Rhizosphere 3 (2017) 53–59

Contents lists available at ScienceDirect

Rhizosphere

journal homepage: www.elsevier.com/locate/rhisph

Diffusion of the maize root signal (*E*)-β-caryophyllene in soils of different textures and the effects on the migration of the entomopathogenic nematode *Heterorhabditis megidis*

Xavier Chiriboga M.[a], Raquel Campos-Herrera[a,b], Geoffrey Jaffuel[a], Gregory Röder[a], Ted C.J. Turlings[a,*]

[a] *Fundamental and Applied Research in Chemical Ecology Laboratory, University of Neuchâtel, Emile-Argand 11, Neuchâtel CH 2000, Switzerland*
[b] *Centro para os Recursos Biológicos e Alimentos Mediterrânicos (MeditBio), Campus Gambelas, Edf. 8, FCT, Universidade do Algarve, Faro 8005-191, Portugal*

ARTICLE INFO

Keywords:
Entomopathogenic nematode
Heterorhabditis megidis
Belowground signaling
Diabrotica virgifera virgifera
(E)-β-caryophyllene
Maize
Soil
Texture

ABSTRACT

Maize roots respond to feeding by larvae of the beetle *Diabrotica virgifera virgifera* by releasing (*E*)-β-caryophyllene (*E*βc). This insect-induced root volatile attracts entomopathogenic nematodes (EPN) and thereby helps to protect the roots against herbivore damage. Previous studies suggest that diffusion of *E*βc occurs through the gaseous rather than the aqueous phase in sand and its diffusion is best at low levels of humidity. However, it remains largely unknown how *E*βc diffuses in typical natural and agricultural soils. To fully understand the function and efficiency of root-produced *E*βc as a belowground signal it is important to know how it spreads in real soils and how soil properties affect its diffusion. Using gas chromatography-mass spectrometry analyses, the diffusion of two doses of synthetic *E*βc (200 ng and 20,000 ng) injected in sand was compared with the diffusion of *E*βc injected in clay, clay-loam and sandy-loam soils, at 3 moisture levels (5, 10 and 20% water), and at two distances (5 and 10 cm) from the *E*βc injection point. The diffusion of the compound was measured with a Solid Phase Micro Extraction (SPME) fiber every 30 minutes over a period of 9 hours. We found that, in contrast to the pattern observed for pure sand, *E*βc diffused best when humidity was high in the three agricultural soils. In subsequent experiments we used glass-trays to create two types of mesocosms to assess the effect of synthetic or root-produced Eβc on the dispersal of the EPN Heterorhabditis megidis and its infection of sentinel hosts in the trays. The presence of synthetic *E*βc did not affect the ability of *H. megidis* to infect the sentinel host. However, under the test conditions, *E*βc released from maize roots influenced the migration behaviour of *H. megidis* depending on soil type. The results suggest that *D. virgifera*-damaged maize plants may recruit *H. megidis* more efficiently in clay loam soils than in other types of soil. These new insights into the diffusion dynamics and attraction efficiency of the root-produced signal *E*βc may help efforts to develop novel strategies for the sustainable management of the maize pest *D. virgifera*.

1. Introduction

Plants produce herbivore-induced plant volatiles (HIPVs) in response to herbivory attack (Karban and Baldwin, 1997; Dicke and Baldwin, 2010). These volatiles, which are not only emitted from leaves (Dicke and Sabelis, 1988; Turlings et al., 1990), but also from roots (Rasmann et al., 2005; Ali et al., 2010) have been proposed to function as an indirect defense to attract natural enemies of the herbivore that attacks the plant. HIPVs mainly comprise terpenoids, fatty acid derivatives, phenyl propanoids and benzenoids (Mumm and Dicke, 2010; Dudareva et al., 2006). Recently, two terpenes have been described as herbivore-induced root signals, the sesquiterpene (*E*)-β-caryophyllene (*E*βc) in maize (Rasmann et al., 2005) and pregeijerene in citrus, specifically Swingle citrumelo (Ali et al., 2010). Both attract entomopathogenic nematodes (EPNs), which infect and kill the root herbivores feeding on the roots of the emitting plants, thereby reducing root damage (Degenhardt et al., 2009; Ali et al., 2012; Hiltpold and Turlings, 2012).

The role of *E*βc as an EPN attractant has been confirmed in laboratory and field experiments. For instance, in experiments with belowground

* Corresponding author.
E-mail addresses: xavier.chiriboga@unine.ch (X. Chiriboga M.), rcherrera@ualg.pt (R. Campos-Herrera), geoffrey.jaffuel@unine.ch (G. Jaffuel), gregory.roeder@unine.ch (G. Röder), ted.turlings@unine.ch (T.C.J. Turlings).

http://dx.doi.org/10.1016/j.rhisph.2016.12.006
Received 2 November 2016; Received in revised form 22 December 2016; Accepted 31 December 2016
Available online 06 January 2017
2452-2198/ © 2017 Elsevier B.V. All rights reserved.

olfactometers, the EPN species *Heterorhabditis megidis* Poinar, Jackson & Klein (Rhabditida: Heterorhabditidae) and *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae) were significantly attracted to damaged maize roots when compared with the attractiveness of undamaged roots (Rasmann et al., 2005; Rasmann and Turlings, 2008). Furthermore, the EPNs *H. megidis* and *Steinernema feltiae* Filipjev (Rhabditida: Steinernematidae) are more efficient in reducing root damaged by *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) in *E*βc emitting maize than in non-emitting variety (Rasmann et al., 2005; Degenhardt et al., 2009; Hiltpold et al., 2010). It has been suggested that the diffusion properties of *E*βc make it a highly suitable belowground signal (Hiltpold and Turlings, 2008).

The diffusion of organic compounds in soil matrices is affected by several factors that are closely related to the sorption properties of the soil (Lindstrom et al., 1967; Steinberg and Kreamer 1993). Sorption of organic compounds in the soil largely depends on its chemical composition (e.g. mineral) and physical properties (e.g. particle size, density, porosity) (Ruiz et al., 1998). Water content (Porter & Kemper, 1960) and organic matter also play key roles in sorption (Li and Werth, 2001). Previous studies with pure sand have demonstrated that *E*βc rapidly diffuses through the gaseous phase of the sand matrix (Rasmann et al., 2005; Hiltpold and Turlings, 2008), which was most evident from the easy and rapid horizontal diffusion of *E*βc at low moisture levels. However, a first attempt to characterize *E*βc diffusion in a sandy soil showed a clear decrease in relation to pure sand (Hiltpold and Turlings, 2008). It therefore remains unclear how *E*βc diffuses in different soil types, and to what extent diffusion depends on sand content. To broaden our knowledge on the signaling function and efficiency of *E*βc as a belowground signal, it is essential to identify key soil characteristics that affect its diffusion.

