measurement at the next measuring point. The measurements were taken 3, 5, 10, and 20 days after capsule application to measure CO_2 production by the capsules. Each of six replicate arenas was sampled once (at all three positions) on each sampling day.

Disruption of host finding (experiment 2)

Experiment 2 tested the application of the capsules only ("Disruption of host location") as a management option in 1-m^2 semi-field plots. These consisted of large plastic containers (120 cm long x 60 cm wide x 80 cm high) that were kept in a greenhouse at 22 ± 2 °C, 65% relative humidity, and ambient light. The containers were filled with *Haplic luvi* soil collected from a field near Göttingen, Germany (51°29`52.88 N, 9°55`38.26 E). The soil was prepared as described in experiment 1, and each semi-field plot was filled to a depth of 40 cm. Corn was sown in plastic trays, and the seedlings were transplanted into the semi-field plots 7 days after sowing. Each plot had two rows of corn with nine plants per row, 60 cm between rows, and a 15-cm within row spacing.

At corn growth stage V7–V8, a trench (approx. 10 cm deep) was dug half way between the corn rows and across the whole plot. Half of the soil that had been removed from the trench was mixed with 450 g of capsules and this mixture of capsules and soil was spread evenly in the trench plot. The remaining soil that had been removed from the trench was used to cover the mixture of capsules and soil.

120 WCR eggs were applied in a single hole 15 cm from the base of each plant at about 7 cm depth and 4 hours after the CEC were applied. As noted earlier, the time of first hatch and the hatching rate were monitored in six Petri dishes, which were placed between the semi-field plots. Twenty-one days after the first larval hatch, the number of larvae/plant was determined by removing a cube of soil (16 cm x 11 cm x 13 cm) around the base of each of six corn plants per semi-field plot (= six cubes/replicate). The soil cubes were placed in a Kempson extraction chamber (Kempson et al. 1968) for 72 h, and the larvae were extracted from the soil with heat (60°C) and counted. The average number of larvae/cube was considered equivalent to the number of larvae/plant. Each of the two treatments (with and without capsules) was represented by four replicate semi-field plots.

Concentrations of soil CO_2 were also determined in experiment 2 (as described in experiment 1) before and after capsules were applied in the semi field plots. CO_2

measurements were done before the capsules were applied to determine corn root respiration. The measurements were done at three points (0, 15, and 30 cm from the plant base) 1 day before corn plants were transplanted into the semi-field plots and 1 day before the capsules were added (= 6 weeks after corn was transplanted). As the semi fields have not been treated with the capsules, these measurements were done in all semi field plots giving eight replicates of each measurement point at each measurement day.

After capsule application soil CO_2 was measured between the plant base and the application trench at three points (0, 15, and 30 cm from the plant base) to measure CO_2 production by the capsules. Note that the 30-cm measurement point was in the trench where capsules had been applied. Soil CO_2 was determined at 3, 5, 10, and 20 days after application to determine the period of time in which CO_2 was emitted. Three replicates of each measurement location and measurement day were carried out.

Attract-and-kill (experiment 3)

An experiment to test the attract-and-kill strategy for control of WCR larvae also used semi-field plots but the container size was reduced to 0.5 m^2 (52 cm x 60 cm x 80 cm) by use of 5-mm-thick plastic sheets (PVC CAW, Germany). *Haplic luvi* soil, prepared according to the same protocol as described for experiment 1 and 2 was used for experiment 3, but this soil was collected from a different field near Göttingen (51°31`16.21 N, 9°57`49.30 E). Corn was sown in plastic trays and after 7 days was transplanted into the semi-field plots. Each plot contained two corn rows with three corn plants per row, 60 cm between rows, and 15 cm within row spacing.

When the corn had advanced to growth stage V7–V8, three treatments were established: CO_2-emitting capsules + Tefluthrin granules; Tefluthrin granules alone; and a control without capsules or granules. The CO_2-emitting capsules +Tefluthrin granules were added at two points between the two rows (and therefore 30 cm from each row) and 15 cm apart; the application points were offset with respect to the seedling locations so that each application point was located equidistance from four seedlings. The Tefluthrin granules alone were evenly dispersed along a 7- to 10-cm-deep trench formed within the two corn rows; during trenching, care was taken not to damage the corn roots, and the trench was filled with soil after the granules were added. The control did not receive capsules or granules and was not trenched. Four

hours after the treatments were applied, 180 WCR eggs per corn plant were inoculated 15 cm from the plant base at 7 cm depth; the eggs were added at a point on a line between the points where the capsules were added and the plant bases. The number of larvae/plant was determined as described in experiment 2 from six plants/semi-field plot (= six samples/replicate).

Experiment 3 had two trials that differed in Tefluthrin application rate. In the two treatments with Tefluthrin granules, Tefluthrin was added at an equivalent of 4.8 mg a.i./m row (= 33% of the standard application rate = High) in trial 1 and at 2.4 mg a.i./m row (= 18% of the standard application rate = Low) in trial 2. For the treatment with CO_2-emitting capsules + Tefluthrin granules (i.e., the attract-and-kill treatment), 240 mg (High) or 120 mg (Low) of Tefluthrin was applied at each application point in trial 1 and trial 2, respectively. For the Tefluthrin granules alone treatment (i.e. the conventional treatment), 240 mg (High) or 120 mg (Low) was evenly applied in each corn row in trial 1 and trial 2, respectively. Each treatment in each trial was represented by three semi-field plots.

Statistical analysis

The CO_2 levels at the CO_2 measurement locations (0, 15, and 30 cm from the plant base) points within soil arenas and semi-field plots were compared with a Friedman ANOVA and subsequent Bonferroni-corrected multiple Wilcoxon matched pairs test.

The larval densities per plant were corrected for larval hatch and calculated as larvae per plant per 100 eggs because hatching rates of the larvae and egg densities differed between the experiments. In experiment 2 (in which disruption of host finding was examined), larval densities were compared with a Mann Whitney U test. In experiment 3 (in which attract-and-kill was examined), larval densities were compared with a Kruskal Wallis test and subsequent multiple comparisons. In an overall comparison of WCR control in experiments 2 and 3, the efficacy of each of the three management options (disruption of host finding, attract-and-kill, and conventional control with an insecticide) was calculated as the reduction of larval densities compared to an untreated control (corrected efficacy % = (100 - (larvae in treatment*100/larvae in control)) (Toepfer et al. 2010b). These values were arcsine transformed before analysis and were then subjected to a Kruskal Wallis test and subsequent multiple comparisons to test for differences between treatments. All statistical tests were carried out using Statistica Version 9 (StatSoft 2011).

Results

CO_2 emission by CO_2-emitting capsules (experiment 1)

In the soil arenas with CO_2-emitting capsules, CO_2 levels relative to those at the plant base tended to drop at 15 cm distance and then increased at 30 cm distance (the 30-cm location was where the capsules were added) on each day of CO_2 measurement (Friedmann ANOVA: day 3: d.f. = 2, X^2 = 10.33, P< 0.01; day 5: d.f. = 2, X^2 = 4.26, P = 0.12; day 10: d.f. = 2, X^2 = 12.00, P< 0.01; day 20: d.f. = 2, X^2 = 7.63, P< 0.05) (Table 1). Although CO_2 levels did not significantly differ at the plant base and at the capsule-addition point (30 cm from the plant base) on any day of measurement, CO_2 levels were higher at the capsule-addition point than at the plant base on day 5 (+ 27 ppm) and 10 (+ 257 ppm) and lower on day 3 (- 24 ppm) and 20 (- 60 ppm) (Table 1) after capsules were applied.

In soil arenas without capsules (controls), CO_2 levels decreased with increasing distance from the plant base on each day of CO_2 measurement (Friedmann ANOVA: day 3: d.f. = 2, X^2 = 10.33, P< 0.01; day 5: d.f. = 2, X^2 = 12.00, P< 0.01; day 10: d.f. = 2, X^2 = 9.48, P< 0.01; day 20: d.f. = 2, X^2 = 9.33, P< 0.01) (Table 1). CO_2 levels were always significantly higher at the plant base (0 cm) than 30 cm from the plant base (P< 0.05).

Table 1. Soil CO_2 levels as affected by addition of CO_2-emitting capsules to soil arenas (capsules were added 30 cm from the plant base), distance from the plant base, and time after capsule addition (experiment 1). CO_2 levels are expressed as a mixing ratio of parts per million. Values are means ±SE (n = 6). Different lowercase letters indicate significant differences between CO_2 levels within an arena without capsules (control) or with capsules after Bonferroni-corrected multiple Wilcoxon matched pair tests with $P < 0.05$.

Distance from plant base (cm)	Days after application of capsules			
	3 days		5 days	
	Without capsules	With capsules	Without capsules	With capsules
0	741 ± 15 a	690 ± 31 a	747 ± 27 a	693 ± 26 a
15	658 ± 21 ab	587 ± 38 a	580 ± 17 ab	598 ± 29 a
30	577 ± 16 b	647 ± 25 a	550 ± 21 b	720 ± 50 a
	10 days		20 days	
	Without capsules	With capsules	Without capsules	With capsules
0	657 ± 13 a	628 ± 30 ab	910 ± 42 a	887 ± 55 a
15	552 ± 12 b	562 ± 30 a	640 ± 30 ab	750 ± 52 b
30	552 ± 4 b	885 ± 71 b	610 ± 31 b	827 ± 49 ab

Disruption of host finding (experiment 2)

Before corn was transplanted in semi-field plots CO_2 levels did not significantly differ between measurement points (Fig. 1, V0) (Friedmann ANOVA: d.f. = 2, X^2= 2.80 P = 0.25). At growth stage V7–V8 (6 weeks after corn was transplanted), CO_2 levels significantly decreased with distance from the plant base (Friedmann ANOVA: V 7 - 8: d.f. = 2, X^2 = 9.25; P< 0.01) (Fig. 1).

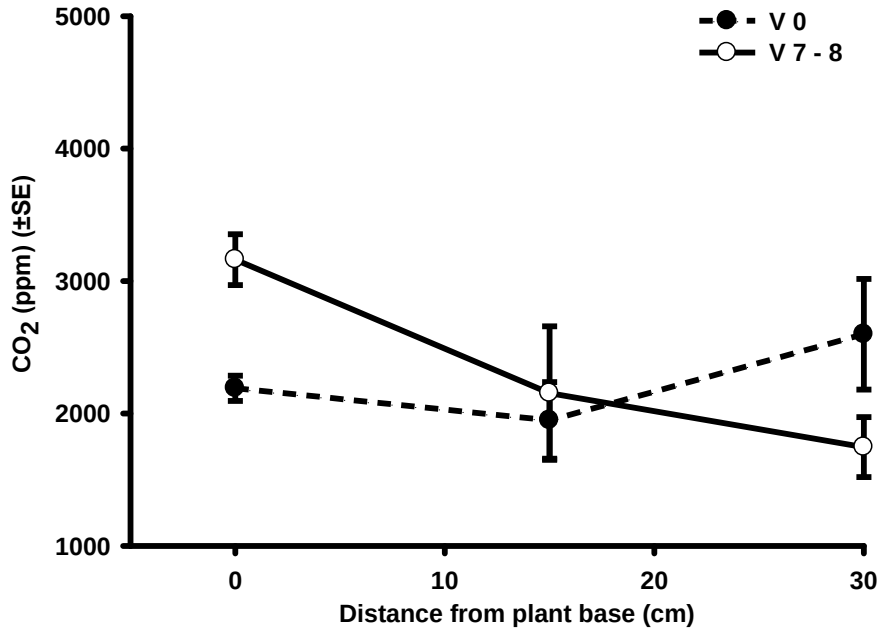


Figure 1.Soil CO_2 levels in semi-field plots without capsules as affected by distance from the plant base and corn growth stage (experiment 2, disruption of host finding). CO_2 levels are expressed as a mixing ratio of parts per million (ppm; means $\pm$ SE) measured 24 h before corn was transplanted (growth stage: V0) and 6 weeks after transplantation (at growth stage V7–V8).

In the semi-field plots with CO_2-emitting capsules, CO_2 levels increased towards the application point (30 cm) on day 3 (Friedmann ANOVA: d.f. = 2, X^2 = 0.67, P = 0. 72) and day 5 (Friedmann ANOVA: d.f. = 2, X^2 = 0.67, P = 0.72) after capsules were applied but the differences were not statistically significant. On day 10, CO_2 levels at the application point were significantly higher than at the plant base (Friedmann ANOVA: d.f. = 2, X^2 = 6.00, P< 0.05). After 20 days, CO_2 levels did not significantly differ between the measurement points but were higher at 15 cm and lower at 30 cm than at the plant base (Friedmann ANOVA: d.f. = 2, X^2 = 4.67, P = 0.09) (Table 2).
In the semi-field plots without capsules, CO_2 levels did not significantly differ between measurement points on day 3 (Friedmann ANOVA: day 3: d.f. = 2, X^2 = 2.00; P = 0.37) and day 20 (Friedmann ANOVA: d.f. = 2, X^2 = 4.67, P = 0.09) (Table 2). On day 5, CO_2 levels were significantly lower at 30 cm than at 0 and 15 cm (Friedmann ANOVA: day 5: d.f. = 2, X^2 = 6.00, P< 0.05). On day 10, CO_2 levels were significantly higher at 15 cm than at 0 cm (Friedmann ANOVA: d.f. = 2, X^2 = 6.00, P< 0.05) (Table 2).

Table 2. Soil CO_2 levels as affected by addition of CO_2-emitting capsules to semi-field plots (capsules were added 30 cm from the plant base), distance from the plant base, and time after capsule addition (experiment 2). CO_2 levels are expressed as a mixing ratio of parts per million (PPM). Values are means ±SE (n = 3). Different lower case letters indicate significant differences between CO_2 levels within a control and capsule arena after Bonferroni corrected multiple Wilcoxon matched pair tests with $P < 0.05$.

Distance from plant base (cm)	Days after application of capsules			
	3 days		5 days	
	Without capsules	With capsules	Without capsules	With capsules
0	2853 ±727 a	3817 ±511 a	3050 ±845 a	3640.0 ±210.1 a
15	3223 ±815 a	3843 ±540 a	3282 ±912 a	3853.3 ±467.0 a
30	3430 ±651 a	3893 ±451 a	2788 ±839 b	4120.0 ±192.9 a
	10 days		20 days	
	Without capsules	With capsules	Without capsules	With capsules
0	2247 ±135 a	2483 ±161 a	4040 ±463 a	4613 ±736 a
15	3040 ±310 b	3397 ±269 b	4103 ±706 a	5727 ±723 a
30	2613 ±146 ab	4007 ±439 b	2770 ±343 a	3813 ±627 a

Addition of capsules did not significantly suppress the number of larvae adjacent to roots. Larval densities (larvae per plant per 100 eggs) were 15.6 ± 1.9 without capsules and 12.9 ± 1.3 with capsules (Mann-Whitney. U test: Z = 0.89; *P* = 0.39) (Fig. 2).

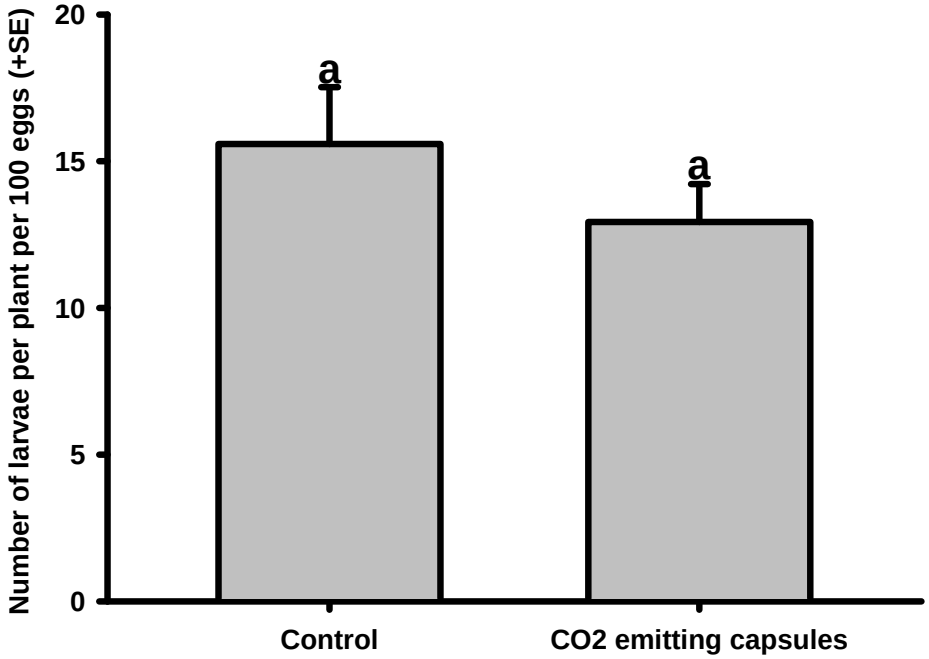


Figure 2. Disruption of host location: Larval density/plant with the application of CO_2 emitting capsules in semi field plots (experiment 2) (Mann Whitney U test; P = 0.39).

Attract-and-kill (experiment 3)

With the high application rate of Tefluthrin in semi-field plots (trial 1), larval density near roots was significantly lower in the conventional treatment than in the control and was lower (but not significantly so) in the attract-and-kill treatment than in the control (Kruskal Wallis test: H = 6.88, $P < 0.05$) (Fig. 3 Trial 1). With the low application rate of Tefluthrin (trial 2), larval density near roots were lower in the conventional treatment and in the attract-and-kill treatment than in the control but the differences were not significant (Kruskal Wallis test: H = 5.42, $P = 0.06$) (Fig. 3 Trial 2).

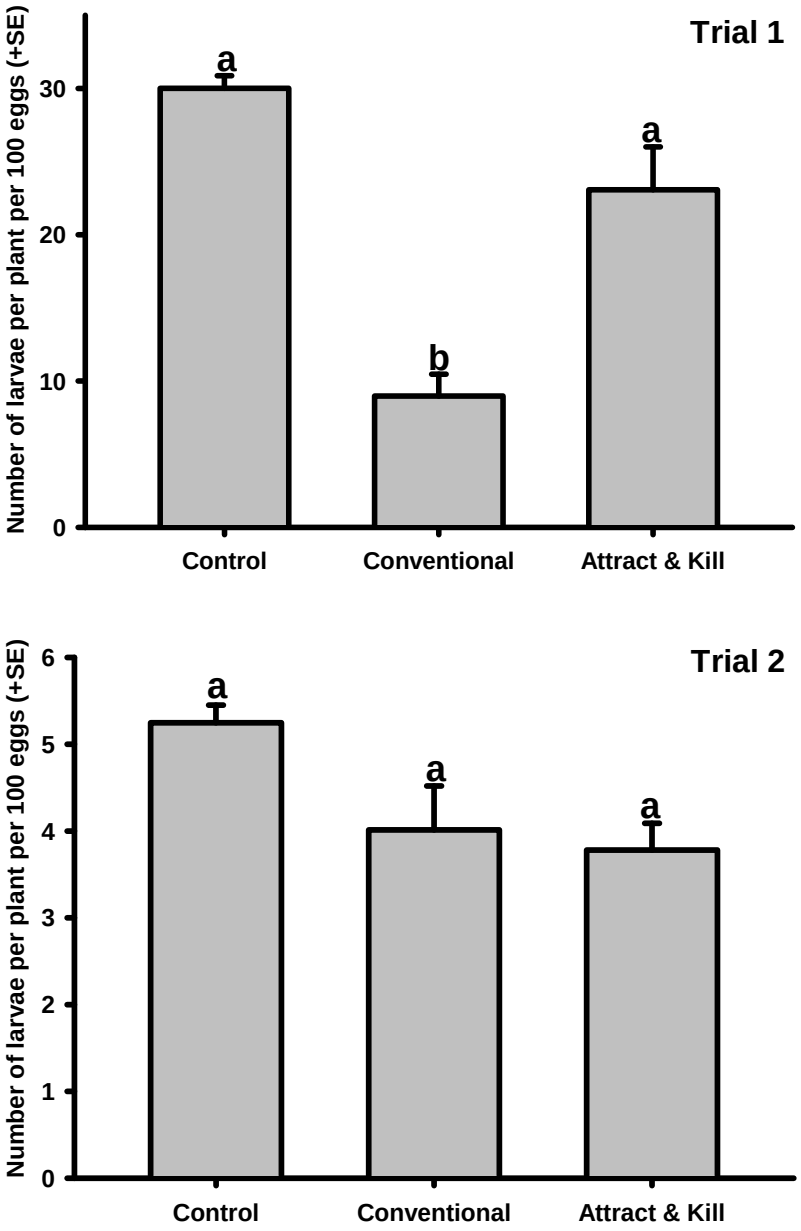


Figure 3. Western corn rootworm larval density/plant in semi-field plots as affected by a conventional treatment (Tefluthin granules alone) and an attract-and-kill treatment (CO_2-emitting capsules + Tefluthin) (experiment 3). The rate of Tefluthrin addition in both treatments was 4.8 mg a.i./100 m row (Trial 1) or 2.4 mg a.i./100 m row (Trial 2). The control was not treated with granules or capsules. Values are means + SE (n = 3). Within each panel, means with different lowercase letters are significantly different (Kruskal Wallis Test; $P < 0.05$ in trial 1, and $P = 0.06$ in Trial 2).

Comparison of WCR suppression by disruption of host location, conventional insecticide, and attract-and-kill (based on data from experiments 2 and 3)

The reduction of larval density (relative to the control) was significantly affected by the treatments tested in the semi-field plots in experiments 2 and 3 (Kruskal Wallis test: H = 8.71; *P* = 0.04) (Fig. 4). This was mainly caused by the substantial reduction (70%) in larval density with the high application rate of Tefluthrin alone in experiment 3. The application of CO_2-emitting capsules alone (to disrupt host location in experiment 2) reduced larval density by only 17%, and this level of control was only marginally increased by addition of Tefluthrin granules with the capsules (to attract-and-kill in experiment 3).

