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Short communication

Persistence of fenhexamid in the nutrient solution of a closed cropping system

Beatrix W. Alsanius[a,*], Karl-Johan Bergstrand[a], Stephen Burleigh[a], Nicolas Gruyer[b], Anna Karin Rosberg[a]

[a] Swedish University of Agricultural Sciences (SLU), Department of Biosystems and Technology, Microbial Horticulture Laboratory, PO Box 103, SE-230 53 Alnarp, Sweden
[b] Horticultural Research Center, Université Laval, G1V 0A6 Québec, Québec, Canada

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ABSTRACT

There are concerns about emissions of plant protection products (PPP) from protected cultivations, including high-tech production systems. Modern high-tech greenhouse horticulture is performed in cropping systems with closed water and nutrient loops, so PPP residues are not leaked to the external environment, but are captured and may accumulate within the recycled greenhouse solution. In this pilot study, the botryticide fenhexamid (FEX; Teldor WG50®) was used as a model PPP and incubated (0.01 times the recommended concentration spray solution) in the colonized nutrient solution from a closed tomato production system in darkness at 20 °C. Samples were taken after 0 h, 24 h, 48 h, 96 h, 192 h and 504 h (0, 1, 2, 4, 8 and 21 days) of incubation to monitor FEX concentration, dissolved organic carbon content (DOC), electrical conductivity, pH and microbial dynamics. Culture-dependent (viable counts for general bacterial, fungal flora and fluorescent pseudomonads on stationary agar, with/without supplement of FEX) and independent (denatured gradient gel electrophoresis, PCR-DGGE) methods were used for microbial analyses.

DOC and FEX concentration did not change during incubation, whereas pH and electrical conductivity increased significantly. Density of culturable fungi decreased from the start of incubation, while density of culturable bacteria declined in late incubation. Fluorescent pseudomonads declined in early incubation but increased significantly at later stages. Under nutrient-deprived conditions, density of culturable bacteria decreased during the course of incubation. Microbial community analysis by DGGE supported these findings. Differences between the density of culturable bacteria assessed on semi-selective media with and without FEX supplementation were low. However, addition of FEX to diluted malt extract agar negatively affected the density of culturable fungi.

1. Introduction

Emissions of plant protection products (PPP) from protected cultivations, including high-tech production systems, have become a cause for concern in recent times (EFSA, 2012). Environmental recipients of PPP emitted from protected horticultural systems include the surrounding air, groundwater and surface water. The quantities emitted are a function of product type, greenhouse construction type, emission route, crop type, design of the cropping system and PPP application technique. Depending on the pest or disease to be controlled and the product's mode of action, PPP may be either applied to the canopy (spray or fogging) or directly to the growing medium or soil by drip irrigation.

In soil-bound protected crops with open water loops, PPP are released to the soil, surface water and groundwater. High-value greenhouse vegetables such as cucumber, tomato and sweet pepper are commonly grown in soilless culture, i.e. either solid inorganic or organic growing medium or liquid hydroponics, for four to eleven months. In these systems, dissolved fertilizers are supplied intermittently via the irrigation water and in order to prevent eutrophication of littoral zones and standing and running waters, the water loop and associated nutrient loop should be closed (Ehret et al., 2001). In closed systems, the nutrient solution is recollected in either open or closed troughs, complemented with nutrients and water in relation to depletion and recycled to the greenhouse crop (Alsanius, 2011). In these systems, PPP residues may be introduced to the closed water loop either through run-off from canopies and deposition on the growing medium or greenhouse floor after aerial application, or through direct injection (Vermeulen et al., 2010). Residual PPPs can thus be expected

* Corresponding author. Tel.: +46 40 415336; mobile: +46 767680496.
E-mail address: beatrix.alsanius@slu.se (B.W. Alsanius).

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to accumulate in the reused nutrient solution unless they are degraded.

The nutrient solution of closed cropping systems is intensively colonized by microbes in the presence of plants (Berkelmann, 1992; Khalil and Alsanius, 2001). Microbial utilization of organic compounds has been demonstrated (Alsanius and Jung, 2004). The starting hypothesis for this study was therefore that pesticides used in closed hydroponic greenhouse systems to counteract foliar diseases do not persist in the nutrient solution beyond the waiting period.

In the study, we monitored the fate of the botryticide fenhexamid (FEX) in microbally-colonized nutrient solution from a mature commercial tomato crop. When applied to greenhouse tomato in Sweden, FEX has a waiting period of three days (Bayer, 2009; Krüger et al., 1999). We monitored abiotic and biotic factors and the occurrence of FEX in the test solution during a period of 21 days under laboratory conditions.

2. Materials and methods

Teldor WG50® (Bayer, 2009) based on the active substance fenhexamid (50%, w/w) (*N*-(2,3-dichloro-4-hydroxyphenyl)-1-methyl-cyclohexanecarboxamide; CAS number: 126833-17-8) was the model botryticide in the present study.

2.1. Preliminary study

Appropriate sampling intervals and concentrations were determined in a preliminary study. Nutrient solution was collected from a 18-week-old commercial tomato crop. Aliquots of 1 L were transferred to sterile 1-L glass flasks and supplemented with 0.075 g FEX L^{-1}; which corresponds to 0.1 times the concentration of the spray solution used in a tomato crop. The solution was statically aerated and incubated at 20 °C in darkness for 21 days (504 h). Samples were taken directly after addition of the pesticide and after 48 h, 96 h, 148 h, 192 h, 240 h and 504 h (0, 2, 4, 8, 10, 21 days). As the degradation of FEX was very low, its concentration was adjusted to 0.01 times the concentration administered in a spray solution.