Soils are composed of particles of different sizes (such as sand, clay and silt) and their relative proportion defines soil texture, which in turn determines pore size. We therefore hypothesized that diffusion of *E*βc and EPN attraction towards the root volatile diminishes when sand content decreases.

The current study aimed to characterize the diffusion of *E*βc through soils of different textures and define how soil humidity affects this diffusion. We used a combination of fibre-based solid phase microextraction and gas chromatography-coupled mass spectrometry (GC–MS) to measure *E*βc diffusion in various soils. In subsequent experiments we used soil-filled glass trays to test how a point source of authentic *E*βc may help guide the EPN *H. megidis* towards sentinel insect hosts in different soil textures. In addition, we tested the attraction of *H. megidis* to insect-damaged maize varieties with distinctly different *E*βc emission rates in these soil types.

2. Materials and methods

2.1. Soils, nematodes, insects, plants, and general procedures

Experiments were performed with pure sand (Migros, Switzerland) and three agricultural soils of different textures (Table 1). We used a Gleyic Cambisol clay soil (C), a clay loam soil (CL) and sandy loam soil (SL) (IUSS Working Group WRB, 2006). Texture analyses were performed by Soil Conseil (Nyon, Switzerland). The soils were collected in the experimental fields of Agroscope, *Institut des Sciences en Production Végétale* (46° 24' N, 6° 14' E, 430 m above sea level, Changins-Nyon, Switzerland) during 2013 and 2014. Following procedures described by Hiltpold and Turlings (2008), soils were ground, sieved in a 2 mm mesh and autoclaved (120 °C) to obtain a sterile substrate. Soils were also ventilated for at least 24 h to eliminate possible odours and volatiles that might interfere with the detection of *E*βc.

A commercial population of the EPN *H. megidis* (Andermatt Biocontrol AG, Switzerland) was used for the glass-tray experiments. The identity of the species was morphologically and molecularly confirmed (ITS rDNA region sequence, GenBank Accession number KJ938577) (Campos-Herrera et al., 2015). New generations of freshly emerged nematodes reared from *Galleria melonella* L. (Lepidoptera: Pyralidae) larvae were used no more than 15 days after emergence. Suspensions of infective juveniles (IJs) were prepared by counting IJs under a stereo-microscope and by adjusting the concentration in distilled water to 2000 IJs/mL. In each soil tray, 2000 IJs of *H. megidis* were inoculated at 10 cm distance from a capillary dispenser (2^{nd} experiment, see below) or the maize plant (3^{rd} experiment).

Larvae of *G. mellonela* (commercial stock, *Au Pêcheur* SARL, Neuchâtel, Switzerland) were used for the nematode rearing and also as sentinel hosts in the first tray experiments to quantify infection success. In the second tray experiments, we infested maize plants with second instar larvae of *Diabrotica virgifera* Le Conte (Coleoptera: Chrysomelidae) obtained from the North Central Agricultural Research Laboratory-USDA (Brookins, USA).

Two maize (*Zea mays* L., Poales: Poaceae) varieties were used: *i*) Graf as the high *E*βC emitting plant and *ii*) Pactol as the non-emitting plant (Gouinguené et al., 2001; Rasmann et al., 2005). Seeds were sown in plastic pots and plants grown in a climate chamber (Grow bank, 24 °C, 14:10 hours light:dark photoperiod, 320 μmo m^{-2} s^{-1}) during 16 days until they reached the 4 leaf-stage.

2.2. Diffusion patterns of synthetic Eβc in various soil types, at different moisture levels, distances and concentrations

A solution of authentic *E*βc (Sigma Aldrich, >98% pure) was prepared at a low (200 ng) and high concentration (20,000 ng) by dissolving the compound in different amounts of pentane. Soil moisture was adjusted by weight/volume to obtain levels corresponding to 5, 10 and 20% with mQ water (Milli-Q Water System, Millipore S.A., Molsheim, France). For the diffusion experiments a Teflon box (12 cm x 10 cm x 4 cm) was filled with one of the soil treatments. For each treatment, we used a soil moisture-soil texture combination with a constant mass of moistened soil or sand, thereby maintaining a homogenous density among all replicates (Supplementary data 1).

Either a low or high concentration of *E*βc was injected in a tray with one of the substrates at different levels of humidity (5%, 10% and 20%) and at different distances from the sampling fibre (10 cm or 5 cm). Each combination of soil, humidity and distance was replicated five times. The Teflon box was placed on a thermal tray, maintaining the temperature at 12 °C. A 0.2 mm diameter cylinder made of ultrafine

Table 1
Characteristics of soils used for diffusion and foraging behaviour experiments.

Nomenclature	Soil texture	% Sand [a]	% Clay [a]	% Silt [a]	pH [a]	% organic matter [a]	Field Capacity [b]
C	Clay	17	48	35	6.7	4.1	25%
CL	Clay loam	29	42	29	8	2.3	21%
SL	Sandy loam	56	18	26	8	2.9	20%
S	Sand (pure)	100	–	–	–	0	10%

[a] Determined at Soil Laboratory (Soil Conseil, Nyon, Switzerland).
[b] Determined following Estimating Soil Moisture by Feel and Appearance, USDA (1998).

metal mesh (2300 mesh, Small Parts Inc., USA) was inserted into the soil, creating a hole in which a Solid Phase Micro Extraction (SPME) fibre (100 µm polydimethylsiloxane, Supelco, Buchs, Switzerland) was introduced at a depth of 3 cm from the soil surface. Automated sampling was done over a total period of 9 or 12 h with a multipurpose sampler (MPS2, GerstelGmbH & Co. KG, Germany) (Hiltpold and Turlings, 2008). After the first 30 min sampling period, the *E*βc solution was injected at 3 cm deep into the soil. Every 30 min, the fibre was retracted automatically (multi-purpose sampler MPS2, Gerstel GmbH & Co. FG, Germany) from the soil matrix and inserted for 3 min into the injector of an Agilent 680 Series gas chromatograph (G1530) coupled to a quadrupole-type mass-selective detector (Agilent 5973, transfer line 230 °C, source 230 °C ionization potential 70 eV). The injector was kept at 230 °C and the desorbed compounds were separated on a polar column (HP1-MS, 30 M, 0.25 MM id, 0.25 µm film; Agilent Technologies, USA) using helium as a carrier gas (constant pressure of 127.9 kPa). Following injection, the temperature of the column was maintained at 40 °C for 1 min and then increased 20 °C min −1 to 250 °C, where it was held for 12 min more.