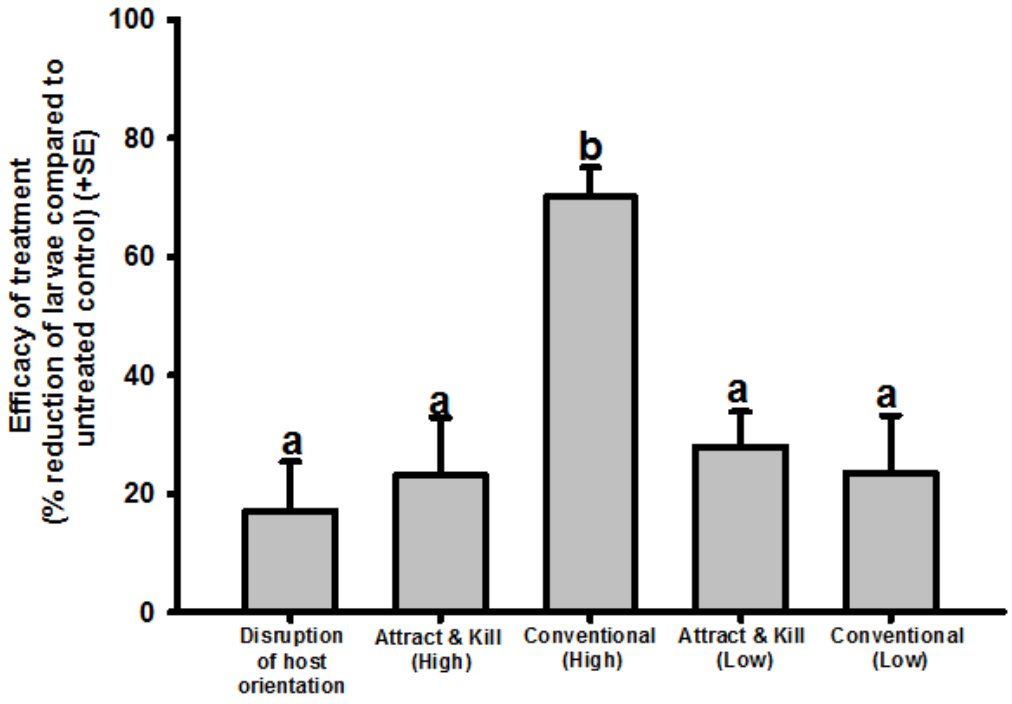


Figure 4. Relative reduction of western corn rootworm larvae 21 days after larval hatch by: disruption of host location (CO_2 emitting capsules alone; experiment 2); an attract-and-kill approach (CO_2-emitting capsules + Tefluthin) with a high rate of Tefluthrin (experiment 3, trial 1) or a low rate of Tefluthrin (experiment 3, trial 2) (experiment 3); and with conventional treatments, which included a high rate of Tefluthrin alone and a low rate of Tefluthrin alone. The high rate and low rates of Tefluthrin were 4.8 mg and 2.4 mg a.i./100 m row, respectively. Values are means + SE (n = 3-4). Means with different lowercase letters are significantly different (Kruskal Wallis test; $P < 0.05$).

Discussion

The application of the CO_2-emitting capsules to the soil increased CO_2 levels around the capsules for up to 20 days. The application of these capsules alone or with a soil insecticide did not sufficiently reduce WCR larval numbers, and control of WCR was much better with a conventional insecticide treatment than with the capsules alone or with capsules plus the insecticide. Thus, an improved formulation of the capsules with an enhanced attractiveness for the larvae needs to be developed for future applications.

CO_2 gradients in the soil in the absence of CO_2 emitting capsules

In addition to reflecting the CO_2 generated by the capsules, the CO_2 levels measured in this study include CO_2 generated by root respiration and microbial respiration (Wang et al. 2005). Consequently, CO_2 levels measured in the semi-field plots (experiment 2) were higher than those in the soil arenas (experiment 1) because the soil volume was larger in the semi-field plots than in the arenas. Presumably this resulted in a much higher microbial biomass and subsequently higher microbial respiration in the semi-field plots. In addition, corn plants were at a more advanced growth stage in experiment 2 (V7–V8) than in experiment 1 (V3–V4) when CO_2 was measured, causing a higher root biomass and a higher CO_2 release from roots (Schwendenmann et al. 2003).

The CO_2 levels in the absence of capsules in experiment 1 decreased with increasing distance from the plant base (Table 1). Decreasing levels of CO_2 with increasing distance from the plant base were also reported for clover roots in a rhizosphere chamber at a much smaller scale (up to 6 cm from clover roots) (Johnson et al. 2006). It can be argued that the CO_2 gradients measured in our experiments are a result of maize root growth because root-derived CO_2 (root and rhizomicrobial respiration (Kuzyakov and Larionova 2005)) can represent as much as 90% of total soil respiration (Hanson et al. 2000). This spatial heterogeneity of CO_2 in the soil, however, may also be influenced by soil bulk density, temperature, humidity, and other soil properties (Schwendenmann et al. 2003). Consequently, any changes in soil properties caused by root growth may have also contributed to higher CO_2 levels in the soil. During the insertion of the measuring probe, we noted that the soil was more compacted around the plant base than at the other measurement points. Because denser soil can reduce CO_2 diffusion (Hinsinger et al. 2003), a higher

accumulation of CO_2 may have been partly caused by soil compaction from root growth rather than from root respiration alone. The same mechanism can be invoked to explain a rise in CO_2 at the plant base in semi-field plots of experiment 2 after 6 weeks of root growth (growth stage V7–V8; Fig. 1).

The CO_2 gradients measured in experiment 2 (Table 2) were inconsistent relative to those in experiment 1 (Table 1). The variability in CO_2 levels was most probably caused by the larger root systems of corn plants in experiment 2 than in experiment 1. CO_2 fluctuations are higher in larger root systems (Pregitzer et al. 1998) as a result of the spatial heterogeneity of respiration rates in different root parts (Hinsinger et al. 2005). Root respiration rates are highest in root tips (Bidel et al. 2001) and in finer roots (Pregitzer et al. 1998), which suggests that researchers should consider the level of root respiration of different root parts when attempting to understand fluctuations in CO_2 gradients in larger corn root systems.

A separation of root respiration from total soil respiration is an essential next step in identifying the quantity of CO_2 in the soil resulting from root respiration. Such a separation has been considered “a challenging task” by soil scientists (Baggs 2006) but is needed to understand the establishment of CO_2 gradients by corn root respiration. A separation of root respiration from total soil respiration could help researchers to calculate effective application rates for CO_2-releasing baits.

CO_2 gradients in the soil with CO_2-emitting capsules

In experiment 1, the CO_2 gradients with the capsules differed compared to the control as CO_2 levels decreased 15 cm from the plant but thenincreased at the capsule site 30 cm from the plant base from day 3 to day 20 after capsules were applied (Table 1). The CO_2 gradients with the capsules in experiment 2 also differed compared to the control on day 5 and 10 as CO_2 levels increased with increasing distance from the plant base. Compared to CO_2 levels measured directly at the plant base, CO_2 levels measured around the capsules in experiments 1 and 2 were higher on day 5 and 10 after capsule application. This rise in CO_2 ranged from 27 to 257 ppm (+4 to 41%) in experiment 1 (Table 1) and from 480 ppm to 1523 ppm (+ 13 to 61%) in experiment 2 (Table 2). Given the ability of 1st instar WCR larvae to detect a 12% difference in CO_2 concentration (Bernklau and Bjostad 1998b), the CO_2 production rates were theoretically high enough (at least on day 10 after capsule application) to lure WCR larvae to the capsules.

An increase in CO_2 levels associated with capsules was mainly evident at the position where the capsules were added, and only small increases in CO_2 levels were evident further than 15 cm from the capsules. We suspect that the soil structure used in our experiments reduced diffusion of CO_2 emissions. Gas diffusion is much lower in a compacted soil than in a porous or sandy soil (Sotta et al. 2006). In the semi-field plots of experiment 2, moreover, we measured CO_2 only in the first 10 cm of the soil depth and not in deeper soil layers. This may have resulted in an underestimation of CO_2 production in the containers because CO_2 tends to sink and thus CO_2 levels increase with depth (Pline and Dusenbery 1987).

For control of the WCR larvae, the emission duration of the CO_2-emitting capsules should be increased. To target WCR larvae during the hatching period, CO_2 release should last for 6–8 weeks when the attractant is applied during sowing (Bernklau et al. 2004) or for at least 4 weeks when the attractant is applied just before larval hatch (Toepfer and Kuhlmann 2006). Field evaluation of the capsules would also be important because CO_2 emission rates in the laboratory/greenhouse can differ from those in the field (Pregitzer et al. 1998), especially with regard to changes in temperature regimes.

Disruption of host location

The application of CO_2-emitting capsules alone failed to substantially disrupt the finding of host roots by larvae in that the capsules reduced larval numbers near host roots by only 17% (Fig. 4). Field experiments described in previous studies have shown that the application of larval attractants alone (e.g. 6 -methoxy 2 -benzoxazolinone (MBOA) (Hibbard et al. 1995)) or various CO_2-releasing products (Bernklau et al. 2004) can be used to manage WCR larvae. The CO_2-emitting capsules used in our study were placed between the corn rows (30 cm from each plant base) to reduce the chance of larvae encountering corn roots as well as possible. We considered this essential when an attractant alone is applied because the neonates must locate roots within 48 h or else they cannot bore into the root and will die of starvation (Strnad and Bergman 1987a). Larvae can disperse up to 1 m through the soil during their development (Short and Luedtke 1970), and larvae also feed on roots in the middle of the corn rows (27–30 cm from the plant base (Strnad and Bergman 1987b, Schumann and Vidal 2012). We speculate that the larvae were initially attracted to the CO_2-emitting capsules in our experiments but were able to

locate and feed on corn roots that were near the capsules. This root feeding may have supported larval development shortly after hatch and enabled larvae to build up sufficient energy reserves to relocate towards roots around the plant base.

Attract-and-kill

The combination of Tefluthrin and CO_2-emitting capsules in an attract-and-kill approach did not result in a level of WCR control that was significantly different from that obtained with capsules alone (Fig. 4). The addition of a pyrethroid with an attractant is generally regarded as an effective combination because of the rapid mortality provided by the pyrethroid (Poullot et al. 2001, Evenden and McLaughlin 2004). Repellent effects of pyrethroids, however, have been reported (Michaelides et al. 1997), and many insects are believed to have evolved to avoid the plant toxin pyrethrum and related pyrethroids (Gould 1991). A repellency of the Tefluthrin granules may have also influenced the attractiveness of the capsules. On the other hand, Tefluthrin did not repel WCR larvae in a laboratory bioassay (Hibbard and Bjostad 1989), and Tefluthrin has been successfully used with CO_2-emitting capsules in an attract-and-kill approach (Schumann et al. 2013).

Reducing the active ingredient of Tefluthrin did not significantly increase the efficacy of the attract-and-kill strategy but did significantly reduce Tefluthrin performance in the conventional treatment (Fig. 4). The latter result may be explained by a lower probability of larvae contacting the insecticide when fewer granules were added. The targeting of WCR larvae in a conventional treatment depends on behavioural preferences or displacement of the larvae (Boetel et al. 2003), both of which determine larval movement into the zone of insecticidal activity. For WCR larvae, the changing preference for different types of roots and for newly developed roots during their development (Chiang 1973) causes a redistribution of the larvae at each instar stage (Strnad and Bergman 1987b, Schumann and Vidal 2012). In an effective attract-and-kill strategy, we hypothesize that the larvae in the soil will be targeted before they move to the plant, increasing the probability of contact with the insecticide even at lower insecticide application rates than used in this study.

If the CO_2-emitting capsules used in this study are to be effective for control of WCR larvae, their attractiveness to the larvae must be increased. This aim may be achieved by adding additional components, apart from CO_2, previously identified as contributing to the orientation of WCR larvae (Bjostad and Hibbard 1992, Hibbard et

al. 1994, Hiltpold et al. 2012, Robert et al. 2012a, Robert et al. 2012b). Host-specific compounds eliciting a localized searching behavior (Bernklau et al. 2009) or acting as feeding stimulants should also be considered. These compounds have been shown to enhance insecticide efficacy against WCR larvae (Bernklau and Bjostad 2005, Bernklau et al. 2011). When selecting these compounds, researchers should consider that the compounds must diffuse well in the soil to contribute to the management of WCR. This is underlined by the fact that CO_2 has been regarded as a good attractant because of its low molecular weight, which allows for rapid diffusion over long distances (Villani and Wright 1990). Volatile signaling molecules like ethylene, which was identified as an WCR attractant of larvae by Robert et al. (2012a), or small uncharged molecules from rhizodeposition like the sugars isolated by Bernklau and Bjostad (2008) are potential candidates because they diffuse well and are not rapidly adsorbed after their release (Jones et al. 2004, Hinsinger et al. 2005).

Overall, the capsules tested in this study have the potential to influence CO_2 gradients in the soil for at least 10 days. A better understanding of how root respiration generates CO_2 gradients in the soil is needed if we are to control WCR larvae with CO_2-releasing attractants. The addition of host-specific compounds to the CO_2-emitting capsules could increase capsule attractiveness and increase the efficacy of the attract-and-kill approach.

Acknowledgements

We thank Bianca Tappe, Magdolna Weller, Daniel Schulze, and Isabella Lübig for their technical assistance; KWS (Einbeck, Germany) for providing the corn seeds; Syngenta (Basel, Switzerland) for supplying Tefluthrin; and Dr. Desiree Jakobs-Schönwandt (University of Applied Sciences, Bielefeld) for the production of the CO_2-emitting capsules. The study was funded by the German Environmental Foundation (DBU).

References

Baggs, E. M. 2006. Partitioning the components of soil respiration: a research challenge. Plant Soil 284: 1-5.

Bernklau, E. J., and L. B. Bjostad. 1998a. Behavioral responses of first-instar western corn rootworm to carbon dioxide in a glass bead bioassay. J. Econ. Entomol. 91: 444-456.

Bernklau, E. J., and L. B. Bjostad. 1998b. Reinvestigation of host location by western corn rootworm larvae (Coleoptera: Chrysomelidae): CO_2 is the only volatile attractant. J. Econ. Entomol. 91: 1331-1340.

Bernklau, E. J., and L. B. Bjostad. 2005. Insecticide enhancement with feeding stimulants in corn for western corn rootworm larvae (Coleoptera: Chrysomelidae). J. Econ. Entomol. 98: 1150-1156.

Bernklau, E. J., and L. B. Bjostad. 2008. Identification of feeding stimulants in corn roots for western corn rootworm (Coleoptera : Chrysomelidae) larvae. J. Econ. Entomol. 101: 341-351.

Bernklau, E. J., E. A. Fromm, and L. B. Bjostad. 2004. Disruption of host location of western corn rootworm larvae (Coleoptera: Chrysomelidae) with carbon dioxide J. Econ. Entomol. 97: 330-339.

Bernklau, E. J., L. B. Bjostad, and B. E. Hibbard. 2011. Synthetic feeding stimulants enhance insecticide activity against western corn rootworm larvae, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). J. Appl. Entomol. 135: 47-54.

Bernklau, E. J., L. B. Bjostad, L. N. Meihls, T. A. Coudron, E. Lim, and B. E. Hibbard. 2009. Localized search cues in corn roots for western corn rootworm (Coleoptera: Chrysomelidae) larvae. J. Econ. Entomol. 102: 558-562.

Bidel, L. P. R., P. Renault, L. Pages, and L. M. Riviere. 2001. An improved method to measure spatial variation in root respiration: application to the taproot of a young peach tree *Prunus persica*. Agronomie 21: 179-192.

Bjostad, L. B., and B. E. Hibbard. 1992. 6-Methoxy-2-benzoxazolinone: A semiochemical for host location by western corn rootworm larvae J. Chem. Ecol. 18: 931-944.

Boetel, M. A., B. W. Fuller, and P. D. Evenson. 2003. Emergence of adult northern and western corn rootworms (Coleoptera : Chrysomelidae) following reduced soil insecticide applications. J. Econ. Entomol. 96: 714-729.

Chiang, H. C. 1973. Bionomics of the northern and western corn rootworms. Annu. Rev. Entomol. 18: 47-72.

Ciosi, M., N. J. Miller, K. S. Kim, R. Giordano, A. Estoup, and T. Guillemaud. 2008. Invasion of Europe by the western corn rootworm, *Diabrotica virgifera virgifera*: multiple transatlantic introductions with various reductions of genetic diversity. Molecular Ecology 17: 3614-3627.

Dillen, K., P. D. Mitchell, and E. Tollens. 2010. On the competitiveness of *Diabrotica virgifera virgifera* damage abatement strategies in Hungary: a bio-economic approach. J. Appl. Entomol. 134: 395-408.

El-Sayed, A. M., D. M. Suckling, C. H. Wearing, and J. A. Byers. 2006. Potential of mass trapping for long-term pest management and eradication of invasive species. J. Econ. Entomol. 99: 1550-1564.

El-Sayed, A. M., D. M. Suckling, J. A. Byers, E. B. Jang, and C. H. Wearing. 2009. Potential of "Lure and Kill" in long-term pest management and eradication of invasive species. J. Econ. Entomol. 102: 815-835.

EPPO (European and Mediterranean Plant Protection Organization). 2011. *Diabrotica virgifera virgifera*. (http://www.eppo.org/QUARANTINE/Diabrotica_virgifera/diabrotica_virgifera.htm).

European Parliament and the Council of the European Union. 2009. Concerning the placing of plant protection products on the market and repealing council directives 79/117/EEC and 91/414/EEC. (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:309:FULL:EN:PDF).

Evenden, M. L., and J. R. McLaughlin. 2004. Factors influencing the effectiveness of an attracticide formulation against the Oriental fruit moth, *Grapholita molesta*. Entomologia Experimentalis Et Applicata 112: 89-97.

Furlan, L., S. Canzi, A. Di Bernardo, and C. R. Edwards. 2006. The ineffectiveness of insecticide seed coatings and planting-time soil insecticides as *Diabrotica virgifera virgifera* LeConte population suppressors. J. Appl. Entomol. 130: 485-490.

Godfrey, L. D., L. J. Meinke, and R. J. Wright. 1993. Vegetative and reproductive biomass accumulation in field corn - Response to root injury by western corn rootworm (Coleoptera, Chrysomelidae). J. Econ. Entomol. 86: 1557-1573.

Gould, F. 1991. Arthropod behaviour and the efficacy of plant protectants. Annu. Rev. Entomol. 36: 305-330.

Gray, M. E., A. S. Felsot, K. L. Steffey, and E. Levine. 1992. Planting time application of soil insecticides and western corn rootworm (Coleoptera, Chrysomelidae) emergence - Implications for long term management programs. J. Econ. Entomol. 85: 544-553.

Gray, M. E., T. W. Sappington, N. J. Miller, J. Moeser, and M. O. Bohn. 2009. Adaptation and invasiveness of western corn rootworm: Intensifying research on a worsening pest. Annu. Rev. Entomol. 54: 303-321.

Hanson, P. J., N. T. Edwards, C. T. Garten, and J. A. Andrews. 2000. Separating root and soil microbial contributions to soil respiration: A review of methods and observations. Biogeochemistry 48: 115-146.

Harris, C. R. 1972. Factors influencing the effectiveness of soil insecticides. Annu. Rev. Entomol. 17: 177-198.

Hibbard, B. E., and L. B. Bjostad. 1988. Behavioral responses of western corn rootworm larvae to volatile semiochemicals from corn seedlings. J. Chem. Ecol. 14: 1523-1539.

Hibbard, B. E., and L. B. Bjostad. 1989. Corn semiochemicals and their effects on insecticide efficacy and insecticide repellency toward western corn rootworm larvae (Coleoptera: Chrysomelidae). J. Econ. Entomol. 82: 773-781.

Hibbard, B. E., E. J. Bernklau, and L. B. Bjostad. 1994. Long-chain free fatty acids: Semiochemicals for host location by western corn rootworm larvae J. Chem. Ecol. 20: 3335-3344.

Hibbard, B. E., F. B. Peairs, S. D. Pilcher, M. E. Schroeder, D. K. Jewett, and L. B. Bjostad. 1995. Germinating corn extracts and 6-Methoxy-2-Benzoxazolinone - western corn rootworm (Coleoptera, Chrysomelidae) larval attractants evaluated with soil insecticides. J. Econ. Entomol. 88: 716-724.

Hibbard, B. E., B. D. Barry, L. L. Darrah, J. J. Jackson, L. D. Chandler, L. K. French, and J. A. Mihm. 1999. Controlled field infestations with western corn rootworm (Coleoptera : Chrysomelidae) eggs in Missouri: Effects of egg

strains, infestation dates, and infestation levels on corn root damage. Journal of the Kansas Entomological Society 72: 214-221.

Hiltpold, I., B. E. Hibbard, B. W. French, and T. C. J. Turlings. 2012. Capsules containing entomopathogenic nematodes as a Trojan horse approach to control the western corn rootworm. Plant Soil 358: 10-24.

Hinsinger, P., C. Plassard, C. X. Tang, and B. Jaillard. 2003. Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: A review. Plant Soil 248: 43-59.

Hinsinger, P., G. R. Gobran, P. J. Gregory, and W. W. Wenzel. 2005. Rhizosphere geometry and heterogeneity arising from root-mediated physical and chemical processes. New Phytol. 168: 293-303.

Johnson, S. N., and P. J. Gregory. 2006. Chemically-mediated host-plant location and selection by root-feeding insects. Physiological Entomology 31: 1-13.

Johnson, S. N., X. X. Zhang, J. W. Crawford, P. J. Gregory, N. J. Hix, S. C. Jarvis, P. J. Murray, and I. M. Young. 2006. Effects of carbon dioxide on the searching behaviour of the root-feeding clover weevil *Sitona lepidus* (Coleoptera : Curculionidae). Bulletin of Entomological Research 96: 361-366.

Jones, D. L., A. Hodge, and Y. Kuzyakov. 2004. Plant and mycorrhizal regulation of rhizodeposition. New Phytol. 163: 459-480.

Kempson, D., M. Lloyd, and R. Ghelardy. 1968. A new extractor for woodland litter. Pedobiologia 3: 1-30.