2.2. Main study

Nutrient solution was sampled from the drainage system of a commercial closed hydroponic greenhouse tomato crop. The nutrient solution had been recycled since the start of the cropping season, 24 weeks earlier. The samples (1 L) were transferred to sterile 1-L bottles and supplemented with 0.0075 g of FEX corresponding to 0.01 times the recommended concentration in a spray solution. The flasks were sealed with sterile caps and connected to a custom-made aeration system consisting of a sterile and a non-sterile part. The sterile part, inside the bottle, consisted of a 152 mm long sterile syringe (⌀, 1.2 mm; 408360, BD Needles, Franklin Lakes, NJ, USA). Outside the bottle, a sterile membrane filter (pore size: 0.22 μm; ⌀, 13 mm; Acrodisc 4454, Pall Corp., Port Washington, NY, USA) was mounted on the flasks and connected to an air pump (Rena 603, Mars Fishcare Inc., Chalfont, PA, USA). Aeration of the nutrient solution during incubation provided steady stirring of the solution and ensured that aerobic conditions prevailed during incubation. The flasks were incubated for 21 days (504 h) at 20 °C in darkness. Samples were taken directly after the start of the experiment and at 24 h, 48 h, 96 h, 192 h and 504 h after addition of the pesticide. The experiment was performed with six independent replicates per sampling event.

2.3. Analyses

Samples were analyzed with respect to FEX content, dissolved organic carbon (DOC), electric conductivity and pH. Furthermore, the dynamics of microbial community structure were analyzed using denaturing gradient gel electrophoresis (DGGE).

2.3.1. Chemical analyses

Fenhexamid was eluted using ammonium formiate solution and methanol. It was detected and quantified using ultra performance liquid chromatography tandem mass spectrometry (UPLC–MSMS; (Waters Aquity UPLC system–API 5000 tandem quadrupole mass spectrometer, Applied Biosystems; column: Acquity UPLC HSS T3 1.8 μm, 2.1 × 150 mm; retention time: 9.72 min) coupled to a five-point matrix matched standard curve.

For quantification of DOC, the samples were membrane-filtered (⌀; 0.2 μm, Acrodisc® 0.2, Pall Corp., USA) and analyzed using the Hach-Lange test kit LCK 385 (Hach-Lange AG, Düsseldorf, Germany). Dissolved CO_2 in the test solution was eliminated before incubation by adding peroxide for 120 min at 100 °C. The DOC content was then determined spectrophotometrically (Hach-Lange Xion).

Electrical conductivity and pH were measured at each sampling event (Mettler-Toledo Seven Multi, Mettler-Toledo Inc., USA).

2.3.2. Microbial analyses

Viable counts were performed on stationary agar with and without 0.01 FEX supplement using diluted malt extract agar (MA; 10 g malt extract (Bacto™ 218630) and 20 g Bacto Agar (Difco 0140-01) per L of ultrapure water), R2A agar (Difco 218263), fluorescent pseudomonad agar (King's Agar B, KB; King et al., 1954) and water agar (WA; 20 g agar (Merck 1614) per L ultrapure water). Samples were serially diluted and triplicate samples were plated by WASP II spiral plater (Microbiology International, USA). Incubation conditions are shown in Table 1. Fluorescent pseudomonads were counted under UV-light (UVC, Spectroline EA 160/FE, Spectronics Corp., Westbury, NY, USA).

2.3.3. Phylogenetic diversity

For molecular characterization of the phylogenetic diversity, 1 L of nutrient solution of each replicate at each sampling event was filtered using a disposable 1-L 0.45 μm PES filter (Cat. No. 514-0605, VWR International AB, Stockholm, Sweden). The filter deposit was suspended in 0.85% salt buffer, transferred to a sterile 1.5-mL microtube, pelleted at 3000 rpm for 3 min using a Minispin microfuge (Eppendorf AG, Hamburg, Germany) and frozen at −80 °C until DNA extraction using a DNeasy Plant Mini Kit (Cat. No. 69104, Qiagen GmbH, Hilden, Germany). To characterize the total bacterial community, 16S rRNA genes were first amplified. The forward primer F968 was AACGCGAAGAACCTTAC and the reverse primer R1378 was CGGTGTGTACAAGGCCCGGGAACG. The PCR reaction consisted of approximately 300 ng of template DNA, measured using a Implen P330 nanophotometer (Implen GmbH, Munich, Germany), 1 × Maxima Hot Start Taq buffer (Cat. No. EP0601, ThermoFisher Scientific AB, Gothenburg, Sweden), 3 mmol L^{-1} $MgCl_2$, 2.5% deionized formamide (Cat. No. F9037, Sigma–Aldrich AB,

Table 1
Incubation conditions and length on different stationary agar media with and without addition of fenhexamid, used in the current assay.

Medium (w and w/o addition of fenhexamid)	Incubation temperature (°C)	Incubation length (h)
King Agar B	25	48
R2A	25	72
Diluted malt extract agar	25	96
Water agar	25	168