Statistical analysis. For soils at 10% of humidity, the curves were fitted according to a Linear Mixed Effects Model (LME) and the parameters for intercept and slope were compared. We exclude sand from the comparison because its diffusion did not fit a linear model. For soils at 20% of humidity, values obtained for abundance of *E*βc were used to model a curve according to the diffusion equation (see equation A.1) (Eqworld, 2015). The parameters of the curve were compared with a Non-linear mixed effects model (NLME). We used R environment (version 3.1.2, 2015) for both analyses.

Equation A.1

$$Abundance(t) = \frac{A}{\sqrt{t}}\exp\left(-\frac{1}{B^{*}t}\right) \quad (1)$$

Where: A represents the slope and B the peak

2.3. *Effects of soil texture on host infection by* Heterorhabditis megidis *in the presence or absence of* E*βC*

Soils of three different textures were prepared as described above. For each substrate one kg of each substrate was adjusted to 20% humidity (weight/volume) and placed in a glass tray (Pyrex, France, 23 cmx15 cmx6 cm) (n=3 per treatment). Four larvae of *G. melonella* were caged in a cylindrical metallic mesh cage (6.5 cm x 2.7 cm, diameter), which was buried at 3 cm distance from one of the edges of the glass container filled with soil. EβC dispensers were made of 1 ml vials containing 10 mg of cotton wool treated with 200 µL of authentic EβC. The vials were closed with a screw cap with a Teflon-covered septum through which a 100 µL glass capillary (Hirschmann Laborgerate ringcaps Duran, GmbH & Co. KG, Germany) was inserted (as described by Hiltpold et al., 2010). The dispensers were inverted and the capillaries inserted into trays with the substrate, behind the cage with larvae and 2 cm from the edge (Fig. 1a). Soil trays without dispensers were used as controls (Fig. 1b). Experiments were conducted at room temperature (22 ± 2 °C, 33% relative humidity) and larval mortality was recorded every 12 hours during 4–5 days. The experiment was repeated three times, each time using fresh nematode inoculum, insect larvae and newly prepared soils.

Data of larval mortality from the three experiments were pooled after testing homogeneity of the results. To compare mean times of death between different treatments statistical analyses were performed with a Generalized Linear Mixed-Effects model, with gamma distribution and replicate as a random effect in R environment (version 3.1.2., 2015). In the second and third experiments, numbers of nematodes that infected each sentinel larva were recorded by dissecting cadavers and digesting the tissues with a pepsin solution 72 h after larval death (Mauleon et al., 1993). Data from these experiments were pooled. The proportion of nematodes that succeeded to infect one larva at each time-point of evaluation was calculated and these data were square root transformed in order to normalize their distribution. Data were analysed with a Two-Way Anova in R environment (version 3.1.2., 2015).

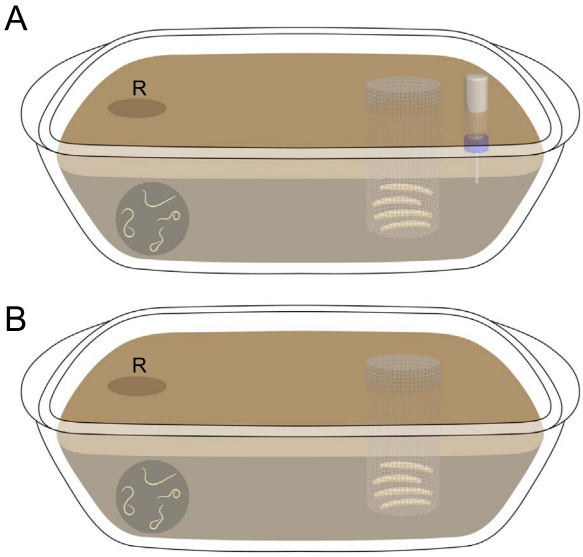

Fig. 1. Set-up used to test the effects of soil type on host infection by *Heterorhabditis megidis*. **(A)** Four *Galleria melonella* larvae caged next to an (*E*)-β-caryophyllene releasing capillary dispenser, **(B)** Four *Galleria melonella* larvae caged without capillary dispenser (control).

2.4. *Effects of soil texture on migration of* Heterorhabditis megidis *in the presence or absence of naturally produced* E*βC*

The effect of plant-produced *E*βC on EPN attraction in different soil types was tested. To provide optimal water conditions for the plants, water content for each soil was adjusted to achieve field capacity based on values provided by the USDA (1998). MilliQ water was added to each substrate in different proportions: C (25%), CL (21%) and SL (20%). In each tray we placed a 16-day old (four leaves) maize plant at 1 cm distance from one of the edges of the tray and the root system was infested with six late second instar *D. virgifera* larvae. The larvae were allowed to feed on the roots for 48 h before releasing the EPNs (Fig. 2). Trays without plants were used as controls. Three replicates per treatment were done simultaneously and the experiment was repeated two times (n=6). Infective juveniles of *H. megidis* were released 48 h after insect infestation, 12 cm away from the maize plant. Fifty-six hours after this release, the numbers of individuals of *H. megidis* were estimated by sampling four positions within each soil tray: 1) 12 cm away from the inoculation point, in the plant/no-plant (P), 2) in the middle of the tray, at 5 cm distance from the inoculation point (M), 3) in the release point (R) and 4) in the sides of the trays (L) (Fig. 2). A sample consisted of two cores of soil of 19.6 cm^3 each one (approximately 50 g of soil), taken with a cylindrical metallic sampler (2.6 cm, diameter). To recover the EPNs from the samples they were placed in Baermann funnels (Hass et al., 1999). Each time the sampler was cleaned with distilled water. After 24 hours, the numbers of *H. megidis* individuals that had fallen from the funnel trap were decanted in 10 mL of water and were counted under a stereo-microscope. The experiment followed a split-plot design with four factors: Replicate, Soil type, Plant presence and Location within the tray. The numbers of nematodes counted at each location were transformed with (log+1) to normalize the data. Data were analysed with a Mixed Procedure with fixed factors (Soil, Plant, Location, Replicate) and random factors (ReplicatexSoil, ReplicatexSoilxPlant in SAS (9.2. Cary, NC, USA). Differences between treatments were obtained by Least Square Means.