Kiss, J., C. R. Edwards, H. K. Berger, P. Cate, M. Cean, S. Cheek, J. Derron, H. Festić, L. Furlan, J. Igrc-Barčić, I. Ivanova, W. Lammers, V. Omelyuta, G. Princzinger, P. Reynaud, I. Sivcev, P. Sivicek, G. Urek, and O. Vahala. 2005. Monitoring of western corn rootworm (*Diabrotica virgifera virgifera* LeConte) in Europe 1992 -2003, pp. 29-41. In S. Vidal, U. Kuhlmann and C. R. Edwards (eds.), Western Corn Rootworm- Ecology and Management. CABI, Wallington,UK.

Kuzyakov, Y., and A. A. Larionova. 2005. Root and rhizomicrobial respiration: A review of approaches to estimate respiration by autotrophic and heterotrophic organisms in soil. J. Plant Nutr. Soil Sci. 168: 503-520.

Levine, E., and H. Oloumi-Sadeghi. 1991. Management of Diabroticite rootworms in corn. Annu. Rev. Entomol. 36: 229-255.

Lilly, J. H. 1956. Soil insects and their control. Annu. Rev. Entomol. 1: 203-222.

Ma, B. L., F. Meloche, and L. Wei. 2009. Agronomic assessment of *Bt* trait and seed or soil-applied insecticides on the control of corn rootworm and yield. Field Crops Research 111: 189-196.

Mayo, Z. B. 1980. Influence of planting dates on the efficacy of soil insecticides applied to control larvae of the western and northern corn rootworm (Coleoptera, Chrysomelidae). J. Econ. Entomol. 73: 211-212.

Mayo, Z. B., and L. L. Peters. 1978. Planting vs cultivation time applications of granular soil insecticides to control larvae of corn rootworms (Coleoptera: Chrysomelidae) in Nebraska. J. Econ. Entomol. 71: 801-803.

Michaelides, P. K., A. L. Cleverly, and D. J. Wright. 1997. Sub-lethal effects of tefluthrin on *Diabrotica undecimpunctata howardi*, Barber: Plant protection and larval development. Crop Protection 16: 423-429.

Moellenbeck, D. J., M. L. Peters, J. W. Bing, J. R. Rouse, L. S. Higgins, L. Sims, T. Nevshemal, L. Marshall, R. T. Ellis, P. G. Bystrak, B. A. Lang, J. L. Stewart, K. Kouba, V. Sondag, V. Gustafson, K. Nour, D. P. Xu, J. Swenson, J. Zhang, T. Czapla, G. Schwab, S. Jayne, B. A. Stockhoff, K. Narva, H. E. Schnepf, S. J. Stelman, C. Poutre, M. Koziel, and N. Duck. 2001. Insecticidal proteins from *Bacillus thuringiensis* protect corn from corn rootworms. Nat. Biotechnol. 19: 668-672.

Patel, A. V., and K. D. Vorlop. 1994. Entrapment of biological control agents applied to entomopathogenic nematodes. Biotechnology Techniques 8: 569-574.

Pimentel, D. 1995. Amounts of pesticides reaching target pests - Environmental impacts and ethics. Journal of Agricultural & Environmental Ethics 8: 17-29.

Pline, M., and D. B. Dusenbery. 1987. Responses of plant parasitic nematode *Meloidogyne incognita* to carbon dioxide determined by video camera computer tracking. J. Chem. Ecol. 13: 873-888.

Poullot, D., D. Beslay, J. C. Bouvier, and B. Sauphanor. 2001. Is attract-and-kill technology potent against insecticide-resistant Lepidoptera? Pest Management Science 57: 729-736.

Pregitzer, K. S., M. J. Laskowski, A. J. Burton, V. C. Lessard, and D. R. Zak. 1998. Variation in sugar maple root respiration with root diameter and soil depth. Tree Physiol. 18: 665-670.

Rasmann, S., T. G. Kollner, J. Degenhardt, I. Hiltpold, S. Toepfer, U. Kuhlmann, J. Gershenzon, and T. C. J. Turlings. 2005. Recruitment of

entomopathogenic nematodes by insect-damaged maize roots. Nature 434: 732-737.

Riedell, W. E., and R. N. Reese. 1999. Maize morphology and shoot CO_2 assimilation after root damage by western corn rootworm. Crop Science 39: 1332-1340.

Robert, C. A. M., M. Erb, M. Duployer, C. Zwahlen, G. R. Doyen, and T. C. J. Turlings. 2012a. Herbivore-induced plant volatiles mediate host selection by a root herbivore. New Phytol. 194: 1061-1069.

Robert, C. A. M., M. Erb, B. E. Hibbard, B. W. French, C. Zwahlen, and T. C. J. Turlings. 2012b. A specialist root herbivore reduces plant resistance and uses an induced plant volatile to aggregate in a density-dependent manner. Functional Ecology 26: 1429-1440.

Schumann, M., and S. Vidal. 2012. Dispersal and spatial distribution of western corn rootworm larvae in relation to root phenology. Agricultural and Forest Entomology 14: 331-339.

Schumann, M., A. Patel, and S. Vidal. 2013. Evaluation of an attract and kill strategy for western corn rootworm larvae. Applied Soil Ecology 64: 178-189.

Schwendenmann, L., E. Veldkamp, T. Brenes, J. J. O'Brien, and J. Mackensen. 2003. Spatial and temporal variation in soil CO_2 efflux in an old-growth neotropical rain forest, La Selva, Costa Rica. Biogeochemistry 64: 111-128.

Short, D. E., and R. J. Luedtke. 1970. Larval migration of western corn rootworm. J. Econ. Entomol. 63: 325-326.

Sotta, E. D., E. Veldkamp, B. R. Guimaraes, R. K. Paixao, M. L. P. Ruivo, and S. S. Almeida. 2006. Landscape and climatic controls on spatial and temporal variation in soil CO_2 efflux in an Eastern Amazonian Rainforest, Caxiuana, Brazil. For. Ecol. Manage. 237: 57-64.

StatSoft, I. 2011. STATISTICA for Windows (data analysis software), Version 10.0. (www.statsoft.com.).

Strnad, S. P., and M. K. Bergman. 1987a. Movement of first instar western corn rootworms (Coleoptera: Chrysomelidae) in soil. Environmental Entomology 16: 975-978.

Strnad, S. P., and M. K. Bergman. 1987b. Distribution and orientation of western corn rootworm (Coleoptera: Chrysomelidae) larvae in corn roots. Environmental Entomology 16: 1193-1198.

Strnad, S. P., M. K. Bergman, and W. C. Fulton. 1986. 1st instar western corn rootworm (Coleoptera: Chrysomelidae) response to carbon dioxide. Environmental Entomology 15: 839-842.

Toepfer, S., and U. Kuhlmann. 2006. Constructing life-tables for the invasive maize pest *Diabrotica virgifera virgifera* (Col.: Chrysomelidae) in Europe. J. Appl. Entomol. 130: 193-205.

Toepfer, S., R. Burger, R. U. Ehlers, A. Peters, and U. Kuhlmann. 2010a. Controlling western corn rootworm larvae with entomopathogenic nematodes: effect of application techniques on plant-scale efficacy. J. Appl. Entomol. 134: 467-480.

Toepfer, S., I. Hatala-Zseller, R. U. Ehlers, A. Peters, and U. Kuhlmann. 2010b. The effect of application techniques on field-scale efficacy: can the use of entomopathogenic nematodes reduce damage by western corn rootworm larvae? Agricultural and Forest Entomology 12: 389-402.

Urias-Lopez, M. A., and L. J. Meinke. 2001. Influence of western corn rootworm (Coleoptera: Chrysomelidae) larval injury on yield of different types of maize. J. Econ. Entomol. 94: 106-111.

Urias-Lopez, M. A., L. J. Meinke, G. H. Leon, and F. J. Haile. 2000. Influence of western corn rootworm (Coleoptera:Chrysomelidae) larval injury on photosynthetic rate and vegetative growth of different types of maize. Environmental Entomology 29: 861-867.

Vaisala. 2012. Vaisala CARBOCAP® sensor for measuring carbon dioxide. (http://www.vaisala.com/Vaisala Documents/Technology Descriptions/CEN-TIA-G-Carbocap-Technology-description-B210780EN-C.pdf).

Van Rozen, K., and A. Ester. 2010. Chemical control of *Diabrotica virgifera virgifera* LeConte. J. Appl. Entomol. 134: 376-384.

Vaughn, T., T. Cavato, G. Brar, T. Coombe, T. DeGooyer, S. Ford, M. Groth, A. Howe, S. Johnson, K. Kolaz, C. Pilcher, J. Purcell, C. Romano, L. English, and J. Pershing. 2005. A method of controlling corn rootworm feeding using a *Bacillus thuringiensis* protein expressed in transgenic maize. Crop Science 45: 931-938.

Villani, M. G., and R. J. Wright. 1990. Environmetal influences on soil macroarthropod behavior in agricultural systems. Annu. Rev. Entomol. 35: 249-269.

Wang, W., K. Ohse, J. J. Liu, W. H. Mo, and T. Oikawa. 2005. Contribution of root respiration to soil respiration in a C-3/C-4 mixed grassland. Journal of Biosciences 30: 507-514.

Wright, R. J., M. E. Scharf, L. J. Meinke, X. G. Zhou, B. D. Siegfried, and L. D. Chandler. 2000. Larval susceptibility of an insecticide-resistant western corn rootworm (Coleoptera : Chrysomelidae) population to soil insecticides: Laboratory bioassays, assays of detoxification enzymes, and field performance. J. Econ. Entomol. 93: 7-13.

Chapter 5

Field evaluation of an attract and kill strategy against western corn rootworm larvae

Authors: Mario Schumann*, Stefan Toepfer, Marina Vemmer, Anant Patel, Uli Kuhlmann and Stefan Vidal

*Corresponding author

Field evaluation of an attract and kill strategy against western corn rootworm larvae

M. Schumann [1], S. Toepfer [2,3], M. Vemmer[4], A. Patel [4], U. Kuhlmann [3] and S. Vidal [1]

[1] Georg-August University, Department of Crop Sciences, Agricultural Entomology, Grisebachstr. 6, 37077 Göttingen, Germany, e-mail: Mario.Schumann@agr.uni-goettingen.de

[2] CABI c/o Plant Protection Directorate, Rarosi ut 110, 6800 Hodmezovasarhely, Hungary

[3] CABI Europe - Switzerland, Rue des Grillons 1, 2800 Delemont, Switzerland

[4] University of Applied Sciences, Engineering and Alternative Fuels, Department of Engineering and Mathematics, Bielefeld, Wilhelm-Bertelsmann-Strasse 10, 33602 Bielefeld, Germany

Abstract

The larvae of the maize pest, western corn rootworm (*Diabrotica virgifera virgifera,* Coleoptera: Chrysomelidae) hatch in the soil in spring and search for maize roots following CO_2 gradients. CO_2 might be used as an attractant towards soil insecticides, a mechanism already shown in laboratory experiments. This study compared the efficacy of several combinations of in or between row applications of different rates of CO_2 - emitting capsules and/or soil insecticides (here Tefluthrin) aimed at preventing root damage by the pest larvae under field conditions.

CO_2 emission of the capsules in the soil lasted up to 28 days with a peak after 21 days coinciding with the first larval hatch. In-row applications of Tefluthrin with or without CO_2 - emitting capsules prevented root damage to a much larger extent (59 to 77 % on the node injury scale) than the between - row applications of Tefluthrin with or without capsules (17 to 31 %). All Tefluthrin applications, regardless of whether at full, half or quarter rates similarly and effectively prevented root damage; thus CO_2 could not significantly further increase this efficacy. In conclusion, further research on the below ground orientation and movement of *D. v. virgifera* larvae, as well as at combinations of very low soil insecticide rates combined with CO_2 - emitting capsules, are needed to potentially develop attract and kill strategies as a management option against this alien maize pest.

Keywords: western corn rootworm; carbon dioxide; Tefluthrin; attract and kill; below ground interaction; *Zea mays*

Introduction

The western corn rootworm (*Diabrotica virgifera virgifera* Le Conte; Coleoptera: Chrysomelidae) is an invasive pest of maize*, Zea mays* in large parts of North America and Europe. It is a univoltine species which overwinters as eggs in the soil (Krysan 1986). After maize has germinated, the larvae hatch and pass through three larval instars (Chiang 1973), almost exclusively feeding on maize roots (Moeser and Hibbard 2005). This leads to a reduced water and nutrient uptake (Gavloski et al. 1992; Kahler et al. 1985) by the maize plant. Serious feeding damage can cause plant lodging (Spike and Tollefson 1991) and economically significant yield losses as plants cannot be harvested (Cox et al. 2008). Adults emerge between mid - June and early August in Central Europe (Toepfer and Kuhlmann 2006), and can occasionally further reduce crop yields through intensive silk feeding which interferes with maize pollination (Chiang 1973).

In the corn belt of the USA costs for the management of this pest and of yield losses are at least 1 billion dollars annually (Spencer et al. 2009). In Europe, populations of this beetle were first detected near Belgrade airport in Serbia in 1992, and a rapid spread in Eastern Europe was monitored the following years (Kiss et al. 2005). Further independent introductions (Ciosi et al. 2008; Miller et al. 2005) and stratified dispersal of the beetle (Bermond et al. 2012; Ciosi et al. 2011) resulted in colonisation of additional Central and Western European countries in the last two decades. Once the beetle will have reached its potential spread in Europe (Kriticos et al. 2012), it is estimated that it can cause 472 million Euro annually when control measures against this pest are not implemented (Wesseler and Fall 2010).

The control of the pest in North America is dominated by the use of *Bacillus thuringiensis* -based transgenic cultivars in combination with soil insecticides (Moellenbeck et al. 2001). Control practices in Europe on the other hand currently mainly involve crop rotation (Dunbar and Gassmann 2013) and chemical control with insecticide seed coatings (Furlan et al. 2006) or granular soil insecticides (Sutter et al. 1989; Sutter et al. 1990). The use of insecticide coated seeds can cause non-target effects, such as neonicotinoids on bees (Girolami et al. 2012, 2013), and are currently debated for potential restrictions in Europe (Cressey 2013). This may make granular soil insecticides the current focus for chemical control of the larvae in Europe. These are usually applied at planting time as a band application over the maize row (Mayo and Peters 1978). A major problem for WCR control with granular

soil insecticides is the variable efficacy which can be influenced by a variety of cultural, environmental and biological factors (Levine and Oloumi-Sadeghi 1991).

The manipulation of biological factors such as the orientation behaviour of the target organism allows to use insecticide more effectively (Harris 1972). A promising option is the use of semiochemicals in host finding (Gould 1991) as they can give the opportunity to enhance control efficacy of toxic ingredients through an attract and kill approach (El-Sayed et al. 2009; Foster and Harris 1997). *Diabrotica v. virgifera* larvae are known to orient towards plant roots following CO_2-gradients (Bernklau and Bjostad 1998b; Bernklau and Bjostad 1998a; Strnad et al. 1986). CO_2 is a ubiquitous volatile released by respiring plant roots (Kuzyakov and Larionova 2005). Its production cannot be switched off (Johnson et al. 2006) and it diffuses over long distances in the soil due to its low molecular weight (Pline and Dusenbery 1987; Villani and Wright 1990). Therefore CO_2 seems to be an appropriate cue for orientation of an insect larva. It is, however, not specific to a certain plant (Johnson and Gregory 2006), and can thus not be used by a larva for detecting a specific host plant. A variety of other larval orientation cues have been suggested such as 6-Methoxy-2-benzoxazolinone (MBOA) (Bjostad and Hibbard 1992), fatty acids (Hibbard et al. 1994; Hiltpold et al. 2012) or E-(β)-*caryophyllene* (Robert et al. 2012a; Robert et al. 2012b), but their importance under field conditions remains unclear. Furthermore compounds such as *caryophyllene* rapidly degrade in the soil (Erb et al. 2012; Rasmann et al. 2005), making it difficult to target the WCR larvae during their hatching period which can last for four weeks (Toepfer and Kuhlmann 2006).

The use of a controlled and slow release of attractants over a longer period of time is mainly known for the control of above ground pests, mainly involving sex pheromones (Heuskin et al. 2011). Recently CO_2 emitting capsules, that releases CO_2 over 14 days at room temperature, was developed (Vemmer and Patel 2011). The capsules also have the potential to attract WCR larvae and could be integrated in an attract and kill strategy with Tefluthrin in laboratory studies (Schumann et al. 2013). This approach might offer the potential for controlling this pest without increasing the toxic active ingredients or may even allow a reduction of active ingredients. The use of this CO_2 attractant for increasing insecticide efficacy against *D. v. virgifera* has, however, never been tested in maize under field conditions.

This study therefore assessed the efficacy of different application types and concentrations of Tefluthrin with or without CO_2 - emitting capsules in preventing root damage by *D. v. virgifera* larvae under field conditions in Hungary in 2011. Results will allow to evaluate whether CO_2 can be used in below ground attract and kill approaches to combat this invasive alien maize pest.

Materials and methods

Study sites

This study was carried out in two conventionally managed maize fields, referred to as field A south of Szeged and field B north of Szeged, in Csongrad County in southern Hungary in 2011(Tab. 1). The soil structure differed between the two study sites and was classified according to soil texture (Atterberg 1905) and soil type terminology (IUSS 2007) . This has been a *D. v. virgifera* - infested region since 1995 (Kiss et al. 2005). Field A hosted a small natural *D. v. virgifera* population whereas field B had no natural population. Pre-crop in 2010 had been maize in field A and winter wheat in field B. Fields had been ploughed in autumn 2010, and were ploughed and harrowed before sowing in 2011. Maize seeds of the hybrid NK Kansas (*Zea mays*, Bayer code ZEAMX, Grain maize hybrid, FAO 300, Syngenta, Budapest, Hungary) were sown 18 and 19 April 2011 (Belarus tractor with Pneumasem sowing machine of Nodet Gugis, Lacaille SA, France). All seeds were fungicide - treated (Fludioxonil + Metalaxyl - M). Individual maize seeds were sown every 180 mm in-rows separated by 750 mm, leading to about 73,000 plants per hectare. Disc waltzing was carried out after sowing. Fields were treated with a post – emergence herbicide at BBCH 11 – 13 early May (5l Lumax / ha + 1 l Dezormin /ha). Additionally mechanical weeding was carried out once in field A in mid - May.

None of the fields had a geographic relief drift; thus soil texture was assumed to be relatively homogenous across fields, but denser along tyre tracks caused by farming machinery. Two one litre soil samples were randomly taken at 50 to 250 mm depth from each field end April to analyse clay, silt and sand content, as well as levels of calcium carbonate, pH (H_2O), and humus by the Soil Conservation Service, Szolnok, Hungary (Tab. 1). Six undisturbed soil cores were taken randomly at 70 to 120 mm depth from each field site once per month from April to July to determine soil moisture and soil bulk density (Copper cylinders, 50 mm deep by 50 mm in diameter, volume 0.1 l). Cylinders with soil were immediately sealed with plastic lids and transported to the laboratory. The samples were weighed, dried at 80 - 120 °C for 24 h, and re – weighed. Gravimetric soil moisture (h_s) and soil bulk density (d_s) was calculated as $h_s\,(weight\ \%) = a - b / b - c * 100$ and $d_s\,(g/cm^3) = m_2 - m_{1/} V$, where: a = wet weight of the soil with ring and lids; m_2 = b = dry weight of the soil with ring and lids; m_1 = c = dry weight of the soil; V = volume of the soil core. Air temperature at

1.5m height and rainfall was recorded hourly from April to September (weather station of Davis Instruments Corp., Hayward, CA, USA) (Tab. 1).

Source and handling of *Diabrotica v. virgifera*

Diabrotica v. virgifera eggs were obtained from a laboratory culture of field - collected beetles in southern Hungary in August and September 2010 (for procedures see (Singh and Moore 1985). Eggs were overwintered at 6 to 8° C in moist sand, and diapause was terminated between 22 and 25 April 2011 by incubating the eggs at 25° C. This spread of incubation dates simulated the long egg hatching period of natural populations in the field (Toepfer and Kuhlmann 2006). At the date of field infestation, the sand was sieved through a 250 - micrometer mesh, and recovered eggs were mixed into a solution of 0.15% aqueous agar.

One set of seven subsequent maize plants of each experimental plot were infested with 200 viable and ready-to-hatch eggs per plant in both fields 2 and 3 May 2011 (BBCH 11-13). The eggs were applied with a standard pipette (5ml, Eppendorf, Hamburg, Germany) in two portions of about 1 ml egg - water - agar into two 100 to 140 mm - deep holes at a distance of 160 to 190 mm from both sides of the maize plant. This is a quarter of the row distance, and is thus simulating eggs halfway between in-row treatments and between-row treatments (for details see below).

A portion of eggs was transferred onto moist filter paper in Petri dishes and incubated at 25 °C in the laboratory to monitor time of first hatch and the hatching rate of the larvae. Larvae started to hatch 16 May over a period of 12 days until 1 June. An average hatching rate of 85 ± 2.12 % was measured. Additionally Petri dishes were buried into field soil at 10 cm depth to determine the time of first hatch at field temperatures. The larvae started to hatch 19 May 2011. According to weather conditions in Hungary in 2011, larval development and adult emergence was neither preceded nor delayed compared with other years (Toepfer and Kuhlmann 2006).

Study design

The efficacy of the larvae killing synthetic insecticide Tefluthrin and larvae attracting CO_2 - emitting capsules at reducing root damage and preventing yield loss were studied in the maize fields A and B in 2011 (Tab. 1 and 2). This study was conducted according to the efficacy evaluation standards PP 1/212 and PP 1/152 (European and Mediterranean Plant Protection Organization (EPPO) 2007, 2011) . Five to eight

plots of 4 rows (3.5 m x 30 m) each were used per treatment and control. A random block design was used for the placement of plots. Seven successive maize plants (≈ 1.3 meters) were randomly chosen among the two middle rows of each 4 row - wide plot for artificial infestation with 200 ready-to-hatch *D. v. virgifera* eggs per plant as described above, as well as for the efficacy data assessments (see below).

Table 1 Characteristics of the two study sites in Csongrad county in southern Hungary in 2011 (Mean temperature = mean daily air temperature at 1.50 m height; Mean soil moisture at 70 to 120 mm depth).