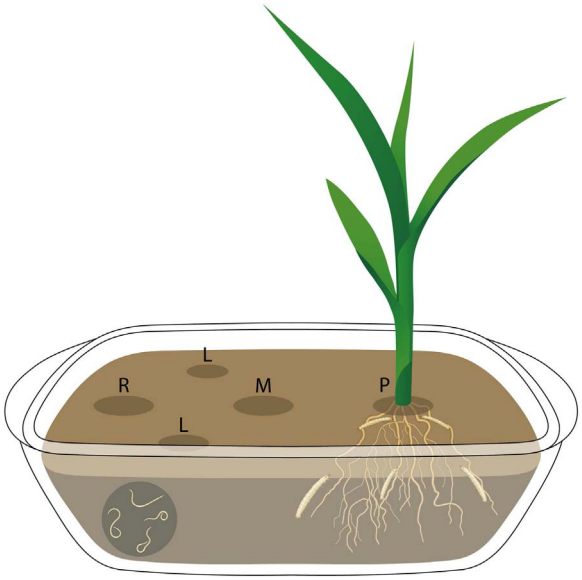

Fig. 2. Set-up used to test the foraging success of *Heterorhabditis megidis* in different soil types. Schematic layout of a tray with a maize plant, rootworm larvae and nematodes: nematode release point (R), lateral (L), middle (M) and plant (P). Control trays did not include maize plants.

3. Results

3.1. Diffusion patterns of synthetic Eβc in various soil types, at different moisture levels, distances and concentrations

The diffusion of *E*βC was strongly reduced in soils with high clay content and 10% moisture level, but markedly improved when soil moisture was increased to 20%. At 10% moisture and 10 cm distance from the source, *E*βC (in low concentrations) diffused readily in sand, as expected (Hiltpold and Turlings, 2008), but was not detected in any of the soils we tested (Supplementary data 2). When the concentration of *E*βC was increased 100-fold, *E*βC rapidly diffused in sand, and we could also detect it at 10 cm distance in clay loam (CL), but not in sandy loam (SL) and clay (C) soils (Supplementary data 3). When reducing the distance from the fibre to the source to 5 cm, with the high dosage of *E*βC and 10% of humidity in the substrates, EβC was detected in considerable amounts immediately after injection in sand, while in all soils a slower but increasing gradient of detection was found over the 9 h of measurements (Fig. 3). The slope and intercept for the gradients of diffusion varied among the soil types. The soils C and CL showed differences in both slope (P=0.03) and intercept (P=0.03), whereas C and SL only differed in slope (P=0.02) and CL and SL

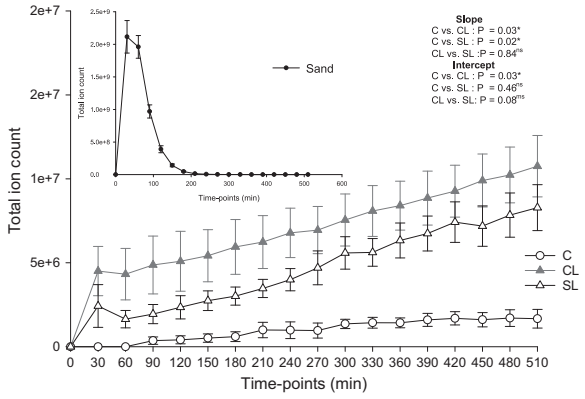

Fig. 3. Diffusion of (*E*)-β-caryophyllene injected at a high concentration (20,000 ng) in pure sand (S) and three different soil types: clay (C), clay loam (CL) and sandy loam (SL) at 10% humidity and at 5 cm distance from the sampling fibre. Data are average ± SEM.

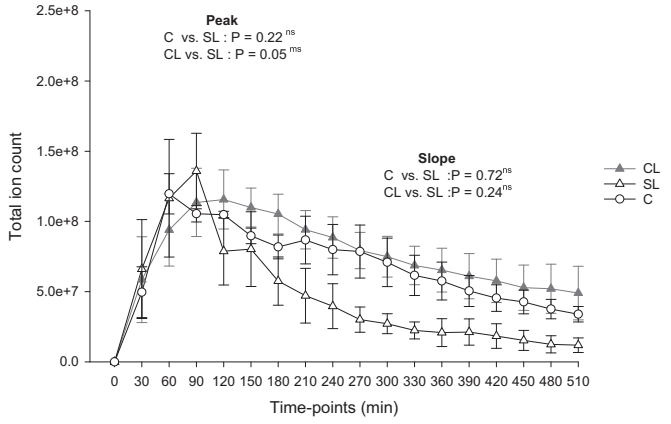

Fig. 4. Diffusion of *E*-(β)-caryophyllene injected at a high concentration (20,000 ng) in three different soil types: clay (C), clay loam (CL) and sandy loam (SL) soil at 20% humidity and at 5 cm distance from the sampling fibre.

differed marginally in the intercept (P=0.09) (Fig. 3).

Interestingly, when soils were tested at 20% humidity, detection of *E*βC improved dramatically (Fig. 4). The highest detection was recorded in sandy loam (SL) within one and half hours after injection. Much lower and similar amounts of *E*βC were measured both in clay loam (CL) and clay (C) within 120 min and 60 min after injection, respectively. Differences between slope/peak in each of the comparison between soils were not significant (P > 0.1). In contrast, in soils at 5% of humidity the diffusion of EβC was marginal and variable in the three soil textures (Supplementary data 4).