Location	South of Szeged, Hungary	North of Szeged, Hungary
Field code	A	B
Field provider	GK Szeged	Agroplanta RT Szeged
Coordinates	N 46° 13.590	N 46° 18` 45.7``
	E 20° 09.035	E 20° 06` 49.2``
Elevation (m)	80	82
Field size (ha)	0.6	15
Trial size (ha)	0.3	0.3
Soil types	mollic fluvisol (clay soil)	chernozem (black soil)
Sand content (%)	27	51
Silt content (%)	32	19
Clay content (%)	41	30
Soil pH (H_2O)	8.2	8.2
Soil $CaCO_3$ (%)	1.2	4.9
Soil Humus (%)	1.6	2.7
Soil bulk density (g/cm^3)	1.0 to 1.2	1.1 to 1.4
Soil moisture (w% = grav%) April / May / June / July	21/ 32/ 17/ 8	15/ 23/ 13/ 7
Mean temperature (C) April / May / June / July	14/17/21.2/21.6	
Sum rainfall (mm) April / May / June / July	2/67/21/34	

Table 2 Treatment characteristics of Tefluthrin (Force™ 1.5G, Syngenta) fine granules and CO_2-emitting capsules, all been applied into the soil against *Diabrotica v. virgifera* larvae in southern Hungary. Hectare rates are calculated for a space of 750 mm between rows; Plots size were 3.5 metres (= 4 rows) x 30 metres. 7 consecutive plants were assessed for root damage and yield per plot. Fields were planted with approximately 73.000 plants per hectare in field A at April 18th, and at field B April 19th 2011; plots were infested with 200 *D. v. virgifera* eggs per plant on May 2nd and 3rd 2011; field A also hosted a natural population*.

Treatments	Application technique	Approx. conc. (kg / ha)	Conc. / row metre (gram)	Conc. / plant (gram)	Field	Plots
In-row applications						
Tefluthrin commercial standard full rate (*FR*)	Insecticide fine granules into sowing row at sowing	15	1.3	0.23	A B	6 7
Tefluthrin half rate (*HR*)	Insecticide fine granules into sowing row at sowing	7.5	0.6	0.11	A B	5 5
Tefluthrin quarter rate (*QR*)	Insecticide fine granules into sowing row at sowing	3.8	0.3	0.05	A B	5 5
Tefluthrin half rate (*HR*) + CO_2 high rate (*H*)	Insecticide fine granules at sowing + CO_2 capsules 2 weeks after sowing into sowing row	7.5 + 3700	0.6 + 277.8	0.11+ 50	A B	5 5
Tefluthrin half rate (*HR*) + CO_2 medium rate (*M*)	Insecticide fine granules at sowing + CO_2 capsules 2 weeks after sowing into sowing row	7.5 + 736	0.6 + 55.6	0.11+ 10	A B	5 5
Tefluthrin half rate (*HR*) + CO_2 low rate (*L*)	Insecticide fine granules at sowing + CO_2 capsules 2 weeks after sowing into sowing row	7.5 + 74	0.6+ 5.6	0.11+ 1	A B	5 5
Tefluthrin quarter rate (*QR*) + CO_2 high rate (*H*)	Insecticide fine granules at sowing + CO_2 capsules 2 weeks after sowing into sowing row	3.8 + 3700	0.3 + 277.8	0.05+ 50	A B	5 5
Tefluthrin quarter rate (*QR*) + CO_2 medium rate (*M*)	Insecticide fine granules at sowing + CO_2 capsules 2 weeks after sowing into sowing row	3.8 + 736	0.3 + 55.6	0.05+ 10	A B	5 5
Tefluthrin quarter rate (*QR*) + CO_2 low rate (*L*)	Insecticide fine granules at sowing + CO_2 capsules 2 weeks after sowing into sowing row	3.8 + 74	0.3+ 5.6	0.05+ 1	A B	5 5

Between-row applications						
CO_2 high rate (*H*)	CO_2 capsules between row 2 weeks after sowing	3700	277.8	50	A B	5 5
Tefluthrin half rate (*HR*) + CO_2 high rate (*H*)	Insecticide fine granules between rows at sowing + CO_2 capsules between rows 2 weeks after sowing	7.5 + 3700	0.6 + 277.8	0.11+ 50	A B	5 5
Tefluthrin half rate (*HR*) + CO_2 medium rate (*M*)	Insecticide fine granules between rows at sowing + CO_2 capsules between rows 2 weeks after sowing	7.5 + 736	0.6 + 55.6	0.11+ 10	A B	5 5
Tefluthrin half rate (*HR*) + CO_2 low rate (L)	Insecticide fine granules between rows at sowing + CO_2 capsules between rows 2 weeks after sowing	7.5 + 74	0.6+ 5.6	0.11+ 1	A B	5 5
Controls						
Untreated artificially infested control	Untreated *D. v. virgifera* – infested control	–	–	–	A B	6 7
Untreated artificially not infested control*	Untreated *D. v. virgifera* – uninfested control	–	–	–	A B	8 8

Product test materials and their application

Tefluthrin fine granules

Fine granules (1 to 2 mm diameter, Formulation type: Fine granule FG of GIFAP code) of the soil insecticide Tefluthrin, i.e. the pyrethroid 2, 3, 5, 6 - Tetrafluoro - 4 - methylbenzyl (Z)-(1RS, 3RS)-3-(2-chloro-3, 3, 3-trifluoro-1-propenyl-2, 2 - methyl-cyclopropanecarboxylate (Force™ 1.5 G, Syngenta, Budapest, Hungary) were applied to control the *D. v. virgifera* larvae. The granules were either applied into the seeding row or between the seeding rows.

As for the in-row applications, a granule applicator (Galdept-10 of Galenika Fitofarmacija, Srem Karlovci, Serbia) was used. Granules were applied into seeding rows at about 80 to 110 mm depth into the soil just prior to seed placement on April 18th and 19th 2011 (Tab. 2). Three different concentrations were applied, i.e. (i), the commercial standard rate of 1.3 gram Tefluthrin per row meter (*FR*), (ii) a half rate of 0.6 gram (*HR*), and (iii) a quarter rate of 0.3 gram (*QR*). For details on per-hectare concentrations refer to Tab. 2.

As for the between-row application of Tefluthrin, a 10 cm wide and 50-110 mm deep trench was created with a hand-held garden cultivator, and the granules were manually dispersed into the trench and covered with soil. Half of the commercial standard rate, i.e. 0.6 gram per row meter (*HR*) was applied. For details see Tab. 2.

CO_2 - emitting capsules

CO_2 - emitting capsules, made up of an artificial CO_2 source (commercially available baker's yeast) encapsulated in Ca-alginate (diameter: 2.3 mm; moisture content: 90%) according to (Patel and Vorlop 1994), were express-shipped to Hungary 28 April 2011. The capsules were kept at 5 - 8 °C until application. The current formulation of the capsules maintains CO_2 gradients for 14 days at room temperature (Vemmer and Patel 2011) The CO_2 - emitting capsules were applied during the same period as the infestation with eggs, i.e. 2 to 5 May 2011 (Maize growth stage: BBCH 11 - 13 (Lancashire et al. 1991)). Capsules were manually applied using teaspoons for 1 gram, tablespoons for 10 gram, and 70 mm diameter kitchen sieves for 25 gram (2 x 5 g) (tab. 2). Capsules were applied at a depth of 70 - 110 mm using an iron hole – maker for small and medium capsule application rates (see for concentrations in table 2), and a 10 cm wide spate for high application rates. The capsules were either applied into the seeding row or between the seeding rows.

As for the in-row applications, holes were made into the maize rows between the young plants, i.e. into the middle of the 18 cm distant plants, to the apply CO_2 - emitting capsules. Three different concentrations of capsules were applied together with the half rate Tefluthrin (*HR*), and three concentrations of capsules with a quarter rate Tefluthrin (*QR*). In detail, either with half or quarter rate Tefluthrin, (i) a high rate of 278 gram capsule were applied per row meter (50 gram per plant) (*H*), or (ii) a medium rate of 56 gram per row meter (10 gram per plant) (*M*), or (iii) a low rate of 5.6 gram per row meter (1 gram per plant) (*L*). For details on per-hectare concentrations see Tab. 2.

As for the between-row applications, capsules were applied in 37 cm distances on each side of a plant row, and into holes ca. 18 cm apart from each other to allow a comparison with the in-row application. A high rate of capsules (*H)*, i.e. 278 g per row meter (50 gram per plant), were applied without insecticide. Moreover, half rate Tefluthrin (*HR*). was applied with (i) a high rate of 278 g capsules (*H)* per row meter (*H*), or (ii) a medium rate of 56 g per row meter (10 gram per plant) (*M*), or (iii) a low

rate of 5.6 g per row meter (1 gram per plant) (*L*) (for details on per-hectare concentrations see table 2.)

Untreated controls

Untreated *D. v. virgifera* egg - infested plots as well as untreated uninfested plots served as controls in both fields.

Assessment of CO_2 emission from capsules in the soil

CO_2 emission was measured in pots (diameter 13 cm) that had been filled with the soil of each field (Tab. 1). Totally, eight pots were set up for each soil type and each of the following CO_2 concentrations:

1. Soil only
2. Soil and 50 g of CO_2 - emitting capsules = high application rate (*H*)
3. Soil and 10 g of CO_2 - emitting capsules = medium application rate (*M*)
4. Soil and 1 g of CO_2 - emitting capsules = low application rate (*L*)

The capsules were applied in the same depth of 70 - 110 mm as in the field 4 May 2011. The pots were slightly irrigated every second day to prevent drying out. CO_2 levels were measured in the soil using a hand held CO_2 meter (CARBOCAP GM 70, Vaisala, Finland) that emits infrared light and uses a non–dispersive infrared sensor for detecting the absorption of light by CO_2 (VAISALA 2010). A 200 mm metal pipe of 3 mm diameter with a hole 30 mm from the tip and combined with a flexible tube was connected to a pump chamber collecting air from the soil into the measuring chamber of the CO_2 meter. The tip of the metal tube was inserted into 100 mm depth so that the drilled holes collected air from about 70 mm depth. Each measurement lasted 15 minutes in which the CO_2 levels typically increased to a peak value in the first 2 - 3 minutes and slowly dropped to a constant level. CO_2 was measured as a mixing ratio of parts per million (PPM), and the maximum and minimum values were averaged. CO_2 levels were measured after 3 and 7 days, and subsequently once per week until no CO_2 emission was detected.

The distribution of the CO_2 level data was visually investigated for normality using histograms. CO_2 levels were compared in a repeated measure ANOVA with time and treatment as independent variables and CO_2 levels as the dependent variable for each soil type.

Assessment of prevention of root damage

In each study field, the root systems of seven infested maize plants were dug out per treatment, plot and field to rate the root damage (soil root systems of ca. 250 x 250 and 200 mm depth) in field A 11 and 12 August, and in field B from 17 to 19 August 2011. This corresponds to totally 35 assessed plants per treatment per field. Loosely adhering soil was removed from the roots by gentle shaking and beating, so as not to break off any of the crown roots. Any remaining soil was removed using a water sprayer. Damage was rated using the linear decimal 0.00 to 3.00 node injury scale (Oleson et al. 2005). The suggested economic threshold level in conventional grain maize is at 0.25 for the corn belt of the USA (Oleson et al. 2005), and 0.75 for irrigated conventional maize growing in northern Italy (R. Edwards, 2011, pers. comm.). To avoid subjective bias on these ratings, root damage was without knowing whether the roots came from treated or untreated plots. Average root damage was calculated for each experimental plot and treatment. The mean efficacy of each treatment was calculated as the reduction in root damage relative to the untreated control across both fields (corrected efficacy % = (100 - (root damage in treated plots * 100 / root damage in the control)).

The distribution of the root damage and efficacy data was visually investigated for normality using histograms. Root data needed square root transformation and efficacy data needed arcsine transformation prior analyses. The influence of the treatments (independent explanatory factor) on the root damage or the efficacy in preventing root damage was tested using univariate ANOVA. Averages of root damage or efficacy data were compared between treatments using Tukey posthoc range test in case of normal distribution of data and equal homogeneity of variances.

Results

CO_2 - emission from capsules in the soil

CO_2 emission from capsules was detected in the soil types of both fields regardless of the application rate. Temporal CO_2 emission patterns were almost identical in both field types (Fig. 1). CO_2 emissions were significantly affected by the application rate (Soil of field A: $F_{3,28} = 41.6$, $P < 0.0001$; Field B: $F_{3, 28} = 27.0$, $P < 0.0001$), the time after CO_2 application (A: $F_{5,140} = 31.0$, $P < 0.0001$; B: $F_{5,140} = 25.8$, $P < 0.0001$), and the interaction of both (A: $F_{15,140} = 14.5$, $P < 0.0001$; B: $F_{15,140} = 8.5$, $P < 0.0001$).

At the highest application rate of 50 g CO_2 - emitting capsules per plant, CO_2 levels were elevated in the soil throughout the measuring period of up to 28 days. The medium application rate of 10 g capsules per plant elevated the CO_2 in the soil between day 7 and 28 after application. The low application rate of 1g CO_2 capsules per plant did not measurably elevate the CO_2 in the soil from field A; but slightly elevated the CO_2 levels in the soil of field B between day 14 and 28.

The main start of CO_2 production coincided with the start of larval hatch of *D. virgifera* in the soil (Fig. 1). CO_2 emission reached a peak around day 21 after application, regardless of the applied rate. The increase in CO_2 emission was likely to be a result of increased temperature (Fig. 1). CO_2 levels started to decline after three weeks until being nearly extinct after five weeks. Heavy rainfall was measured at the 11^{th} and 19^{th} day after the application, but had no measurable effect on CO_2 levels in the soil.

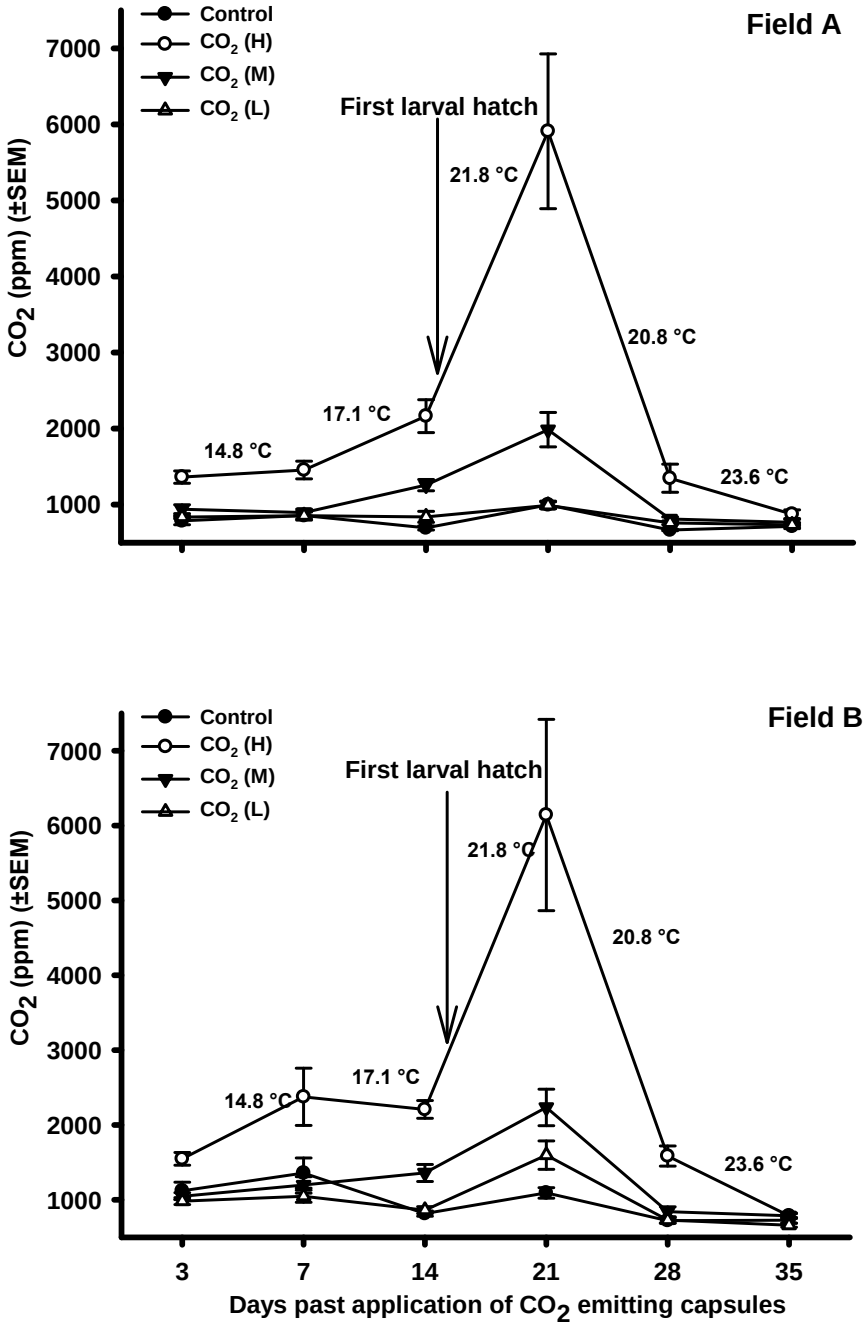


Fig. 1 Elevated CO_2 levels in potted soil from two maize fields (A, B) in southern Hungary in 2011, as a result of the application of CO_2 - emitting capsules at high (H: 50 g per plant, 277.8g per row meter), medium (M: 10 g per plant, 55.6 g per row meter) or low (L: 1 g per plant, 5.56 g per row meter) rates. Capsules were applied into a depth of 7 - 10 cm into the soil on May 4th 2011, and first larval hatch commenced about 15 days after application of the capsules; PPM = mixing ratio of parts per million. Temperatures show the mean air temperatures between CO_2 measurements

Root damage caused by *Diabrotica v. virgifera* larvae

Root damage in the infested but untreated control plots was found to be close to the economic threshold of 0.25 for conventional corn (Oleson et al. 2005) (Node injury scale: Field A: 0.17 ± 0.04; Field B: 0.16 ± 0.02). Uninfested control plots were slightly damaged by a small natural infestation in field A (Node injury scale: 0.08 ± 0.04). Few feeding scares were detected in the uninfested control plots of field B, and are likely not originating from *D. v. virgifera.* Data from the uninfested control were not included in further data analysis.

Prevention of root damage by treatments

The treatment type significantly affected the prevention of root damage, i.e. the pest larvae, across all treatments ($F_{11,110} = 3.96$, $P < 0.001$; Fig. 2). This effect was mainly caused by the lower efficacies of the between row application of the products (between 17 - 30%) compared to those of the in-row applications (between 59 – 76 %). At the lowest in-row application rate of Tefluthrin (QR), the additional application of CO_2 capsules across the different rates increased efficacies from 59% (Tefluthrin only) up to 73 % (Tefluthrin+CO_2 (L)). A similar pattern was also measured when CO_2 capsules were added to a between row applications of half rate Tefluthrin and efficacy increased from 17% (Tefluthrin only) up to 31% (Tefluthrin + CO_2 (M)) .

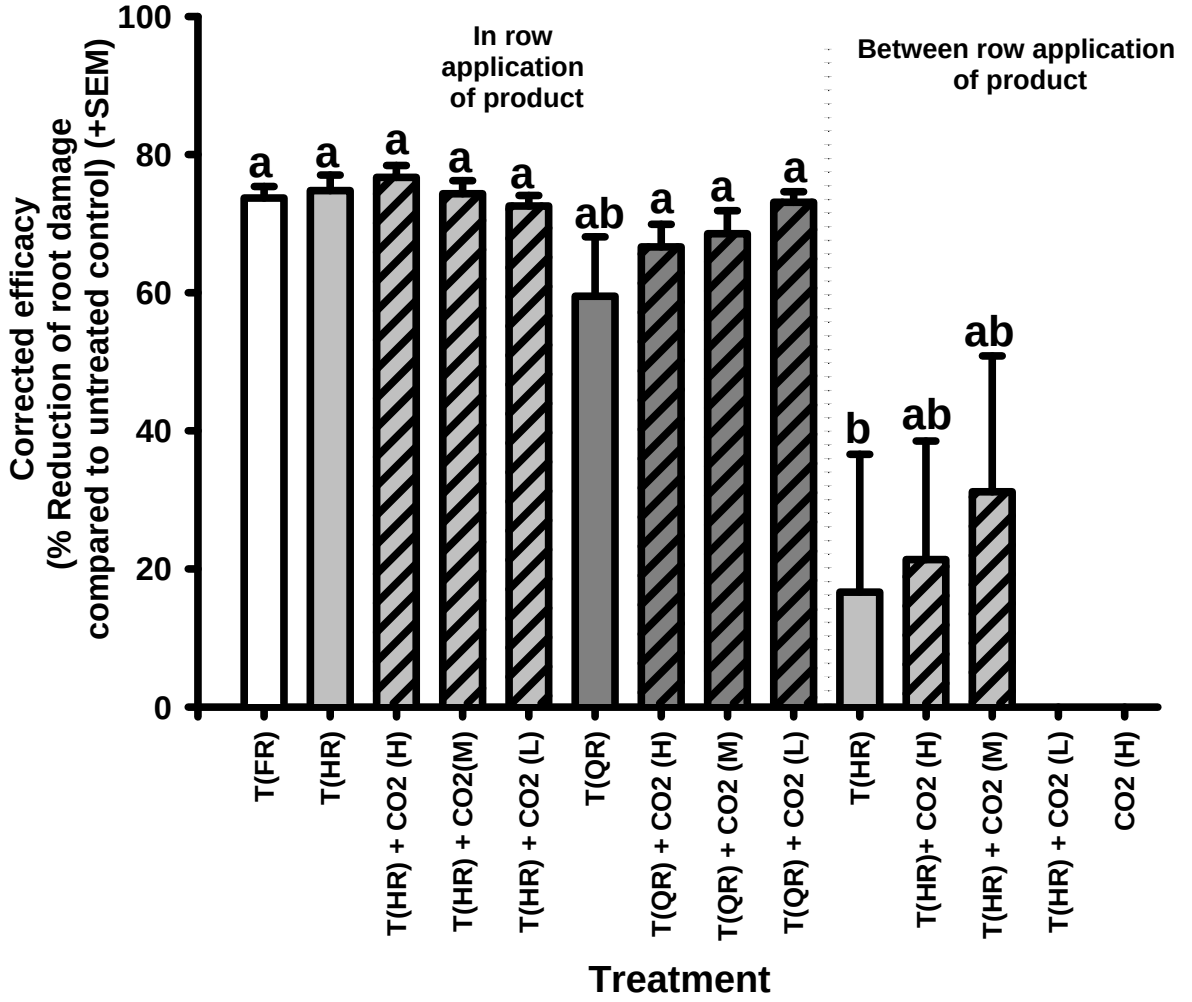


Fig. 2 Mean percent reduction of root damage by *D. v. virgifera* from the full (FR), half (HR) and quarter (QR) application rate of Tefluthrin with the addition of a high (H), medium (M) and low (L) concentration of CO_2 emitting capsules (hatched bars) in and between the maize rows across two maize fields in southern Hungary in 2011. Roots were infested with 200 eggs per plant. Root damage was assessed by the 0 - 3 node injury scale (= corrected efficacy). Error bars = SEM; letters above bars indicate significant differences according to Tukey post hoc test at $P < 0.05$

Prevention of root damage by in-row application of Tefluthrin

All in-row Tefluthrin treatments without CO_2 - emitting capsules prevented root damage by 73.7 ± 1.7 % at full rate, 74.8 ± 2.3 % at half rate, 59.9 ± 8.6 % at quarter rate of Tefluthrin across both fields (Fig. 2). In field A all application rates of Tefluthrin significantly reduced root damage from 0.17 ± 0.04 in the untreated infested control to 0.05 ± 0.001, 0.05 ± 0.004 and 0.05 ± 0.002 at a full, half and quarter rate, respectively ($F_{3,18}$ = 8.6, $P < 0.001$). No significant differences between the application rates could be measured. In field B, also all application rates of Tefluthrin

significantly reduced root damage from 0.16 ± 0.02 in the control to 0.04 ± 0.005, 0.04 ± 0.006 and 0.08 ± 0.03 at the full, half and quarter rate, respectively ($F_{3, 20}$ = 17.9, P < 0.001). Root damage with a quarter rate application rate of Tefluthrin appeared slightly higher, but no significant difference versus the half or full application rate could be detected (P > 0.05) (Fig. 3).

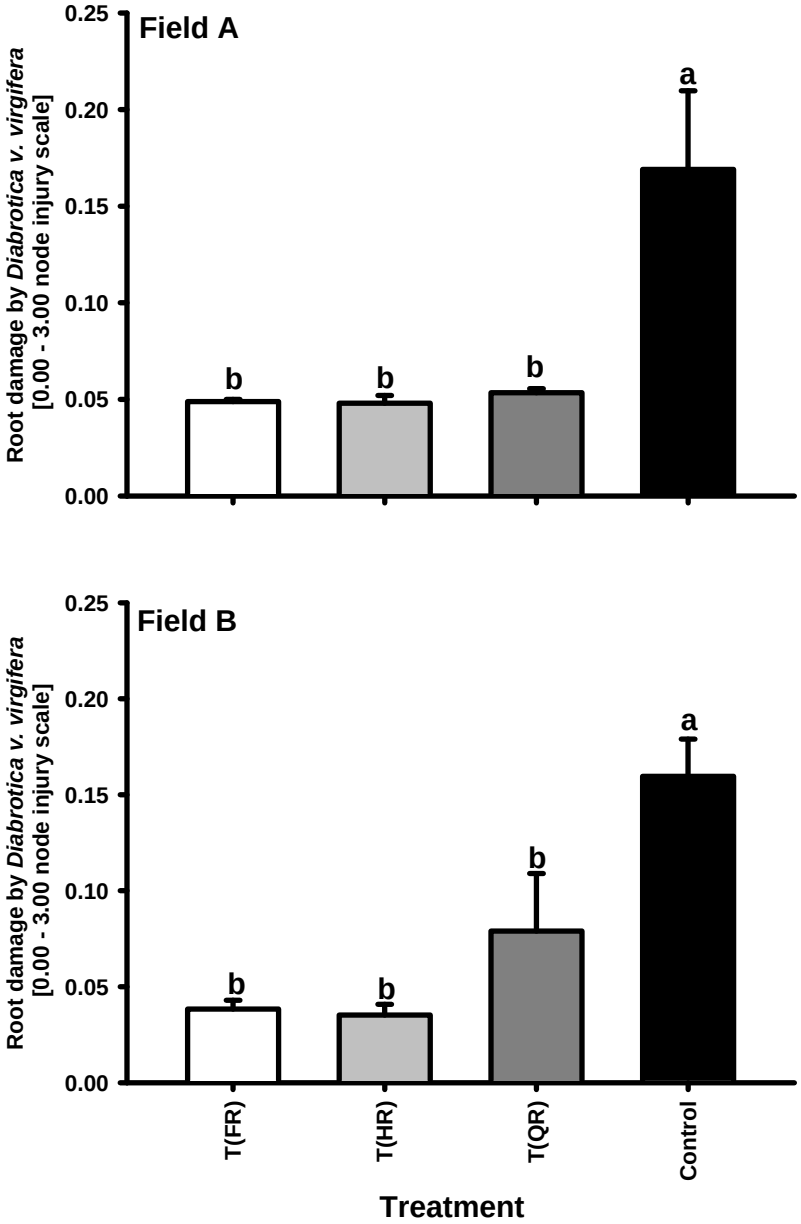


Fig. 3 Root damage of *Diabrotica v. virgif*era larvae in maize plots treated with Tefluthrin (T) fine granules at full rate (FR), half rate (HR) and quarter rate (QR) into the maize rows in two maize fields (A and B) in southern Hungary in 2011. Roots artificially infested with 200 eggs per plant. Root damage were assessed using the 0.00 - 3.00 node injury scale. 5 x 7 consecutive plants were assessed per treatment and field. Error bars = SEM; Letters above bars indicate significant differences according to Tukey post hoc test at P < 0.05

Prevention of root damage by in-row application of half rate Tefluthrin and CO_2 - emitting capsules

Treatments of half rate Tefluthrin with different rates of CO_2 emitting capsules prevented root damage in both fields by 72.6 ± 1.5 % at a low CO_2 and up to 76.7 ± 1.7 % at high CO_2 rate. The prevention of root damage with full or half rate Tefluthrin alone (see above) had been so effective that no significant influence was detected with regard to the prevention of root damage when adding CO_2 emitting capsules to half rate Tefluthrin (Fig. 2). In field A, all CO_2 rates added to half rate Tefluthrin reduced root damage according to the node injury scale from 0.17 ± 0.04 in the untreated infested control to 0.04 ± 0.003 at high CO_2, 0.04 ± 0.006 at medium CO2, and 0.04 ± 0.004 at low CO_2 application rate compared to a half Tefluthrin rate without CO_2 (0.05 ± 0.004) ($F_{5, 26}$ = 9.42, P < 0.0001; Fig. 4). In field B, all CO_2 rates added to half rate Tefluthrin reduced root damage from 0.16 ± 0.02 in the control to 0.04 ± 0.004 at a high CO_2, 0.05 ± 0.002 at medium CO_2, and 0.05 ± 0.002 at low CO_2 application rate, compared to 0.04 ± 0.006 at half Tefluthrin rate without CO_2 ($F_{5, 28}$ = 27.79, P < 0.0001; Fig. 4).

Prevention of root damage by in-row application of quarter rate Tefluthrin and CO_2 - emitting capsules

The CO_2 capsules increased the efficacy of quarter rate Tefluthrin from 59.5 ± 8.6 % (Tefluthrin alone) to 66.6 ± 3.3 %, 68.6 ± 3.3 % and 73.1 ± 1.5 % at the high, medium and low application rate of CO_2, respectively; however, no significant difference in the increase in efficacy could be detected (Fig. 2). In field A all CO_2 rates added to quarter rate Tefluthrin reduced root damage according to the node injury scale from 0.17 ± 0.04 in the untreated infested control to 0.05 ± 0.008 at high CO_2, 0.06 ± 0.011 at medium CO_2, and 0.04 ± 0.004 at low CO_2 application rate, compared with 0.05 ± 0.002 quarter Tefluthrin rate without CO_2 ($F_{5,26}$ = 7.19, P < 0.001; Fig. 5). In field B, all CO_2 rates added to quarter rate Tefluthrin reduced root damage from 0.16 ± 0.02 in the control to 0.06 ± 0.008 at high CO_2, 0.05 ± 0.003 at medium CO_2, and 0.05 ± 0.002 at low CO_2 rate, compared to 0.08 ± 0.03 at quarter and 0.04 ± 0.005 at full Tefluthrin rate without CO_2 ($F_{5,28}$ = 14.29, P < 0.001; Fig. 5).

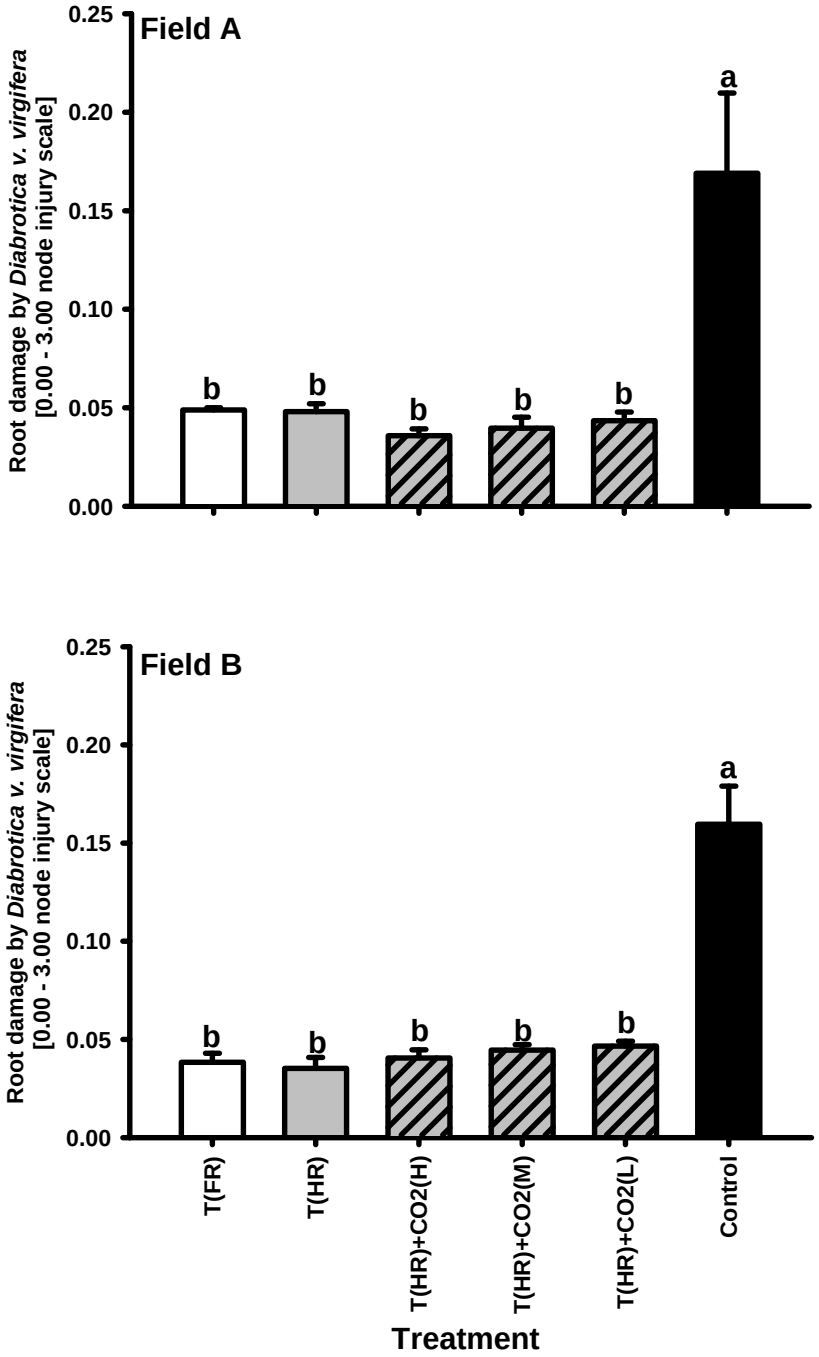


Fig. 4 Root damage of *Diabrotica v. virgif*era larvae in maize plots treated with Tefluthrin (T) fine granules at full rate (FR), and half rate (HR) and at half rate (HR) with a high (H), medium (M) and low (L) concentration of CO_2-emitting capsules (hatched bars) into the maize rows in two maize fields (A and B) in southern Hungary in 2011. Roots artificially infested with 200 eggs per plant

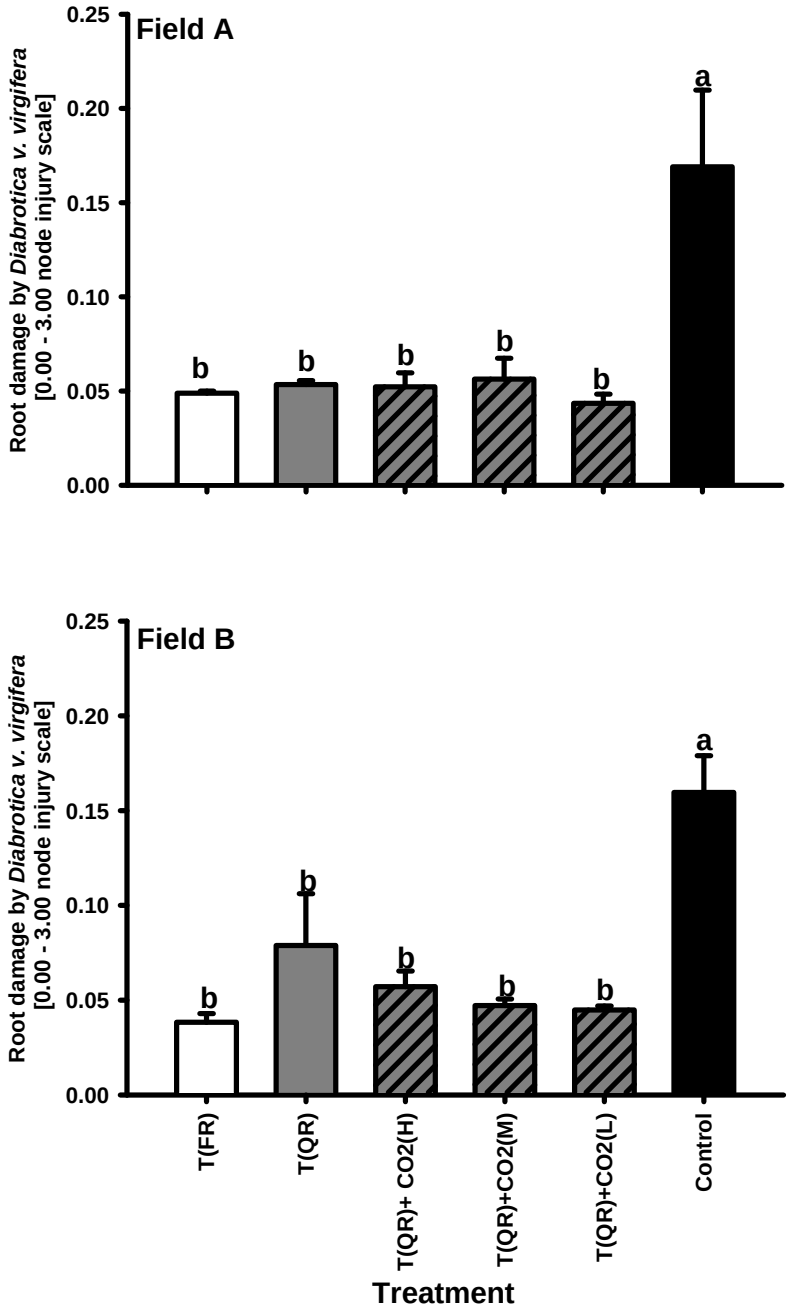


Fig. 5 Root damage of *Diabrotica v. virgif*era larvae in maize plots treated with Tefluthrin (T) fine granules at full rate (FR), and quarter rate (QR) and at quarter rate with a high (H), medium (M) and low (L) concentration of CO_2-emitting capsules (hatched bars) into the maize rows in two maize fields (A and B) in southern Hungary in 2011. Roots were artificially infested with 200 eggs per plant

Prevention of root damage by between - row application of half rate Tefluthrin enhanced by CO_2 - emitting capsules

The between - row application of Tefluthrin with a medium rate of CO_2 prevented 31.2 ± 19.7 % root damage followed by Tefluthrin and high rate CO_2 with 21.3 ± 17.2 %, compared with 16.7 ± 19.9 % root damage prevention when half rate Tefluthrin was applied without CO_2. (Fig. 2) In field A, none of between-row applications of CO_2 rates added to half rate Tefluthrin as well as half rate Tefluthrin alone reduced root damage compared with the untreated infested control (node injury scale) and only the in-row application of Tefluthrin reduced root damage ($F_{7, 34} = 5.3$, $P < 0.001$). At a high CO_2, 0.17 ± 0.05, 0.17 ± 0.06 at medium CO_2, or 0.23 ± 0.04 half rate Tefluthrin rate without CO_2 versus 0.17 ± 0.04 in the untreated infested control. With CO_2 only and a low CO_2 rate with Tefluthrin root damage even increased to 0.44 ± 0.12 and 0.34 ± 0.12 (Fig. 6). In field B, the between-row application of medium CO_2 rate added to half rate Tefluthrin as well as the half rate Tefluthrin alone reduced root damage from 0.16 ± 0.02 in the control to 0.05 ± 0.005 and 0.06 ± 0.009 for both treatments, respectively. None of the other between-row applications, i.e. low and high CO_2 rates added to half rate Tefluthrin or high rate CO_2 alone significantly reduced root damage compared with the untreated infested control (node injury scale: 0.09 ± 0.03 at high CO_2 with half rate Tefluthrin, 0.10 ± 0.03 at low CO_2 with half rate Tefluthrin, and 0.10 ± 0.03 at high CO_2 rate only) (Fig. 5) ($F_{7, 36} = 6.8$, $P < 0.001$) (Fig. 6)

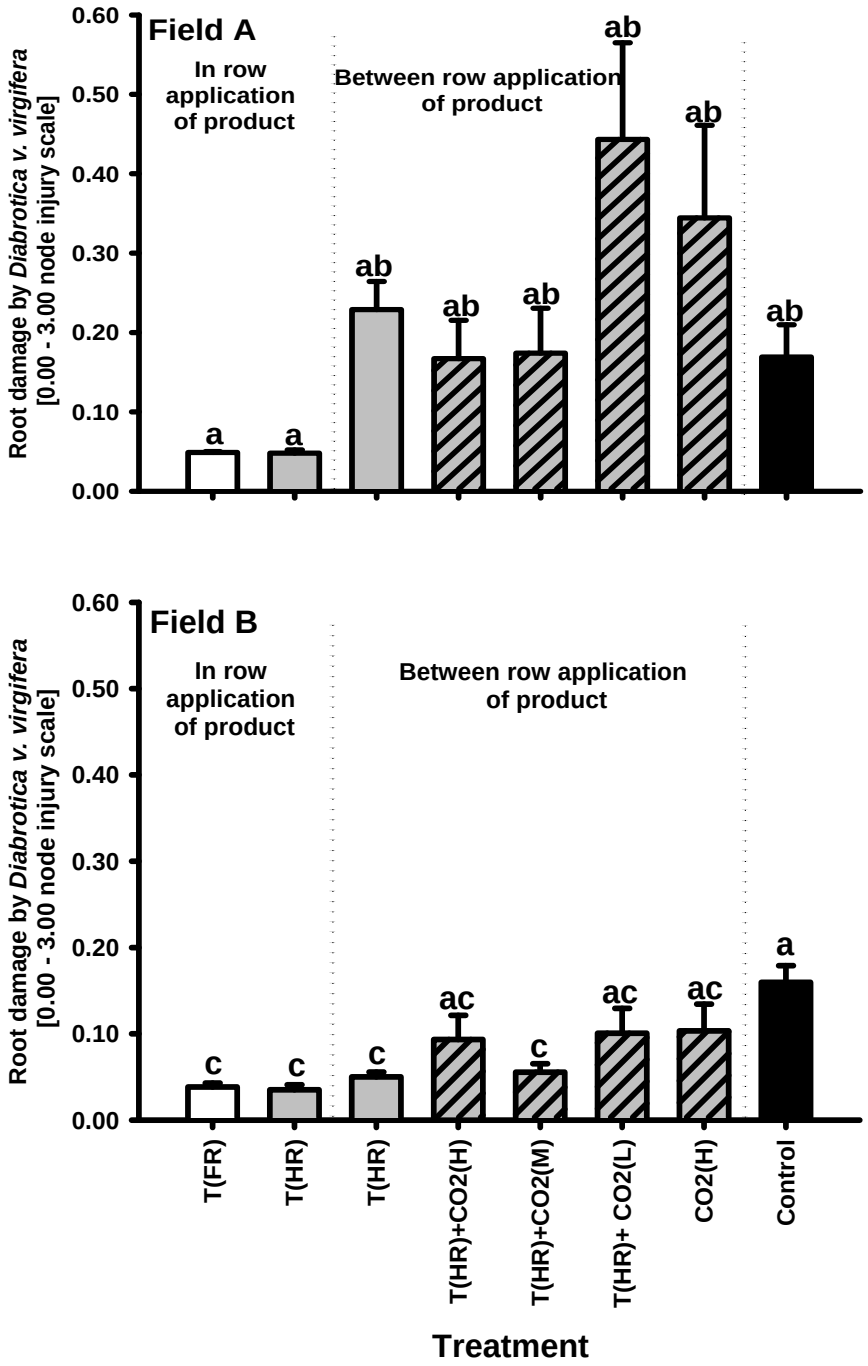


Fig. 6 Root damage of *Diabrotica v. virgifera* larvae in maize plots treated with Tefluthrin (T) fine granules at full rate (FR), half rate (HR) in the maize rows and at half rate (HR) with a high (H), medium (M) and low (L) concentration of CO_2 - emitting capsules (hatched bars) between the maize rows in two maize fields (A and B) in southern Hungary in 2011

Discussion

This study showed that an encapsulated CO_2 source applied during maize sowing provides CO_2 for a sufficient time period to target western corn larvae during their hatching period that typically starts two to three weeks after sowing and lasts for several weeks. Most of the tested combinations of CO_2 emitting capsule formulations with the soil insecticide Tefluthrin as well as the insecticide alone were able to effectively prevent a large proportion of root damage that would have been caused by *D. v. virgifera* larvae. The efficacies of in-row applications of the different rates of Tefluthrin only as well as of different rates of CO_2 - emitting capsules with half rate Tefluthrin appeared all to be sufficiently high that hardly any extra effects of the addition of CO_2 - emitting capsules could be detected. In other words, CO_2 was not able, under the here-presented conditions and setup, to further increase the already high efficacy of Tefluthrin. Between-row treatments were much less effective than in-row treatments, regardless of whether CO_2 capsules were added or not.