3.2. Effects of soil texture on host infection by Heterorhabditis megidis in the presence or absence of EβC

Overall, the mean time of death after release of *H. megidis* was affected by soil type (P < 0.01; Fig. 5). Mean time of death was also affected by the presence or absence of an *E*βC-releasing capillary dispenser (P < 0.05; Supplementary data 5) but this effect only accounted for 27% of the variation in the experiment. The interaction between soil and *E*βC was not significant (P > 0.1) and we therefore used an additive model to analyse the data. The model showed that the observed mean times of death are lower for sand, sandy loam and clay

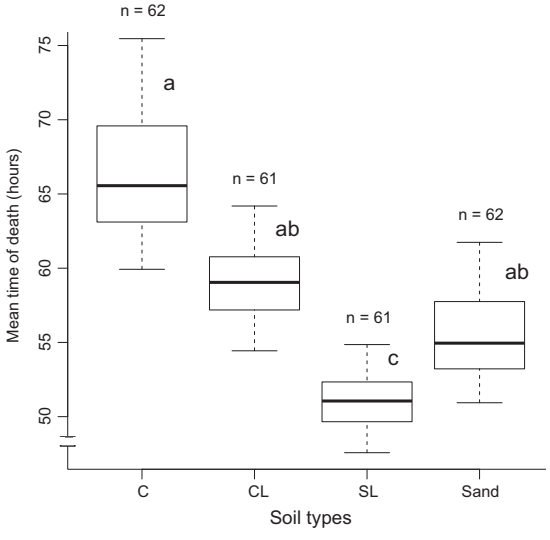

Fig. 5. Mean times (hours) until the death of *Galleria melonella* larvae infected by *Heterorhabditis megidis* that foraged in: sand (S), clay (C), clay loam (CL) and sandy soil (SL). Values represent means in presence of an *E*βC-capillary dispenser or absence of an *E*βC-capillary dispenser. Different letters represent significant differences. Data are averaged and the box plots represent the SEM.

Table 2
Observed means time of death for *G. melonella* larvae in each type of soil.

Type of Soil	Observed mean time of death (hours)	Predicted mean time of death (hours)
Sand	55,5	55.4
Sandy loam	51,6	51,4
Clay	66,8	66,3
Clay loam	60,0	59,7

loam soils compared with clay soil (Table 2). Numbers of IJs that succeed to infect one larvae of *G. melonella* were different between soil types ($F_{3,163} = 10.7$, $P < 0.01$) and the presence of a *E*βC-capillary dispenser had a marginal effect ($F_{1,163} = 2,7$, $P = 0.09$). The interaction between soil type and presence of *E*βC was not significant (Supplementary data 6a and 6b).

3.3. *Effects of soil texture on migration of* Heterorhabditis megidis *in the presence or absence of naturally produced* E*βC*

The migration of IJs of *H. megidis* was different for the two maize varieties ($F_{2,84} = 10.42$, $P < 0.0001$) but was not affected by soil type ($F_{3,84} = 0.67$, $P = 0.67$). However, there was a significant interaction Plant x Soil ($P = 0.002$) affecting EPN migration in the trays. In trays with pure sand and in trays with clay loam soil more IJs of *H. megidis* were recruited in the rhizosphere of maize plants var. Graf (high emitter of *E*βC) as compared to var. Pactol (low emitter of *E*βC) (Fig. 6a and b). This was not the case for trays with sandy loam and clay soil, where there were no differences between the nematodes recruited by the two maize varieties (Fig. 6c-d). In all cases, many more EPN were recovered near the plants than in the same place in control trays without plants (Fig. 6). The numbers of nematodes that were recovered from the other sampling spots in the trays showed not clear patterns (Fig. 6).

4. Discussion

4.1. *Diffusion of* E*-(β)-caryophyllene in different soil textures*

Our findings confirm the hypothesis that sand content in a soil affects the diffusion of *E*βC. In soils with 10% moisture, *E*βC diffused better in both a clay loam soil and a sandy loam soil (29.2 and 55.9% sand) compared to a clay soil (17% sand). A previous study by Hiltpold and Turlings (2008) showed that diffusion of *E*βC was significantly decreased in a sandy loam soil comparing to diffusion in pure sand. Moreover, when studying *E*βC diffusion in relation to water content in pure sand, diffusion of *E*βC was almost two-fold larger at 1% of humidity than at 10% humidity (Hiltpold and Turlings, 2008). In sharp contrast, in our study the best diffusion of *E*βC occurred in soils at 20% of humidity, which is around the field capacity of these soils (see Table 1). The quantity of *E*βC (100-fold higher than in the Hiltpold and Turlings (2008) study) used in our experiments was far outside of the natural range of *E*βC production by maize roots, but it allowed us to detect the volatile with the employed methodology and to reveal how *E*βC behaves in different soils and under different humidity conditions. In soils with 10% water, *E*βC was detected in clay loam soil, but not in sandy loam and clay soils, which is in agreement with the pattern observed in soils at 20% moisture. However, in soils with 5% water, *E*βC diffused better in sandy loam than in clay loam soil. This may be explained by the fact that pores in clay loam soils are easily saturated with small quantities of water in contrast to sandy loam soils.

*E*βC is a non-polar compound that dissolves poorly in water and is assumed to disperse through the gaseous phase of the soil (Hiltpold and Turlings, 2008; Turlings et al., 2012). It has been well documented that diffusion of volatile organic compounds in soils is highly dependent on soil adsorption properties (Lindstrom et al., 1967; Steinberg and Kreamer 1993), and is largely determined by the mineral composition of soils (Ruiz et al., 1998). It has been suggested that since the water molecule is a strong dipolar molecule, it competes for adsorption sites in the soil mineral particles and may displace non-