CO_2 - emitting capsules

In both tested soil types, an elevated CO_2 production by the capsules was measured at the time when larvae started to hatch in the field (Fig. 1); thus a possibility for larval attraction was given. Still on day 21, elevated CO_2 levels of 1597 ppm were measured and this even for the lowest, i.e. 1g, application rate of CO_2 (versus 1093 ppm in soil of the untreated control). Given the sensitivity of 1^{st} instar larvae to detect already a 12% difference between CO_2 concentrations (Bernklau and Bjostad 1998a), the CO_2 production by the capsules of the application rates used in this study were likely to be sufficiently high to attract *D. v. virgifera* larvae. There appeared to be a relationship between CO_2 production and air temperatures, with the highest of CO_2 emission 21 days after capsule application, coinciding with larval hatching as well as with increasing temperatures (> 21 °C). Before larval hatching, CO_2 production only slowly built up in the soil most likely due to lower temperatures measured during this time period. Further studies on the synchronization of CO_2 emission and larval hatching rates in relation to outside temperatures would be necessary to develop an appropriate formulation of the capsules. Moreover, a formulation with an extended CO_2 emission by another 2 weeks might ensure that larvae can be targeted during their hatching period even under the yearly varying climatic conditions (Toepfer and Kuhlmann 2006).

In-row applications

In-row Tefluthrin treatments without CO_2 - emitting capsules prevented 59 to 75% of the root damage according to the node injury damage rating scale. The reduction level at a full application rate of Tefluthrin (= European standard) are in line with published efficacy data (Pilz et al. 2009). Applications of Tefluthrin at lower rates did not significantly affect efficacy (Fig. 3), indicating that quarter, half and full rates were all effective, masking an potential dose - efficacy response. This is surprising, as it implies that growers can apply lower insecticide application rates for a sufficient control of WCR larvae. These results might, however, also be primarily due to the placement of the product into the target zone which can be affected by calibration method or environmental conditions during application, especially under reduced insecticide rates (Fuller et al. 1997). As a slight reduction was observed at a quarter application rate compared with half and full rate, we suggest that future studies on enhancing this soil insecticide may better be conducted with a maximum of 25% of the standard application rates.

As a consequence of the high efficacies of all in-row application rates of Tefluthrin, conclusive statements about potential enhancement through CO_2 are difficult to make. The slightly lower efficacy at a quarter application rate of Tefluthrin (63%) compared to the full rate (85%) seemed to be compensated (i.e. enhanced up to 15%) by adding CO_2 - emitting capsules. This points to an attract and kill mechanism by an in-row application of the of CO_2 - emitting capsules, and we would expect the effect to become higher at even less insecticide rates. It was unexpected to see indications for such effects in the maize rows. This is because CO_2 is only considered as a general non-specific semiochemical for the WCR larvae to locate roots over a longer range (Johnson and Nielsen 2012). The larvae are assumed to change to a more localised searching behaviour only upon close encounter/ contact with a root (Bernklau et al. 2009; Strnad and Dunn 1990), switching their orientation behaviour to a more host specific semiochemical (Johnson and Gregory 2006). Given this larval behaviour, CO_2 can be regarded, in contrast to our results, a less important compound for orientation. The potential role of CO_2 in combination with host specific compounds has never been considered before, but may play a role close to or in the maize root system. Previous studies have shown that neonate larvae initially prefer root tips when they start feeding in a maize root system (Clark et al. 2006). Root tips are the point of new root cell formation, and also a point of higher CO_2 production

(Bidel et al. 2001). Thus CO_2 might act as an indicator for fresher and more suitable root material, when larvae start feeding in a root system. Moreover, chemical defences are expected to be low in new root tissues (van Dam 2009), also helping the larvae to overcome defence systems of the plant. Considering this, CO_2 would become important close to or in maize root systems, and may therefore account for the patterns found in this study.

Between – row applications

A between-row applications of Tefluthrin or CO_2/Tefluthrin combinations prevented root damage to a much lesser extent than in-row applications of the products. Given the methods and rates used in this study, such applications cannot control WCR larval populations sufficiently enough from an economic point of view. Across both fields, up to 31% root damage (node injury scale) was prevented with a CO_2/Tefluthrin combination compared to 17% using Tefluthrin alone (Fig. 2). A 10 g application rate of capsules per plant resulted in slightly better damage prevention than 50 g. It is possible that the CO_2 emission from the 50 g capsules at time of larval hatch was too high which can cause a repellent effect on the larvae (Bernklau and Bjostad 1998b). Between – row application may prove a bit more effective when *D. v. virgifera* eggs are more equally or randomly distributed over the field, and not, as in this study, half way between the attract and kill components and the maize row. The natural infestation found in field A in this study would have simulated such conditions but the larval distribution appeared however so much clumped across the field that the five plots per treatment did not sufficiently account for this variability. In the future a more careful selection for previously uninfested fields is needed as natural infestation with unknown egg densities are inadequate to evaluate efficacy of insecticide formulations (Mayo 1986).

Apart from the results of the insecticide, a CO_2 capsule application between rows, as tested in our study, cannot be regarded as a treatment method resulting in a high control efficacy against WCR larvae. CO_2 applications had already been tested for a disruption of host location by (Bernklau et al. 2004) for a range of different CO_2 releasing products. This concept cannot be supported for this pest species by our results. It has been shown that WCR larvae are able to move up to 1 m through the soil during their development (Short and Luedtke 1970) and disperse to the middle of the maize rows to feed on the fine root material (Schumann and Vidal 2012; Strnad

and Bergman 1987). Therefore, we speculate that larvae, which were initially attracted to the applied CO_2 sources, were later on attracted to roots in the proximity of the capsules, supporting larval development. Additionally, under natural infestations (as in field A of this study), it is possible that larvae from the natural population were attracted to the capsules, and moved to the nearest plants roots resulting in even higher damage. The marginally better control results obtained in field B with no natural infestations might be considered as support for this hypothesis. On the other hand, larval movement might also be limited by soil conditions. Soil parameters could have influenced the attraction of CO_2 as the soil between some rows was dense, for example through tyre tracks, thus limiting CO_2 diffusion and larval movement. Moreover, larval orientation in wet soils towards carbon dioxide can be hampered as soils, saturated with moisture, lower the speed and distance of CO_2 diffusion (Villani and Wright 1990). A combination of these factors might have led to larvae not moving to a CO_2 source over longer distances when applied between the maize rows.

Future of below ground attract and kill

To improve efficacy of attract and kill strategies, the attractiveness of the here-used CO_2 - emitting capsules need to be improved by adding specific host volatiles that elicit a localized larval searching behavior (Bernklau et al. 2009) or act as feeding stimulants (Bernklau and Bjostad 2005; Bernklau et al. 2011). Several studies have identified specific compounds that act as attractants for WCR larvae (Bjostad and Hibbard 1992; Hibbard et al. 1994; Hiltpold et al. 2012; Robert et al. 2012a; Robert et al. 2012b). An integration of feeding stimulants into capsule formulations will allow to use killing agents that need to be taken up by ingestion, such as Thiamethoxam-based products (Bernklau and Bjostad 2005). However pyrethroids have generally been regarded as most effective in attract and kill strategies due to their rapid knock down effects to the target organisms (Evenden and McLaughlin 2004; Poullot et al. 2001).

As far as the experimental design is concerned, an assessment of both, the adult beetle density and root damage is essential, as root damage alone might not be a reliable predictor to evaluate insecticide efficacies (Sutter et al. 1990). This is because a reduction in WCR larval densities does not necessarily relate to a similar reduction in root damage. The reduction of beetle densities would rely on the

behavioural preference and displacement of the larvae (Boetel et al. 2003) so that they feed on roots in the zone of insecticidal activity (Villani and Wright 1990). Studies on larval distribution in the root system (Schumann and Vidal 2012; Strnad and Bergman 1987) showed that the larvae constantly re-distribute themselves, thus potentially feeding outside the catchment zone of insecticidal activity. Furthermore larvae prefer insecticide untreated soil and physical soil properties, such as a lack of soil moisture in the upper soil layer, which prevent contact with insecticidal material (Sutter et al. 1989). All these factors may contribute to a reduced root damage but not lower beetle densities.

Overall, the results of this study showed that an attract and kill strategy based on a CO_2 attractant alone does not significantly increase the efficacy of soil insecticides. However, a full appreciation of this strategy may only be illustrated when conducting studies at lower insecticide application rates than the ones used here, or when combining the artificial CO_2 emitting sources with other orientation cues. Further knowledge on the below ground orientation and movement of *D. v. virgifera* larvae, especially in the root system, is also needed in this regard, as well as an improved formulation of larval attractants.

Acknowledgements

This work was possible due to the technical support of the Plant Protection and Soil Conservation Directorate of Csongrad County in Hodmezovasarhely in Hungary, offered by Ibolya Hatala – Zseller, Laszlo Hodi, and others. We like to thank the Cereal Research Station of Szeged and the Agroplanta RT for providing field space, Endre Szell and his team (Cereal Research Station of Szeged, Hungary) for technical support and field management, as well as our summer students Rajmond Stuber and Ferenc Koncz (Hodmezovasarhely, Hungary) and Nikolin Karapancsi (AGRINET Foundation, Korce, Albania) for their help in field work. We thank Stephan Dannert and Sebastian Fechner (FH Bielefeld, Germany) for the assistance in the production of CO_2 capsules. This study was funded by Syngenta Crop Protection AG, Basel, Switzerland.

References

Atterberg A (1905) Die rationelle Klassifikation der Sand und Kiese. Chemiker Zeitung 29:195–198.

Bermond G, Ciosi M, Lombaert E, Blin A, Boriani M, Furlan L, Toepfer S, Guillemaud T (2012) Secondary contact and admixture between independently invading populations of the Western corn rootworm, *Diabrotica virgifera virgifera* in Europe. PLoS One 7 (11).

Bernklau EJ, Bjostad LB (1998a) Behavioral responses of first-instar western corn rootworm to carbon dioxide in a glass bead bioassay. J Econ Entomol 91 (2):444-456.

Bernklau EJ, Bjostad LB (1998b) Reinvestigation of host location by western corn rootworm larvae (Coleoptera: Chrysomelidae): CO_2 is the only volatile attractant. J Econ Entomol 91 (6):1331-1340.

Bernklau EJ, Bjostad LB (2005) Insecticide enhancement with feeding stimulants in corn for Western corn rootworm larvae (Coleoptera: Chrysomelidae). J Econ Entomol 98 (4):1150-1156.

Bernklau EJ, Bjostad LB, Hibbard BE (2011) Synthetic feeding stimulants enhance insecticide activity against western corn rootworm larvae, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). J Appl Entomol 135 (1-2):47-54.

Bernklau EJ, Bjostad LB, Meihls LN, Coudron TA, Lim E, Hibbard BE (2009) Localized search cues in corn roots for western corn rootworm (Coleoptera: Chrysomelidae) larvae. J Econ Entomol 102 (2):558-562.

Bernklau EJ, Fromm EA, Bjostad LB (2004) Disruption of host location of western corn rootworm larvae (Coleoptera: Chrysomelidae) with carbon dioxide J Econ Entomol 97 (2):330-339.

Bidel LPR, Renault P, Pages L, Riviere LM (2001) An improved method to measure spatial variation in root respiration: application to the taproot of a young peach tree *Prunus persica*. Agronomie 21 (2):179-192.

Bjostad LB, Hibbard BE (1992) 6-Methoxy-2-benzoxazolinone: A semiochemical for host location by western corn rootworm larvae J Chem Ecol 18 (7):931-944.

Boetel MA, Fuller BW, Evenson PD (2003) Emergence of adult northern and western corn rootworms (Coleoptera : Chrysomelidae) following reduced soil insecticide applications. J Econ Entomol 96 (3):714-729.

Chiang HC (1973) Bionomics of the northern and western corn rootworms. Annu Rev Entomol 18:47-72.

Ciosi M, Miller NJ, Kim KS, Giordano R, Estoup A, Guillemaud T (2008) Invasion of Europe by the western corn rootworm, *Diabrotica virgifera virgifera*: multiple transatlantic introductions with various reductions of genetic diversity. Molecular Ecology 17 (16):3614-3627.

Ciosi M, Miller NJ, Toepfer S, Estoup A, Guillemaud T (2011) Stratified dispersal and increasing genetic variation during the invasion of Central Europe by the western corn rootworm, *Diabrotica virgifera virgifera*. Evolutionary Applications 4 (1):54-70.

Clark PL, Vaughn TT, Meinke LJ, Molina-Ochoa J, Foster JE (2006) *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) larval feeding behavior on transgenic maize (MON 863) and its isoline. J Econ Entomol 99 (3):722-727.

Cox WJ, Shields E, Cherney DJR (2008) Western corn rootworm damage subtly affects corn growth under moderate environmental stress. Crop Science 48 (3):1164-1169.

Cressey D (2013) Reports spark row over bee-bothering insecticides. Nature News. doi:10.1038/nature.2013.12234

Dunbar W, Gassmann AJ (2013) Abundance and distribution of western and northern corn rootworm (*Diabrotica* spp.) and prevalence of rotation resistance in eastern Iowa . J Econ Entomol 106 (1):168-180.

El-Sayed AM, Suckling DM, Byers JA, Jang EB, Wearing CH (2009) Potential of "Lure and Kill" in long-term pest management and eradication of invasive species. J Econ Entomol 102 (3):815-835.

Erb M, Glauser G, Robert CAM (2012) Induced Immunity Against Belowground Insect Herbivores- Activation of Defenses in the Absence of a Jasmonate Burst. J Chem Ecol 38 (6):629-640.

European and Mediterranean Plant Protection Organization (EPPO) (2007) Efficacy evaluation of plant protection products: Design and analysis of efficacy evaluation trials PP 1/152 (3). EPPO Bulletin 37 11-24.

European and Mediterranean Plant Protection Organization (EPPO) (2011) Evaluation of insecticides: *Diabrotica v. virgifera*. EPPO Standards for the efficacy evaluation of plant protection products PP1/212(2).

Evenden ML, McLaughlin JR (2004) Factors influencing the effectiveness of an attracticide formulation against the Oriental fruit moth, *Grapholita molesta*. Entomologia Experimentalis Et Applicata 112 (2):89-97.

Foster SP, Harris MO (1997) Behavioral manipulation methods for insect pest-management. Annu Rev Entomol 42:123-146.

Fuller BW, Boetel MA, Walgenbach DD, Grundler JA, Hein GL, Jarvi KJ, Keaster AJ, Landis DA, Meinke LJ, Oleson JD, Ostlie KR, Tollefson JJ, Wedberg JL, Wilde GE, Evenson PD (1997) Optimization of soil insecticide rates for managing corn rootworm (Coleoptera: Chrysomelidae) larvae in the north central United States. J Econ Entomol 90 (5):1332-1340.

Furlan L, Canzi S, Di Bernardo A, Edwards CR (2006) The ineffectiveness of insecticide seed coatings and planting-time soil insecticides as *Diabrotica virgifera virgifera* LeConte population suppressors. J Appl Entomol 130:485-490.

Gavloski JE, Whitfield GH, Ellis CR (1992) Effect of larvae of western corn rootworm (Coleoptera: Chrysomelidae) and of mechnical root pruning on sap flow and growth of corn. J Econ Entomol 85 (4):1434-1441.

Girolami V, Marzaro M, Vivan L, Mazzon L, Greatti M, Giorio C, Marton D, Tapparo A (2012) Fatal powdering of bees in flight with particulates of neonicotinoids seed coating and humidity implication. J Appl Entomol 136 (1-2):17-26.

Girolami V, Marzaro M, Vivan L, Mazzon L, Greatti M, Giorio C, Marton D, Tapparo A (2013) Aerial powdering of bees inside mobile cages and the extent of neonicotinoid cloud surrounding corn drillers. J Appl Entomol 137:35-44.

Gould F (1991) Arthropod behaviour and the efficacy of plant protectants. Annu Rev Entomol 36:305-330.

Harris CR (1972) Factors influencing the effectiveness of soil insecticides. Annu Rev Entomol 17:177-198.

Heuskin S, Verheggen FJ, Haubruge E, Wathelet JP, Lognay G (2011) The use of semiochemical slow-release devices in integrated pest management strategies. Biotechnol Agron Soc 15 (3):459-470.

Hibbard BE, Bernklau EJ, Bjostad LB (1994) Long-chain free fatty acids: Semiochemicals for host location by western corn rootworm larvae J Chem Ecol 20 (12):3335-3344.

Hiltpold I, Hibbard BE, French BW, Turlings TCJ (2012) Capsules containing entomopathogenic nematodes as a Trojan horse approach to control the western corn rootworm. Plant Soil 358 (1-2):10-24.

IUSS (2007) World reference base for soil resources. World resource reports, vol 103. FAO, Rome, Italy.

Johnson SN, Gregory PJ (2006) Chemically-mediated host-plant location and selection by root-feeding insects. Physiological Entomology 31 (1):1-13.

Johnson SN, Nielsen UN (2012) Foraging in the dark - Chemically mediated host plant location by belowground insect herbivores. J Chem Ecol 38 (6):604-614.

Johnson SN, Zhang XX, Crawford JW, Gregory PJ, Hix NJ, Jarvis SC, Murray PJ, Young IM (2006) Effects of carbon dioxide on the searching behaviour of the root-feeding clover weevil *Sitona lepidus* (Coleoptera : Curculionidae). Bulletin of Entomological Research 96 (4):361-366.

Kahler AL, Olness AE, Sutter GR, Dybing CD, Devine OJ (1985) Root damage by western corn rootworm and nutrient in maize. Agronomy Journal 77 (5):769-774.

Kiss J, Edwards CR, Berger HK, Cate P, Cean M, Cheek S, Derron J, Festić H, Furlan L, Igrc-Barčić J, Ivanova I, Lammers W, Omelyuta V, Princzinger G, Reynaud P, Sivcev I, Sivicek P, Urek G, Vahala O (2005) Monitoring of western corn rootworm (*Diabrotica virgifera virgifera* LeConte) in Europe 1992 -2003. In: Vidal S, Kuhlmann U, Edwards CR (eds) Western Corn Rootworm-Ecology and Management. CABI, Wallington,UK, pp 29-41.

Kriticos DJ, Reynaud P, Baker RHA, Eyre D (2012) Estimating the global area of potential establishment for the western corn rootworm (*Diabrotica virgifera virgifera*) under rain-fed and irrigated agriculture. Bulletin OEPP/EPPO Bulletin 42 (1):56–64.

Krysan JL (1986) Introduction: Biology, distribution and identification of Pest Diabrotica. In: Krysan JL, Miller AM (eds) Methods for the Study of Pest *Diabrotica* Springer Verlag, New York, pp 1-23.

Kuzyakov Y, Larionova AA (2005) Root and rhizomicrobial respiration: A review of approaches to estimate respiration by autotrophic and heterotrophic organisms in soil. J Plant Nutr Soil Sci 168 (4):503-520.

Lancashire PD, Bleiholder H, Vandenboom T, Langeluddeke P, Stauss R, Weber E, Witzenberger A (1991) A Uniform Decimal Code for Growth-Stages of Crops and Weeds. Annals of Applied Biology 119 (3):561-601.

Levine E, Oloumi-Sadeghi H (1991) Management of Diabroticite rootworms in corn. Annu Rev Entomol 36:229-255.

Mayo ZB (1986) Field evaluation of insecticides for control of larvae of corn rootworm. In: Krysan JL, Miller TA (eds) Methods for the study of Pest *Diabrotica*. Springer Verlag, pp 183-204.

Mayo ZB, Peters LL (1978) Planting vs cultivation time applications of granular soil insecticides to control larvae of corn rootworms(Coleoptera: Chrysomelidae) in Nebraska. J Econ Entomol 71 (5):801-803.

Miller N, Estoup A, Toepfer S, Bourguet D, Lapchin L, Derridj S, Seok Kim K, Renaud P, Furlan L, Guillemaud T (2005) Multiple transatlantic introductions of the Western corn rootworm. Science 310:992.

Moellenbeck DJ, Peters ML, Bing JW, Rouse JR, Higgins LS, Sims L, Nevshemal T, Marshall L, Ellis RT, Bystrak PG, Lang BA, Stewart JL, Kouba K, Sondag V, Gustafson V, Nour K, Xu DP, Swenson J, Zhang J, Czapla T, Schwab G, Jayne S, Stockhoff BA, Narva K, Schnepf HE, Stelman SJ, Poutre C, Koziel M, Duck N (2001) Insecticidal proteins from *Bacillus thuringiensis* protect corn from corn rootworms. Nat Biotechnol 19 (7):668-672.

Moeser J, Hibbard BE (2005) A synopsis of the nutritional ecology of larvae and adults of *Diabrotica virgifera virgifera* (Le Conte) in the New and Old World – Nouvelle cuisine for the invasive maize pest *Diabrotica virgifera virgifera* in Europe? In: Vidal S, Kuhlmann U, Edwards CR (eds) Western Corn Rootworm- Ecology and Management. CABI, Wallington,UK, pp 41-65.

Oleson JD, Park YL, Nowatzki TM, Tollefson JJ (2005) Node-injury scale to evaluate root injury by corn rootworms (Coleoptera : Chrysomelidae). J Econ Entomol 98 (1):1-8.

Patel AV, Vorlop KD (1994) Entrapment of biological control agents applied to entomopathogenic nematodes. Biotechnology Techniques 8 (8):569-574.

Pilz C, Keller S, Kuhlmann U, Toepfer S (2009) Comparative efficacy assessment of fungi, nematodes and insecticides to control western corn rootworm larvae in maize. Biocontrol 54 (5):671-684.

Pline M, Dusenbery DB (1987) Responses of plant parasitic nematode *Meloidogyne incognita* to carbon dioxide determined by video camera computer tracking. J Chem Ecol 13 (4):873-888.

Poullot D, Beslay D, Bouvier JC, Sauphanor B (2001) Is attract-and-kill technology potent against insecticide-resistant Lepidoptera? Pest Management Science 57 (8):729-736.

Rasmann S, Kollner TG, Degenhardt J, Hiltpold I, Toepfer S, Kuhlmann U, Gershenzon J, Turlings TCJ (2005) Recruitment of entomopathogenic nematodes by insect-damaged maize roots. Nature 434 (7034):732-737.

Robert CAM, Erb M, Duployer M, Zwahlen C, Doyen GR, Turlings TCJ (2012a) Herbivore-induced plant volatiles mediate host selection by a root herbivore. New Phytol 194 (4):1061-1069.

Robert CAM, Erb M, Hibbard BE, French BW, Zwahlen C, Turlings TCJ (2012b) A specialist root herbivore reduces plant resistance and uses an induced plant volatile to aggregate in a density-dependent manner. Functional Ecology 26 (6):1429-1440.

Schumann M, Patel A, Vidal S (2013) Evaluation of an attract and kill strategy for western corn rootworm larvae. Applied Soil Ecology 64:178-189.

Schumann M, Vidal S (2012) Dispersal and spatial distribution of western corn rootworm larvae in relation to root phenology. Agricultural and Forest Entomology 14 (4):331-339.

Short DE, Luedtke RJ (1970) Larval migration of western corn rootworm. J Econ Entomol 63 (1):325-326.

Singh P, Moore RF (1985) Handbook of insect rearing. Elsevier science, Amsterdam.

Spencer JL, Hibbard BE, Moeser J, Onstad DW (2009) Behaviour and ecology of the western corn rootworm (*Diabrotica virgifera virgifera* LeConte). Agricultural and Forest Entomology 11 (1):9-27.

Spike BP, Tollefson JJ (1991) Yield response of corn subjected to western corn rootworm (Coleoptera, Chrysomelidae) infestation and lodging. J Econ Entomol 84 (5):1585-1590.

Strnad SP, Bergman MK (1987) Distribution and orientation of western corn rootworm (Coleoptera: Chrysomelidae) larvae in corn roots. Environmental Entomology 16 (5):1193-1198.

Strnad SP, Bergman MK, Fulton WC (1986) 1st instar western corn rootworm (Coleoptera: Chrysomelidae) response to carbon dioxide. Environmental Entomology 15 (4):839-842.

Strnad SP, Dunn PE (1990) Host Search Behavior of Neonate Western corn rootworm (*Diabrotica virgifera virgifera*). Journal of Insect Physiology 36 (3):201-205.

Sutter GR, Branson TF, Fisher JR, Elliott NC, Jackson JJ (1989) Effect of insecticide treatments on root damage ratings of maize in controlled infestations of western corn rootworms (Coleoptera: Chrysomelidae). J Econ Entomol 82 (6):1792-1798.

Sutter GR, Fisher JR, Elliott NC, Branson TF (1990) Effect of insecticide treatments on root lodging and yields of maize in controlled infestations of western corn rootworms (Coleoptera: Chrysomelidae). J Econ Entomol 83 (6):2414-2420.

Toepfer S, Kuhlmann U (2006) Constructing life-tables for the invasive maize pest *Diabrotica virgifera virgifera* (Col.: Chrysomelidae) in Europe. J Appl Entomol 130 (4):193-205.

VAISALA (2010) How to Measure Carbon Dioxide. http://www.vaisala.com/Vaisala%20Documents/Application%20notes/CEN-TIA-Parameter-How-to-measure-CO2-Application-note-B211228EN-A.pdf. Accessed 19.02.2013

van Dam NM (2009) Belowground herbivory and plant defenses. Annual Review of Ecology Evolution and Systematics 40:373-391.

Vemmer M, Patel A (2011) Development of CO_2 releasing beads to control soil borne insect pest. Paper presented at the 19.International Conference on Bioencapsulation, Amboise, France,

Villani MG, Wright RJ (1990) Environmetal influences on soil macroarthropod behavior in agricultural systems. Annu Rev Entomol 35:249-269.

Wesseler J, Fall EH (2010) Potential damage costs of *Diabrotica virgifera virgifera* infestation in Europe - the 'no control' scenario. J Appl Entomol 134 (5):385-394.

General discussion

The increasing spread of invasive pest species as a result of climate change (Cannon 1998; Baker et al. 2000) and new introduction pathways (Hulme et al. 2008) will impact on any nation's economy in the future. With regard to invasive insect pests, new management strategies for their control would need to be implemented. The invasion of the western corn rootworm (WCR) in Europe resulted in the higher adaptation of crop rotation (Gray et al. 2009). Such an approach however is economically less feasible and chemical control for the larvae will become a significant control option in the future (Van Rozen & Ester 2010). One major key drawback for a successful management of the larvae is that the cryptic feeding habit of such a soil dwelling organism makes it difficult to assess their behaviour non invasive and destructively (Johnson et al. 2006). Consequently targeting below ground organisms results in excessive input of chemical control compared to their above ground counter parts (Blackshaw & Kerry 2008). Thus studies on the behavioural ecology of a target organism can deliver fundamental aspects of the success or failure for new management tactics. For this study non destructive methodologies were developed to examine WCR larval spatial ecology. Based on these findings the development of a new strategy that aims at reducing chemical control against WCR larvae through the combination of an encapsulated CO_2 attractant ("Attract & Kill") could be implemented.

Spatial ecology of WCR larvae

Studies of WCR larval distribution on a plant scale level are rare (e.g. Strnad & Bergman 1987) but could help to support a more targeted management approach as the feeding location of the larvae is not the target location of the insecticide. Through the use of a non destructive methodology (Chapter 1) and semi field scale soil stratification (Chapter 3), this study indentified patterns in the dispersal and temporal and spatial distribution of WCR larvae in the root system. Comparing the spatial distribution pattern across both methodologies it could be shown that larvae follow a certain sequence of distribution changes in the root system: The spatial distribution pattern of the larvae initially showed an "establishment phase" where the larvae started to feed in one major cluster close to their point of insertion in the root system and then actively dispersed in the root system over time. Despite the increasing dispersal the distribution of the larvae always remained aggregated, a feature

common for many soil dwelling insects (Brown & Gange 1990). Thus the majority of larvae were found at specific locations in the root system. The preference for different root types and newly emerging roots is most likely to be a trigger for distribution changes.

The study on spatial distribution at a semi field scale (Chapter 3) has shown that larval development time rather than growth stage of maize affected the distribution changes of the larvae. A wider range of host phenologies would need to be tested to achieve a more dominant effect on larval development (Hibbard et al. 2008) and hence differences in distribution changes. Understanding the effect of plant phenology on host – herbivore interactions can help to improve and develop more sustainable pest management strategies (Leather 2010). First insights from the spatial distribution of the larvae at different growth stages of maize implies that chemical control could be less effective at a higher growth stage of maize as a higher proportion of larvae that feed beyond the zone of insecticidal activity will survive (Gray et al. 1992). Consequently larvae should be targeted at the beginning of their development before they start to distribute in the root system.

Implementation of CO_2 emitting capsules to attract WCR larvae

CO_2 as a cue for the orientation towards the host plant is common across many orders of soil dwelling larvae (Johnson et al. 2006). Since its first identification as an orientation cue to WCR larvae by Strnad et al. (1986), numerous studies have identified it as one of the major cues for orientation (Hibbard & Bjostad 1989; Bernklau & Bjostad 1998b) with attempts to incorporate it into WCR control by disrupting their host location (Bernklau et al. 2004). Besides the fact that such an approach has the potential to be integrated into the control of the larvae, the formulation of CO_2 releasing products has been proposed to be encapsulated to guarantee a slow CO_2 release over a longer period of time. Such an encapsulation has been described by Cheong (1993) and tested for WCR larvae by Moeser et al. (unpublished) but has never been evaluated for an integration into chemical control of WCR larvae.

The encapsulated CO_2 product (CO_2 emitting capsules) used in this study increased CO_2 levels in the soil around the CO_2 source for up to 20 days with a peak CO_2 emission 5 to 10 days after their application at temperatures under greenhouse conditions (chapter 4). Elevated CO_2 levels were mainly measured up to 15 cm from

the CO_2 source. This could be due to the soil structure which might have reduced the distance of CO_2 emission as a more compacted soil would not allow diffusion as well as more porous or sandy soil (Sotta et al. 2006). This is important for future studies as different soil types and structures need to be taken into account to evaluate CO_2 attractants.

Under field conditions CO_2 emission was considerably different as CO_2 levels, after an initial low CO_2 emission, peaked about 3 weeks after their application (chapter 5). There appeared to be a correlation between the level of CO_2 emission and outside temperature so that CO_2 emission increased as temperature increased. Compared to the temperatures under greenhouse conditions (~ 23 – 24°C), the outside temperature under field conditions were initially much lower (~ 14 – 17°C). Thus the product has been used up faster under greenhouse conditions than at the outside temperature and CO_2 was emitted for a longer period of time. The peak emission of CO_2 under field conditions did coincide with larval hatch so future studies should investigate the correlation between larval hatch and CO_2 emission taking changes in temperature into account.

The spatial and quantitative analyses of WCR distribution in the observation device (chapter 1) showed that the capsules initially diverted a proportion of larvae from the plant but larvae eventually moved away from the capsules again. It is very likely that the larvae didn't find any food and kept on moving, potentially following a new CO_2 gradient (Strnad & Dunn, 1990) that was emitted by the maize roots. Through the fine resolution of the spatial distribution in the observation device, larval activity could be measured directly at the capsules. This is an essential consideration for an integration of the capsules in an A&K approach. Larvae should not just be diverted from the plant and need to be attracted directly to the CO_2 source as this is where the killing agent would be placed in an "Attract & Kill" strategy.

Due to the larvae's oligophagous nature (Moeser & Hibbard, 2005), it was assumed that CO_2 would not play a role for the orientation of the larvae in the root system. The spatial and quantitative analyses of larval distribution when larvae were inserted in the root system (chapter 2) indicate that CO_2 attraction might still play a role in the orientation of WCR larvae in the root system. Potential mechanisms for this behaviour could be the explained with the sequence of rootworm attack in the root system: Spatial distribution patterns of the larvae showed that larvae started to aggregate by feeding on root tips on the periphery of the root system shortly after

insertion (chapter 1). Similar observations were also made by Clark et al. (2006) with neonate larvae on maize seedlings in a transparent gel medium. Root tips are the point of new root cell formation, preferred by larvae when they start feeding in the root system (Clark et al., 2006) and also a point of higher CO_2 production (Bidel et al 2001). Thus CO_2 might be an indicator for fresher and more suitable root material, when they start feeding in a root system. Also when larvae switch to a new root as a result of heavy damage at one root (Hibbard et al., 2003), they could follow a new CO_2 gradient to locate this root material (Strnad & Dunn, 1990). Chemical defences are also expected to be low in new root tissue (Van Dam, 2009), thus feeding at a root tip might help the larvae to overcome the defence systems of the plant. Overall CO_2 as a cue to orientation could be an important pre requisite to manipulate larval behaviour in the root system.

Under semi field and field conditions the application of the capsules as a disruption of host orientation only resulted in low reduced larval densities and root damage (chapter 4 & 5) and would not be suitable as a management tool for WCR larvae. Considering the mechanism of larvae moving away from the capsules after initial attraction as identified in chapter 1, could also be applied for field situations. It is known that larvae can cover a distance of up to 1 m from larval hatch to adult emergence (Short & Ludtke 1970) and 1st instar larvae can still establish in the root system when they hatch 60 cm from the plant base (Schumann, unpublished). Additionally under the conditions in the semi field trials the distribution patterns of the larvae (chapter 3) identified two mechanisms that could explain the low reduction in larval densities with the application of the capsules only: 1. Larvae fed in close proximity of their hatch. Thus they are surrounded by roots that they can feed on and CO_2 is not the major cue to divert a sufficient proportion of larvae away from the roots. 2. Larvae moved into parts of the soil where the capsules were applied (27 – 30 cm from the plant base) to feed on the fine root material (Strnad & Bergman 1987; Schumann & Vidal, 2012). It is possible that the larvae were initially attracted to the CO_2 source but were able to locate roots in close proximity of the capsules. This might have supported larval development and the larvae were able to build up sufficient energy resources to move to the root further away from the CO_2 source, possibly towards roots around the plant base. Data from natural infested plots in the field (chapter 5) showed that root damage can even be higher with CO_2, again probably because larvae were initially attracted to the CO_2 source and, as they were

not killed off, moved to the nearest plant. Overall this indicates that the addition of a killing agent (= Attract & Kill) would be necessary for a reliable control of the larvae with the capsules.

"Attract & Kill" against WCR larvae

The use of "Attract & Kill" (A&K) against WCR larvae has first been examined by Hibbard et al. (1995) with 6 – methoxy 2 – benzoxazolinone (MBOA), previously identified as a host location semiochemical (Bjostad & Hibbard, 1992), in combination with the experimental insecticide chlorethoxyphos. To give the A&K effect the potential to be applied with a currently registered insecticide in the field, the soil granule Force 1.5G (active ingredient: Tefluthrin) was taken as the kill component in this study. Due to its repellent nature as a pyrethroid (Michaelides et al., 1997) it was important to determine whether it can be integrated into an A&K strategy. Larvae recovered at the capsules (Fig. 1) in the observation device (chapter 1) showed typical knock down symptoms such as writhing and curling caused by tefluthrin toxification (Bernklau et al., 2011) indicating an A&K effect

The mortality of the larvae with A&K, however, increased by decreasing the active ingredient of tefluthrin. Thus it can be argued that the attractive effect of the capsules was bigger masking the potential repellent effect of the granules. A repellent effect would not be noticed until after contact when it is too late for escape without death (Brockerhoff & Suckling 1999). This potential masking effect becomes more pronounced at lower application rates of the killing agent, resulting in reduced repellent effects (Michaelides et al. 1997) and higher mortality rates of WCR larvae. This mechanism was an important step in the evaluation for A&K as an insecticide should not compromise the function of the attractant (El - Sayed et al. 2009) and the ratio of the A&K components needs to be considered in the future. Furthermore at the lower insecticide application rates a decrease in mortality over time was measured. This indicates a sub lethal effect on the larvae and is also an essential consideration when reducing the active ingredient for A&K (Krupke et al., 2002).

Whereas mortality increased by decreasing the active ingredient with A&K, it decreased in a conventional treatment . This could be because the application of an insecticide in a conventional treatment relies on the behavioural preference and displacement of larvae (Boetel et al., 2003) so that larvae feed on roots in the zone of insecticidal activity (Villani & Wright, 1990). Consequently the spatial and temporal

distribution changes of the larvae in the root system determine the mortality of the larvae in a conventional treatment. The spatial analysis of larval distribution (chapter 1) showed that larvae started to aggregate outside the zone of insecticidal activity so that initially only a minority of larvae was eventually affected by the insecticide. In subsequent measurements larvae started to distribute in the root system with more larvae moving into this zone, reflected by an increase in mortality over time. The size of the zone of insecticidal activity was not defined or measured in this study. It can, however, be hypothesized that the probability of larvae moving into this zone decreases with reduced application rates, lowering the overall mortality of the larvae. Latter argument can also be applied to the conventional treatment under semi field conditions (Chapter 4) where the reduction in larval density was affected by the application rate of Tefluthrin in a conventional treatment but not in an A&K treatment. With these mechanisms in mind A&K could enhance insecticide efficacy compared to a conventional treatment at lower application rates of the insecticide.

With the currently used application method under both semi field and field conditions, the between – row applications of A&K did not control larval populations sufficient enough from an economic point of view (Chapter 4 & 5). However between – row application may prove more effective when WCR eggs are more equally or randomly distributed over the field, and not, as tested in these studies, half way between the between – row application and the maize row. An in row application of the A&K components under field conditions (Chapter 5) on the other hand did seem to increase the efficacy of soil insecticides at lower application rates of Tefluthrin. Thus a CO_2 attraction in A&K close to the root system is more feasible than between row application and supports findings that CO_2 might still play a role for orientation of the larvae in the root system (Chapter 2).

a)  b) 

Figure 1 - Larvae with knock down symptoms and dead larvae in a) an “Attract & Kill” strategy at the capsules and b) in the root system in a conventional treatment. Insecticide granules are marked red.

Future development

From an experimental point of view for further evaluation of such behavioural based approaches for WCR larvae, the use of 2nd instar larvae (Chapter 1 & 2) as the test organism is one major drawback to test larval orientation, especially because behaviour of the larvae after first contact with the root can change (Bernklau et al., 2009). For a potential field application it is expected that 1st instar larvae will be targeted as they are the most important life stage for host plant selection (Bernklau & Bjostad, 1998b) so any behavioural based management evaluation should be carried with this larval stage. Behavioural observations of neonate larvae whilst maintaining geotropic and thigmotatic cues, however, provide a bigger challenge. The development of techniques with transparent media (Clark et al., 2006) creates new potential to evaluate their behaviour non destructively.

The A&K components tested in this study have the potential to target WCR larvae. Overall the attractiveness of the capsules needs to be improved to deliver better control of the larvae. The integration of host specific cues such as feeding stimulants (Bernklau and Bjostad 2005) or compounds that elicit localised search cues (Bernklau et al. 2009) could help to attract more larvae. These compounds can also help to use A&K with a wider range of insecticides, for example ones that need to be taken up by ingestion such as thiamethoxam products. Currently pyrethroids such as Tefluthrin mixed with an attractant are regarded as an effective combination due to

rapid knock down effect to the target organism (Evenden and McLaughlin 2004; Poullot et al. 2001).

Unlike previously assumed a CO_2 attraction still seems to have an effect on the larvae when they move in the root system. Furthermore an A&K approach seemed to enhance insecticide efficacy under field conditions when the components were applied in the maize row i.e. close to the root system. Such an approach is, from a practical point of view, a better option as root protection is still provided and farmers can use standardise application equipment and thus should be the focus of A&K against WCR larvae in the future.

References

Baker, R.H.A., Sansford, C.E., Jarvis, C.H., Cannon, R.J.C., MacLeod, A., & Walters, K.F.A. (2000) The role of climatic mapping in predicting the potential geographical distribution of non-indigenous pests under current and future climates. Agriculture Ecosystems & Environment, 82, 57-71.

Bernklau, E.J. & Bjostad, L.B. (1998a) Behavioral responses of first-instar western corn rootworm to carbon dioxide in a glass bead bioassay. Journal of Economic Entomology, 91, 444-456.

Bernklau, E.J. & Bjostad, L.B. (2005) Insecticide enhancement with feeding stimulants in corn for western corn rootworm larvae (Coleoptera: Chrysomelidae). J. Econ. Entomol., 98, 1150-1156.

Bernklau, E.J. & Bjostad, L.B. (1998b) Reinvestigation of host location by western corn rootworm larvae (Coleoptera: Chrysomelidae): CO_2 is the only volatile attractant. Journal of Economic Entomology, 91, 1331-1340.

Bernklau EJ, Bjostad LB & Hibbard BE (2011) Synthetic feeding stimulants enhance insecticide activity against western corn rootworm larvae, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). Journal of Applied Entomology 135: 47–54.

Bernklau, E.J., Bjostad, L.B., Meiiils, L.N., Coudron, T.A., Lim, E., & Hibbard, B.E. (2009) Localized search cues in corn roots for western corn rootworm (Coleoptera: Chrysomelidae) larvae. Journal of Economic Entomology, 102, 558-562.

Bernklau, E.J., Fromm, E.A., & Bjostad, L.B. (2004) Disruption of host location of western corn rootworm larvae (Coleoptera: Chrysomelidae) with carbon dioxide Journal of Economic Entomology, 97, 330-339.

Bidel, L. P. R., Renault P., Pages L., and Riviere L. M. (2001). An improved method to measure spatial variation in root respiration: application to the taproot of a young peach tree *Prunus persica*. Agronomie, 21, 179-192.

Bjostad, L.B. & Hibbard, B.E. (1992) 6-Methoxy-2-benzoxazolinone: A semiochemical for host location by western corn rootworm larvae Journal of Chemical Ecology, 18, 931-944.

Blackshaw, R.P. & Kerry, B.R. (2008). Root herbivory in agricultural ecosystems. In Introduction: Root Feeders – An Ecosystem Perspective (eds S.J. Johnson & P.J. Murray), pp. 35 - 53. CABI, Wallingford,UK.

Boetel, M.A., Fuller, B.W., & Evenson, P.D. (2003) Emergence of adult northern and western corn rootworms (Coleoptera : Chrysomelidae) following reduced soil insecticide applications. Journal of Economic Entomology, 96, 714-729.

Brockerhoff, E.G. & Suckling, D.M. (1999) Development of an attracticide against light brown apple moth (Lepidoptera: Tortricidae). Journal of Economic Entomology, 92, 853-859.

Brown, V.K. & Gange, A.C. (1990) Insect herbivory below ground. Advances In Ecological Research, 20, 1-58.

Cannon, R.J.C. (1998) The implications of predicted climate change for insect pests in the UK, with emphasis on non-indigenous species. Global Change Biology, 4, 785-796.

Chege, P.G., Clark, T.L., & Hibbard, B.E. (2009) Initial larval feeding on an alternate host enhances western corn rootworm (Coleoptera: Chrysomelidae) beetle emergence on Cry3Bb1-expressing maize. Journal of the Kansas Entomological Society, 82, 63-75.

Cheong, S.H., Park, J.K., Kim, B.S., & Chang, H.N. (1993) Microencapsulation of yeast- cells in the calcium alginate membrane. Biotechnology Techniques, 7, 879-884.

Ciosi, M., Miller, N.J., Kim, K.S., Giordano, R., Estoup, A., & Guillemaud, T. (2008) Invasion of Europe by the western corn rootworm, *Diabrotica virgifera virgifera*: multiple transatlantic introductions with various reductions of genetic diversity. Molecular Ecology, 17, 3614-3627.