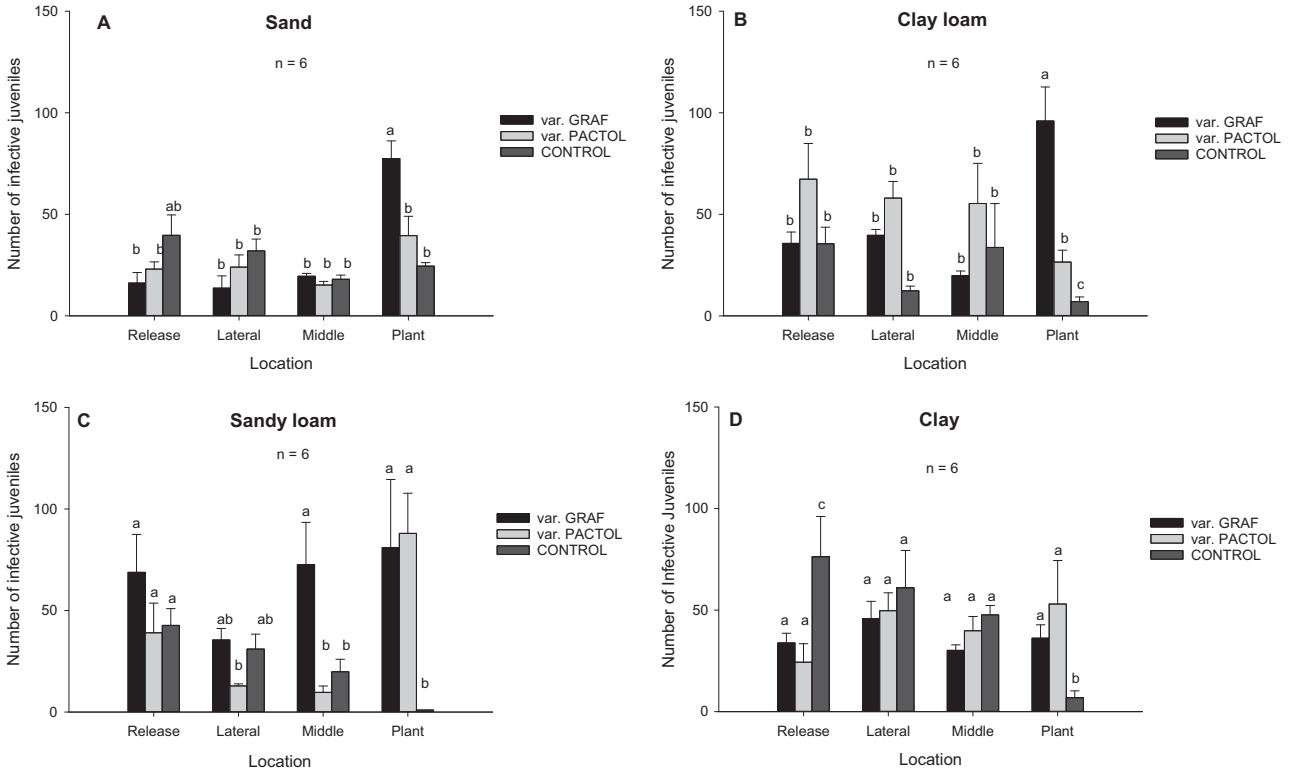

Fig. 6. Numbers of infective juveniles (IJs) of *Heterorhabditis megidis* in samples taken from different spots in the experimental tray (Fig. 2) in: **A.** pure sand **B.** clay loam **C.** sandy loam **D.** clay. Data are average ± SEM and pooled for two experiments. Different letters show significant differences.

polar organic molecules (Ruiz et al., 1998). Our results are in agreement with this hypothesis and imply that in soil water prevents the interaction of *E*βC with soil particles of high adsorption capacity (i.e. clay). It is also interesting to note that the detectability of the *E*βC in a sandy loam soil decreased rapidly in comparison to a clay loam or clay soil (Fig. 4), this might be explained by the fact that adsorption is relatively low in sand (Ruiz et al., 1998) and as a result the root volatile may be partially lost by vertical diffusion, as shown by Rasmann et al. (2005).

Soil porosity also plays a role in the diffusion of volatile organic compounds. Pore size affects and is affected by several factors, such as the movement of water, air and other fluids, the transport and reaction of chemicals, and the residence of roots and other biota (Nimmo, 2004). In general, sandy soils have a larger particle and pores sizes (Plant and Soil Sciences elibrary, 2014), which may favour the diffusion of root produced volatiles through the gaseous phase. Indeed, we found better diffusion of *E*βC in clay loam and sandy loam soils, which have a relatively higher content of sand and larger pores in comparison to a clay soil.

Overall, the results confirm that EβC is a suitable belowground signal in real cropping conditions, but different soil types may differ in the action-radius of *E*βC for EPN attraction.

4.2. *Effects of soil texture on host infection by* Heterorhabditis megidis *in presence or absence of synthetic* E*βC*

Our results support previous conclusions about the effects of soil texture on virulence and infectivity of EPNs. We recorded earlier mortality of *G. melonella* larvae in pure sand and sandy loam than in clay and clay loam soils, probably due to reduced motility of the *H. megidis* in the clay-rich soils, as Kaya (1990) suggests that nematode motility generally decreases as soil pores become smaller. Indeed, the rates of movement and infection by nematodes are strongly correlated with the amount of soil pore openings of dimensions similar to or greater than the diameters of the nematodes (Portillo-Aguilar et al., 1999). Small soil pores, particularly in combination with higher soil moisture also limit oxygen levels and with that activity and survival (Kung et al., 1990) of EPNs. Indeed, motility and persistence are influenced by numerous interacting intrinsic (e.g. behavioural, physiological and genetic characteristics) and extrinsic factors [e.g. temperatures, soil moisture, soil texture, relative humidity and UV radiation (Kaya, 1990; Smits, 1996; Stuart et al., 2006, 2015).

The *E*βC releasing capillary had only a small effect on the infectivity and virulence of *H. megidis*. This result may have several explanations. Possibly, under the experimental conditions, the CO_2 expelled by *G. melonella* larvae was more readily detected by the IJs of *H. megidis*, before they were able to detect *E*βC. Turlings et al. (2012), showed that CO_2 works in synergy with the EβC and propose CO_2 predominantly serves as a response activator that alerts EPNs to the general presence of living organisms and may enhance their responsiveness to more specific and more reliably inducible plant cues. In the current study we also did not find any differences between the number of IJs that succeed to infect one *G. melonella* larvae in treatments with an *E*βC releasing capillary and without it, in contrast to when we used *E*βC-releasing maize plants.

4.3. *Effects of soil texture on migration of* Heterorhabditis megidis *in presence or absence of natural* E*βC*

In agreement with observations in pure sand by Rasmann et al. (2005) and Hiltpold et al. (2009), IJs of *H. megidis* were significantly more attracted to maize plants that release *E*βC than to plants with low production or controls without plants. We also confirmed the important role of root cues for EPNs attraction (Wang and Gaugler, 1998), independently of the fact that roots produce *E*βC or not, they recruited more EPNs than empty trays, possibly because of root-produced CO_2, which may serve as a universal host cue (Gaugler et al., 1980; Dillman et al., 2012; Turlings et al., 2012). The attraction towards the roots is also evident from the fact that fewer nematodes remained at the original point of release in trays with plants.