Clark, P.L., Vaughn, T.T., Meinke, L.J., Molina-Ochoa, J., & Foster, J.E. (2006) *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) larval feeding behavior on transgenic maize (MON 863) and its isoline. Journal of Economic Entomology, 99, 722-727.

Dawson, L.A. & Byers, R.A. (2008). Methods for studying root herbivory. In Introduction: Root Feeders – An Ecosystem Perspective (eds S.J. Johnson & P.J. Murray), pp. 3 - 19. CABI, Wallingford,UK.

Ellsbury, M.M., Clay, A.C., Clay, E.D., & Malo, D.D. (2005). Within - field spatial variation of northern corn rootworm distributions. In Western Corn Rootworm-Ecology and Management (eds S. Vidal, U. Kuhlmann & C.R. Edwards), pp. 145 - 153. CABI, Wallington,UK.

El-Sayed, A.M., Suckling, D.M., Byers, J.A., Jang, E.B., & Wearing, C.H. (2009) Potential of "lure and kill" in long term pest management and eradication of invasive Species. Journal of Economic Entomology, 102, 815-835.

EPPO (2011) www.eppo.org/QUARANTINE/Diabrotica_virgifera/diabrotica_virgifera

European Union. (2009) Concerning the placing of plant protection products on the market and repealing council directives 79/117/EEC and 91/414/EEC.

Evenden, M.L. & McLaughlin, J.R. (2004) Factors influencing the effectiveness of an attracticide formulation against the Oriental fruit moth, *Grapholita molesta*. Entomologia Experimentalis Et Applicata, 112, 89-97.

Furlan, L., Canzi, S., Di Bernardo, A., & Edwards, C.R. (2006) The ineffectiveness of insecticide seed coatings and planting-time soil insecticides as *Diabrotica virgifera virgifera* LeConte population suppressors. Journal of Applied Entomology, 130, 485-490.

Gould, F. (1991) Arthropod behaviour and the efficacy of plant protectants. Annual Review of Entomology, 36, 305-330.

Gray, M.E., Felsot, A.S., Steffey, K.L., & Levine, E. (1992) Planting time application of soil insecticides and western corn rootworm (Coleoptera, Chrysomelidae) emergence - Implications for long term management programs. Journal of Economic Entomology, 85, 544-553.

Gray, M.E., Sappington, T.W., Miller, N.J., Moeser, J., & Bohn, M.O. (2009) Adaptation and invasiveness of western corn rootworm: Intensifying research on a worsening pest. Annual Review of Entomology, 54, 303-321.

Harris, C.R. (1972) Factors influencing the effectiveness of soil insecticides. Annual Review of Entomology, 17, 177-198.

Harris, D.G. & Vvan Bavel, C.H.M. (1957) Root respiration of tobacco,corn, and cotton plants. Agronomy Journal, 49, 182-184.

Harrison, R.D., Gardner, W.A., Tollner, W.E., & Kinard, D.J. (1993) X-ray computed-tomography studies of the burrowing behavior of 4th-Instar pecan weevil (Coleoptera, Curculionidae). Journal of Economic Entomology, 86, 1714-1719.

Hibbard, B.E. & Bjostad, L.B. (1988) Behavioral-responses of western corn rootworm larvae to volatile semiochemicals from corn seedlings. Journal of Chemical Ecology, 14, 1523-1539.

Hibbard, B.E., Duran, D.P., Ellersieck, M.R., & Ellsbury, M.M. (2003) Post-establishment movement of western corn rootworm larvae (Coleoptera:

Chrysomelidae) in Central Missouri corn. Journal of Economic Entomology, 96, 599-608.

Hibbard, B.E., El Khishen, A.A., & Vaughn, T.T. (2009) Impact of MON863 transgenic roots is equivalent on western corn rootworm larvae for a wide range of maize phenologies. Journal of Economic Entomology, 102, 1607-1613.

Hibbard, B.E., Peairs, F.B., Pilcher, S.D., Schroeder, M.E., Jewett, D.K., & Bjostad, L.B. (1995) Germinating corn extracts and 6-Methoxy-2-Benzoxazolinone - western corn rootworm (Coleoptera, Chrysomelidae) larval attractants evaluated with soil insecticides. Journal of Economic Entomology, 88, 716-724.

Hibbard, B.E., Schweikert, Y.M., Higdon, M.L., & Ellersieck, M.R. (2008) Maize phenology affects establishment, damage, and development of the western corn rootworm (Coleoptera: Chrysomelidae). Environmental Entomology, 37, 1558-1564.

Huang, X.P. & Mack, T.P. (2001) Artificial carbon dioxide source to attract lesser cornstalk borer (Lepidoptera : Pyralidae) larvae. Journal of Economic Entomology, 94, 860-867.

Hulme, P.E., Bacher, S., Kenis, M., Klotz, S., Kuhn, I., Minchin, D., Nentwig, W., Olenin, S., Panov, V., Pergl, J., Pysek, P., Roques, A., Sol, D., Solarz, W., & Vila, M. (2008) Grasping at the routes of biological invasions: a framework for integrating pathways into policy. Journal of Applied Ecology, 45, 403-414.

Hunter, D.H. (2001) Out of sight, out of mind: the impacts of root-feeding insects in natural and managed systems. Agricultural and Forest Entomology, 3, 3-9.

Johnson, S.N. & Gregory, P.J. (2006) Chemically mediated host-plant location and selection by root-feeding insects. Physiological Entomology, 31, 1-13.

Johnson, S.N., Zhang, X.X., Crawford, J.W., Gregory, P.J., Hix, N.J., Jarvis, S.C., Murray, P.J., & Young, I.M. (2006) Effects of carbon dioxide on the searching behaviour of the root-feeding clover weevil *Sitona lepidus* (Coleoptera : Curculionidae). Bulletin of Entomological Research, 96, 361-366.

Kiss J, Edwards CR, Berger HK, Cate P, Cean M., Cheek S, Derron J, Festic H, Furlan L, Igrc-Barcˇic´ J, Ivanova I, Lammers W, Omelyuta V, Princzinger G, Reynaud P, Sivcev I, Sivicek P, Urek G, and Vahala G (2005). Monitoring of Western Corn Rootoworm (Diabrotica virgifera virgifera LeConte) in Europe 1992 - 2003. In Western Corn Rootworm- Ecology and Management (eds S. Vidal, U. Kuhlmann & C.R. Edwards), pp. 29-41. CABI, Wallington, UK.

Krupke, C.H., Roitberg, B.D., & Judd, G.J.R. (2002) Field and laboratory responses of male codling moth (Lepidoptera : Tortricidae) to a pheromone-based attract-and-kill strategy. Environmental Entomology, 31, 189-197.

Krysan, J.L. (1986). Introduction: Biology, distribution and identification of Pest Diabrotica. In Methods for the Study of Pest Diabrotica (eds J.L. Krysan & A.M. Miller), pp. 1-23. Springer Verlag, New York.

Leather, S.E. (2010) Precise knowledge of plant growth stages enhances applied and pure research. Annals of Applied Biology, 157, 159-161.

Lefko, S.A., Nowatzki, T.M., Thompson, S.D., Binning, R.R., Pascual, M.A., Peters, M.L., Simbro, E.J., & Stanley, B.H. (2008) Characterizing laboratory colonies of western corn rootworm (Coleoptera : Chrysomelidae) selected for survival on maize containing event DAS-59122-7. Journal of Applied Entomology, 132, 189-204.

Levine, E. & Oloumi-Sadeghi, H. (1991) Management of diabroticite rootworms in corn. Annual Review of Entomology, 36, 229-255.

Losel, P.M., Penners, G., Potting, R.P.J., Ebbinghaus, D., Elbert, A., & Scherkenbeck, J. (2000) Laboratory and field experiments towards the development of an attract and kill strategy for the control of the codling moth, *Cydia pomonella*. Entomologia Experimentalis Et Applicata, 95, 39-46.

Ma, B.L., Meloche, F., & Wei, L. (2009) Agronomic assessment of Bt trait and seed or soil-applied insecticides on the control of corn rootworm and yield. Field Crops Research, 111, 189-196.

Mankin RW, Johnson SN, Grinev DV & Gregory PJ (2008) New experimental techniques for studying root Herbivores. Root Feeders – An Ecosystem Perspective (ed. by SJ Johnson & PJ Murray), pp. 3 – 19. CABI, Wallingford, UK.

Mayo, Z.B. (1980) Influence of planting dates on the efficacy of soil insecticides applied to control larvae of the western and northern corn rootworm (Coleoptera, Chrysomelidae). Journal of Economic Entomology, 73, 211-212.

Mayo, Z.B. & Peters, L.L. (1978) Planting Vs cultivation time applications of granular soil insecticides to control larvae of corn rootworms (Coleoptera:Chrisomelidae) in Nebraska. Journal of Economic Entomology, 71, 801-803.

Meinke, L.J., Sappington, T.W., Onstad, D.W., Guillemaud, T., Miller, N.J., Judith, K., Nora, L., Furlan, L., Jozsef, K., & Ferenc, T. (2009) Western corn rootworm

(*Diabrotica virgifera virgifera* LeConte) population dynamics. Agricultural and Forest Entomology, 11, 29-46.

Michaelides, P.K., Cleverly, A.L., & Wright, D.J. (1997) Sub-lethal effects of tefluthrin on *Diabrotica undecimpunctata howardi*, Barber: Plant protection and larval development. Crop Protection, 16, 423-429.

Moeser, J. & Hibbard, B.E. (2005). A Synopsis of the nutritional ecology of larvae and adults of *Diabrotica virgifera virgifera* (Le Conte) in the new and old World – nouvelle cuisine for the invasive maize pest *Diabrotica virgifera virgifera* in Europe? In Western Corn Rootworm- Ecology and Management (eds S. Vidal, U. Kuhlmann & C.R. Edwards), pp. 41-65. CABI, Wallington,UK.

Pimentel, D., Zuniga, R., & Morrison, D. (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics, 52, 273-288.

Pline, M. & Dusenbery, D.B. (1987) Responses of plant parasitic nematode *Meloidogyne incognita* to carbon dioxide determined by video camera computer tracking. Journal of Chemical Ecology, 13, 873-888.

Poullot, D., Beslay, D., Bouvier, J.C., & Sauphanor, B. (2001) Is attract-and-kill technology potent against insecticide resistant Lepidoptera? Pest Management Science, 57, 729-736.

Rasmann, S., Kollner, T.G., Degenhardt, J., Hiltpold, I., Toepfer, S., Kuhlmann, U., Gershenzon, J., & Turlings, T.C.J. (2005) Recruitment of entomopathogenic nematodes by insect-damaged maize roots. Nature, 434, 732-737.

Reinecke, A., Muller, F., & Hilker, M. (2008) Attractiveness of CO_2 released by root respiration fades on the background of root exudates. Basic and Applied Ecology, 9, 568-576.

Sakai, A.D. (2001) The population biology of invasive species. Annual Review of Ecology and Systematics, 32, 305-322.

Schumann, M and Vidal, S (2012). Dispersal and spatial distribution of western corn rootworm larvae in relation to root phenology. Agricultural and Forest Entomology, 14, 331-339.

Short, D.E. & Luedtke, R.J. (1970) Larval migration of western corn rootworm. Journal of Economic Entomology, 63, 325-326.

Sotta, E. D., Veldkamp E., Guimaraes B. R., Paixao R. K., Ruivo M. L. P., and Almeida S. S. (2006). Landscape and climatic controls on spatial and temporal

variation in soil CO_2 efflux in an Eastern Amazonian Rainforest, Caxiuana, Brazil. For. Ecol. Manage., 237, 57-64.

Spencer, J.L., Hibbard, B.E., Moeser, J., & Onstad, D.W. (2009) Behaviour and ecology of the western corn rootworm (*Diabrotica virgifera virgifera* LeConte). Agricultural and Forest Entomology, 11, 9-27.

Strnad, S.P. & Bergman, M.K. (1987) Distribution and orientation of western corn rootworm (Coleoptera: Chrysomelidae) larvae in corn roots. Environmental Entomology, 16, 1193-1198.

Strnad, S.P., Bergman, M.K., & Fulton, W.C. (1986) 1st Instar western corn rootworm (Coleoptera, Chrysomelidae) response to carbon dioxide. Environmental Entomology, 15, 839-842.

Strnad, S.P. & Dunn, P.E. (1990) Host search behavior of neonate western corn-rootworm (*Diabrotica virgifera virgifera*). Journal of Insect Physiology, 36, 201-205.

Szalai, M., Komaromi, J.P., Bazok, R., Barcic, J.I., Kiss, J., & Toepfer, S. (2011) Generational growth rate estimates of *Diabrotica virgifera virgifera* populations (Coleoptera: Chrysomelidae). Journal of Pest Science, 84, 133-142.

Toepfer, S., Burger, R., Ehlers, R.U., Peters, A., & Kuhlmann, U. (2010) Controlling western corn rootworm larvae with entomopathogenic nematodes: effect of application techniques on plant-scale efficacy. Journal of Applied Entomology, 134, 467-480.

Toepfer, S., Ellsbury, M.M., Eschen, R., & Kuhlmann, U. (2007) Spatial clustering of *Diabrotica virgifera virgifera* and *Agriotes ustulatus* in small-scale maize fields without topographic relief drift. Entomologia Experimentalis Et Applicata, 124, 61-75.

Van Dam, N.M. (2009) Belowground herbivory and plant defenses. Annual Review of Ecology Evolution and Systematics, 40, 373-391.

Van Rozen, K. & Ester, A. (2010) Chemical control of *Diabrotica virgifera virgifera* LeConte. Journal of Applied Entomology, 134, 376-384.

Villani, M.G. & Wright, R.J. (1990) Environmetal influences on soil macroarthropod behavior in agricultural systems. Annual Review of Entomology, 35, 249-269.

Vincenzo, G., Marzaro,M., Vivan,L., Mazzon,L., Greatti,M., Giorio,C., Marton,D. & Tapparo,A. (2012). Fatal powdering of bees in flight with particulates of neonicotinoid seed coating and humidity implications. In press (doi: 10.1111/j.1439-0418.2011.01648.x).

Wagner, D.L. & Van Driesche, R.G. (2010). Threats posed to rare or endangered insects by invasions of nonnative species. Annual Review of Entomology, 55, 547-568.

Wensler, R.J. (1971) Locomotor and feeding activity of larvae of *Scarabaeid sericesthis geminata* (Coleoptera). Entomologia Experimentalis Et Applicata, 14, 270-278.

Wesseler, J. & Fall, E.H. (2010) Potential damage costs of *Diabrotica virgifera virgifera* infestation in Europe - the 'no control' scenario. Journal of Applied Entomology, 134, 385-394.

Publications

Peer reviewed articles

Schumann M. and S. Vidal. 2012. Dispersal and spatial distribution of western corn rootworm larvae in relation to root phenology. *Agricultural and Forest Entomology, 14, 331–339*

Schumann M., Patel A. and Vidal S. 2013. Evaluation of an attract & kill strategy for western corn rootworm larvae. *Applied Soil Ecology, 64, 178 – 189*

Schumann M., Patel A., Vemmer M. and Vidal S. 2013. The role of carbon dioxide as an orientation cue for western corn rootworm larvae within the maize root system - implications for an attract and kill approach. *Pest Management Science (Accepted)*

Non peer reviewed articles

Schumann M., Jakobs-Schönwandt D., Vemmer M., Patel A. und Vidal S. (2013) „Attract & Kill" - Eine neue Strategie zur *Diabrotica*-Bekämpfung, *Mais, 2, 92-93* („Attract & Kill" – a new strategy for *Diabrotica* management)

Presentations at conferences or workshops

Schumann, M., Kurtz, B. , Möser, J., Hibbard B. and Vidal S. 2008. Interactions between phytopathogenic fungi and western corn rootworm larvae. DIABR-ACT Symposium "Harmonise the strategies for fighting *Diabrotica virgifera virgifera*", Göttingen

Schumann, M. and Vidal S. 2009 Changes in western corn rootworm below ground distribution during larval development. DGAAE (deutsche Gesellschaft für allgemeine und angewandte Entomologie) conference, Göttingen

Schumann,M. and Vidal S. 2009 Changes in western corn rootworm below ground distribution during larval development 23rd IWGO Conference & 2nd International Conference of Diabrotica Genetics, Munich

Schumann, M., Patel, A. and Vidal, S. 2010. Einsatz künstlicher CO2-Kapseln als Lockstoff für die Larven des Westlichen Maiswurzelbohrers (Use of artificial CO_2-capsules as an attractant for western corn rootworm larvae). 57th Conference of the German Plant Protection Society (DPG), Berlin

Schumann, M. 2011. Entwicklung einer „Attract & Kill" Strategie zur Kontrolle der Larven des Westlichen Maiswurzelbohrers . 11. DBU Fachkolloquium , Göttingen

Schumann, M., Patel, A. and Vidal, S. 2011. Management of western corn rootworm larvae with artificial CO_2 sources. DGAAE (Deutsche Gesellschaft für allgemeine und angewandte Entomologie) conference, Berlin.

Schumann, M., Patel, A. and Vidal, S. 2011. Evaluation of an "Attract & Kill" strategy for western corn rootworm larvae. 24th IWGO Conference & 3rd International Conference of Diabrotica Genetics, Freiburg

Schumann, M. 2011. Development of an A&K strategy for the control of western corn rootworm larvae – from the quarantine to the field. Phytomedicine Colloquium, Göttingen

Posters at conferences or workshops

Schumann, M. and Vidal S. 2009 Noninvasive methods for evaluating western corn rootworm larval behaviour and maize root growth 23rd IWGO Conference & 2nd International Conference of Diabrotica Genetics, Munich

Schumann, M., Patel, A. and Vidal, S. 2010. Entwicklung einer Attract and Kill-Methode für die Larven des Westlichen Maiswurzelbohrers (Development of an Attract and Kill-strategy against Western corn rootworm larvae). 57th Conference of the German Plant Protection Society (DPG), Berlin

Schumann, M., Patel, A. and Vidal, S. 2011. „Attract & Kill“against western corn rootworm larvae. DGAAE (Deutsche Gesellschaft für allgemeine und angewandte Entomologie) conference, Berlin.

Schumann, M., Reibe, K. and Vidal, S. 2011. Evaluation of western corn rootworm larval performance on conventional maize cultivars in a soil - less bioassay 24th IWGO Conference & 3rd International Conference of Diabrotica Genetics, Freiburg.

Danksagung/ Acknowledgements

Herrn Prof. Dr. Stefan Vidal für das interessante Thema meiner Dissertation, die tolle Betreuung und die Möglichkeit meine Ideen und Interessen zu entwickeln und zu verwirklichen.

Herrn Prof. Dr. Stefan Schütz für die bereitwillige Übernahme des Korreferats.

Der Deutschen Bundesstiftung Umwelt (DBU) für die finanzielle Unterstützung und die Möglichkeit, auf Seminaren so viele tolle Menschen kennnzulernen. Frau Dr. Schlegel Starmann für die Betreuung von Seiten der DBU und für die Unterstützung bei der Organisation des DBU Workshops in Göttingen.

Prof. Anant Patel, Dr. Desiree Jakobs Schönwandt und Marinna Vemmer (FH Bielefeld) für die schöne Zusammenarbeit und Produktion der CO_2 Kapseln.

Allen Mitarbeiter der *Diabrotica* Gruppe: Bianca Tappe, Magdolna Weller, Benedikt Kurtz, Anne Wilstermann, Joachim Moeser und Christine Venjakob für ihre Hilfe und Unterstützung bei der Durchführung der Versuche.

Allen Zivis (Thorston Ogon, Daniel Schütz) und Auszubildenen (Jacqueline Platzek, Katja Fischer, Isabella Rübig) für ihre Hilfe bei den Versuchen.

Meinen Kollegen der Agrarentomologie für die tolle, freundliche und lustige Zusammenarbeit mit unterhaltsamen Kaffee – und Mittagspausen …. und natürlich dem “Feierabendbier”.

Stefan Töpfer, Ferenc Koncz and Nikolin Karapancsi (Plant Protection and Soil Conservation Directorate of Csongrad County in Hodmezovasarhely in Hungary) for their help during the field work.

Meiner Familie und Freunden besonders meiner Steffi, die mich mit ihrer Liebe in allen Lebenslagen während der Promotion unterstützt und motiviert hat.

Curriculum vitae

Date/place of birth: 21st of February 1983 in Nuremberg

2002	A-level (Abitur), King's School Bruton, England
2002 – 2003	Military Service, Berchtesgaden, Germany
2003 – 2006	B.Sc. Zoology, Cardiff University, Wales (Bachelor Thesis: "The effects of human-induced climate change on sea ice-associated species - Sentinels of global warming")
2005	Field work in Cadiz, Spain on the aggregation formation of the common garden snail *Thebia pisana*
2006	Insect Investigations Ltd., Cardiff. Trials officer testing various insecticides against household and agricultural pests
2006 – 2007	M.Sc. Applied Entomology, Imperial College, London (Master Thesis: "Fungus colonization in relation to the subterranean feeding of the western corn rootworm (Coloeptera: Chrysomelidae) in conventional and transgenic maize")
2007 – 2008	Research assistant at the Georg August University, Göttingen
2011	Project assistant on enhancement of soil insecticides in Hodmezovarsahely, Hungary
2008 – 2012	PhD in the Department for Crop Sciences, Agricultural Entomology, Georg-August University, Göttingen. Title: "Development of an "Attract & Kill" strategy for the control of western corn rootworm larvae". (German Federal Environmental Foundation (DBU) scholarship)

1. Hiermit erkläre ich, dass diese Arbeit weder in gleicher noch in ähnlicher Form bereits anderen Prüfungsbehörden vorgelegen hat.

   Weiter erkläre ich, dass ich mich an keiner anderen Hochschule um einen Doktorgrad beworben habe.

   Göttingen, den .....

   .....
   (Unterschrift)

2. Hiermit erkläre ich eidesstattlich, dass diese Dissertation selbständig und ohne unerlaubte Hilfe angefertigt wurde.

   Göttingen, den .....

   .....
   (Unterschrift)