The migration of *H. megidis* toward *E*βC producing plants was dependent on soil type and was most effective in clay loam soil. In this soil, IJs migrated more toward *E*βC producing plants (var. Graf) than toward non-producing maize plants (var. Pactol). This result is in agreement with a Hungarian field trial in clay loam soil, where plants that produced *E*βC were found to attract more EPNs than plants that did not produced *E*βC (Rasmann, 2006). In our tray experiments with non-releasing Pactol plants, the majority of nematodes remained at the release point or in the middle of the tray, which was not the case for the trays with Graf plants (Fig. 6). In contrast, in sandy loam soil, at the time we evaluated the experiment, there was not difference between Graf and Pactol plants in the number of nematodes that reached the plant area. However, in the middle of the tray we found a significantly higher number of IJs in the trays with Graf plants, suggesting that IJs were moving toward the plant releasing the EPN attractant. If this is the case, we can conclude that recruitment of *H. megidis* by *E*βC producing plants takes a longer time in a sandy loam soil than in a clay loam soil. This corresponds well with the diffusion of *E*βC in the different soils: *E*βC detection after applying it in the diffusion experiments reached its maximum level at the same time for both soils. However, the detection of the *E*βC was more sustained over time in the clay loam soil than in the sandy loam soil (Fig. 4). This suggests that *E*βC may be perceived more easily by the EPN *H. megidis* in clay loam soil and may be rapidly lost in a sandy loam soil due to upward volatilization.

In clay soil, the migration of *H. megidis* toward *E*βC producing and non-producing plants was much less than in the other soil types. The diffusion of *E*βC in clay soil was comparable to its diffusion in clay loam soil. Yet, the IJs took longer to reach the roots. This suggests that, even though the IJs might readily detect the *E*βC signal, their movement is impaired in this type of soil because of low porosity (Kaya, 1990; Stuart et al., 2006, Stuart et al., 2015).

5. Conclusions

The diffusion of the root signal *E*βC was found to strongly depend on soil texture and soil humidity. At low water content (10%), its diffusion was reduced specially in clay soil but it was improved when water content was increased to 20% in clay loam, sandy loam and clay soils. Moreover, the gradient of CO_2 produced by insect-caged hosts may be established faster than the one of the synthetic *E*βC, favouring the location of the hosts by the nematodes independently of the presence of other cues. However, in soil-filled glass trays, under laboratory conditions, the production of *E*βC by maize roots was found to influence the migration behaviour of *H. megidis* depending on the soil texture. Under real agricultural conditions, clay loam soils facilitate the recruitment of *H. megidis* IJs towards the rhizosphere of *E*βC-producing plants. Orientation by EPN in sandy loam soils may be less efficient and in clay soils the *E*βC signal may not at all help maize plants to recruit IJs. Further research on the dynamics of the *E*βC root signal in relation to other soils factors such as: pH, organic matter, moisture and temperature is needed to elucidate how to better exploit this HIPV to control *D. virgifera* and other soil-borne pests.

Acknowledgements

We thank Radu Slobodeanu and Xoaquín Moreira for their support with the statistical analyses. We are thankful to Thomas Degen for drawing the illustrations, to Rubén Blanco Pérez and Juan Traine for the technical support, and to the members of the Soil and Vegetation Laboratory (Université de Neuchâtel) for sharing their equipment and facilities. This study was supported by the NRP68 program

"Sustainable use of soil as a resource" (project no. 143065) from the Swiss National Science Foundation awarded to TCJT. XC was endowed with an Excellence Scholarship of the Swiss Confederation and GJ was supported by an economic stimulus grant from the Swiss National Foundation.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.rhisph.2016.12.006.

References

Ali, J.G., Alborn, H.T., Campos-Herrera, R., Kaplan, F., Duncan, L.W., Rodriguez-Saona, C., Koppenhöfer, A.M., Stelinski, L.L., 2012. Subterranean, herbivore-induced plant volatile increases biological control activity of multiple beneficial nematode species in distinct habitats. PLoS One 7, e38146. http://dx.doi.org/10.1371/journal.pone.0038146.

Ali, J.G., Alborn, H.T., Stelinski, L.L., 2010. Subterranean herbivore-induced volatiles released by citrus roots upon feeding by *Diaprepes abbreviatus* recruit entomopathogenic nematodes. J. Chem. Ecol. 36, 361–368. http://dx.doi.org/10.1007/s10886-010-9773-7.

Campos-Herrera, R., Jaffuel, G., Chiriboga, X., Blanco-Pérez, R., Fesselet, M., Půža, V., Mascher, F., Turlings, T.C.J., 2014. Traditional and molecular detection methods reveal intense interguild competition and other multitrophic interactions associated with native entomopathogenic nematodes in Swiss tillage soils. Plant Soil 389, 237–255. http://dx.doi.org/10.1007/s11104-014-2358-4.

Degenhardt, J., Hiltpold, I., Kollner, T.G., Frey, M., Gierl, A., Gershenzon, J., Hibbard, B.E., Ellersieck, R., Turlings, T.C.J., 2009. Restoring a maize root signal that attracts insect-killing nematodes to control a major pest. Proc. Natl. Acad. Sci. USA 106, 13213–13218. http://dx.doi.org/10.1073/pnas.0909073106.

Dicke, M., Sabelis, M.W., 1988. How plants obtain predatory mites as bodyguards. Neth. J. Zool. 38, 148–165. http://dx.doi.org/10.1163/156854288X00111.

Dicke, M., Baldwin, I.T., 2010. The evolutionary context for herbivore-induced plant volatiles: beyond the "cry for help". Trends Plant Sci. 15, 167–175. http://dx.doi.org/10.1016/j.tplants.2009.12.002.

Dillman, A.R., Guillermin, M.L., Ha, J., Kim, B., Sternberg, P.W., Hallem, E. a, 2012. Olfaction shapes host – parasite interactions in parasitic nematodes. Proc. Natl. Acad. Sci. USA 109, E2324–E2333, (doi:10.1073/pnas.1211436109/-/DCSupplemental.www.pnas.org/cgi/doi/10.1073/pnas.1211436109).

Dudareva, N., Negre, F., Nagegowda, D.A., Orlova, I., 2006. Plant Volatiles: recent advances and future perspectives. CRC Crit. Rev. Plant Sci. 25, 417–440. http://dx.doi.org/10.1080/07352680600899973.

Gaugler, R., Lebeck, L., Nakagaki, B., Boush, G.M., 1980. Orientation of the entomogenous nematode Neoaplectana carpocapsae to carbon dioxide. Environ. Entomol. 9, 649–652. http://dx.doi.org/10.1093/ee/9.5.649.

Gouinguené, S., Degen, T., Turlings, T.C.J., 2001. Variability in herbivore-induced odour emissions among maize cultivars and their wild ancestors (teosinte). Chemoecology 11, 9–16. http://dx.doi.org/10.1007/PL00001832.

Hass, B., Griffin, C.T., Downes, M.J., 1999. Persistence of heterorhabditis infective juveniles in soil: comparison of extraction and infectivity measurements. J. Nematol. 31, 508–516.

Hiltpold, I., Toepfer, S., Kuhlman, U., Turlings, T.C.J., 2009. How maize root volatiles affect the efficacy of entomopathogenic nematodes in controlling the western corn rootworm? Chemoecology 20, 155–162. http://dx.doi.org/10.1007/s00049-009-0034-6.

Hiltpold, I., Baroni, M., Toepfer, S., Kuhlmann, U., Turlings, T.C.J., 2010. Selection of entomopathogenic nematodes for enhanced responsiveness to a volatile root signal helps to control a major root pest. J. Exp. Biol. 213, 2417–2423. http://dx.doi.org/10.1242/jeb.041301.

Hiltpold, I., Turlings, T.C.J., 2012. Manipulation of chemically mediated interactions in agricultural soils to enhance the control of crop pests and to improve crop yield. J. Chem. Ecol. 38, 641–650. http://dx.doi.org/10.1007/s10886-012-0131-9.

Hiltpold, I., Turlings, T.C.J., 2008. Below ground chemical signaling in maize: when simplicity rhymes with efficiency. J. Chem. Ecol. 34, 628–635. http://dx.doi.org/10.1007/s10886-008-9467-6.

IUSS Working Group WRB, 2006. World reference base for soil resources 2006. World Soil Resources Reports No 103. FAO, Rome

Karban, R., Baldwin, I.T., 1997. Induced Responses to Herbivory. University of Chicago Press, Chicago, USA.

Kaya, H.K., 1990. Soil ecology. In: Gaugler, R., Kaya, H.K. (Eds.), Entomopathogenic Nematodes in Biological Control.. CRC, Boca Raton, Florida, 93–116.

Kung, S.-P., G.R, K.H, 1990. Soil type and entomopathogenic nematode persistence. J. Invertebr. Pathol. 22, 440–445.

Li, J., Werth, C.J., 2001. Evaluating competitive sorption mechanisms of volatile organic compounds in soils and sediments using polymers and zeolites. Environ. Sci. Technol. 35, 568–574.

Lindstrom, F.T., Haque, R., Freed, V.H., Boersma, L., 1967. Theory on the movement of some herbicides. Linear diffusion and convection of chemicals in soils. Environ. Sci. Technol. 1, 561–565.

Mauleon, H., Briand, S., Laumond, C., Bonifassi, E., 1993. Short notes. J. Appl. Nematol. 16, 185–191.

Mumm, R., Dicke, M., 2010. Variation in natural plant products and the attraction of bodyguards involved in indirect plant defense. Can. J. Zool. 88, 628–667. http://dx.doi.org/10.1139/Z10-029.

Nimmo, J.R., 2004. Porosity and pore size distribution. Encycl. Soils Environ. 3, 295–303. http://dx.doi.org/10.1081/E-ESS-120042734.

Porter, L., Kemper, W., Jackson, R., Stewart, B., 1960. Chloride diffusion in soils as influenced by moisture content. Soil Sci. Soc. Proc., 460–463.

Portillo-Aguilar, C., Villani, M., Tauber, M., Tauber, C., Nyrop, J., 1999. Entomopathogenic nematode (Rhabditida: heterorhabditidae and steinermatidae) response to soil texture and bulk density. Environ. Entomol. 28, 1021–1035.

Rasmann, S., Köllner, 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. http://dx.doi.org/10.1038/nature03451.

Rasmann, S., Turlings, T.C.J., 2008. First insights into specificity of belowground tritrophic interactions. Oikos 117, 362–369. http://dx.doi.org/10.1111/j.2007.0030-1299.16204.x.

Ruiz, J., Bilbao, R., Murillo, M.B., 1998. Adsorption of different VOC onto soil minerals from gas phase: influence of mineral, type of VOC, and air humidity. Environ. Sci. Technol. 32, 1079–1084. http://dx.doi.org/10.1021/es9704996.

Smits, P.H., 1996. Post-application persistence of entomopathogenic nematodes. Biocontrol Sci. Technol. 6 (3), 379–388. http://dx.doi.org/10.1080/09583159631352.

Steinberg, S.M., Kreamer, D.K., 1993. Evaluation of the sorption of volatile organic compounds by unsaturated calcareous soil from Southern Nevada using inverse gas chromatography. Environ. Sci. Technol. 27, 883–888. http://dx.doi.org/10.1021/es00042a010.

Stuart, R.B., Barbercheck, M.E., Grewal, P.S., 2015. Entomopathogenic nematodes in the soil environment: distributions, interactions and the influence of biotic and abiotic factors. In: Nematode Pathogenesis of Insects and other Pests. Raquel Campos-Herrera (Ed). Springer International Publishing, Switzerland. doi:10.1007/978-1-4419-7931-5.

Stuart, R.J., Barbercheck, M.E., Grewal, P.S., Taylor, R. a J., Hoy, C.W., 2006. Population biology of entomopathogenic nematodes: concepts, issues, and models. Biol. Control 38, 80–102. http://dx.doi.org/10.1016/j.biocontrol.2005.09.019.

Turlings, T.C.J., Hiltpold, I., Rasmann, S., 2012. The importance of root-produced volatiles as foraging cues for entomopathogenic nematodes. Plant Soil 358, 51–60. http://dx.doi.org/10.1007/s11104-012-1295-3.

Turlings, T.C.J., Tumlinson, J.H., Lewis, W.J., 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. Science 250, 1251–1253. http://dx.doi.org/10.1126/science.250.4985.1251.

Wang, Y., Gaugler, R., 1998. Host and penetration site location by entomopathogenic nematodes against japanese beetle larvae. J. Invertebr. Pathol. 72, 313–318.

Web References

EqWorld 2015 (accessed in March 2015) ⟨http://eqworld.ipmnet.ru/en/solutions/lpde/diffusion.pdf⟩.

Plant and Soil Sciences elibrary, 2014 (accessed in August 2014) ⟨https://passel.unl.edu/pages/informationmodule.php?Idinformationmodule=1130447039 & topicorder=2 & maxto=10 & minto=1⟩.