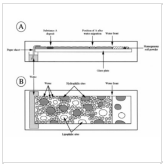

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Soil thin-layer chromatography

Andreea S[1]

[1]University of Groningen

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W.H. Molenkamp

ABSTRACT

In order to get a relative estimation of the diffusion of our neuropeptide in the soil we can use a soil thin-layer chromatography. In this experiment, developed by Helling & Turner in 1968, a thin-layer chromatography apparatus is utilized to mimic the environment conditions to a certain extent. Using C14 labeled glycines we can use X-ray to determine the distance traveled by the peptide. Use of a small his-tag is unreliable due to the small length of the peptide. As a control we will use two C14 labeled compounds common in the exudate of the plant: Glucose and Glutamic acid. Glucose is considered to diffuse well in the soil whereas glutamic acid has a lower diffusion coefficient (cm/h).

Protocol is adapted from Ravanel and colleagues (1999).

P. Ravanel, M.H. Liégeois, D. Chevallier, M. Tissut (1999). Soil thin-layer chromatography and pesticide mobility through soil microstructures. New technical approach. Journal of Chromatography.
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chromatography. In this experiment, developed by Helling & Turner in 1968, a thin-layer chromatography apparatus is utilized to mimic the environment conditions to a certain extent. Using C14 labeled glycines we can use X-ray to determine the distance traveled by the peptide. Use of a small his-tag is unreliable due to the small length of the peptide. As a control we will use two C14 labeled compounds common in the exudate of the plant: Glucose and Glutamic acid. Glucose is considered to diffuse well in the soil whereas glutamic acid has a lower diffusion coefficient (cm/h).

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1 **Preparation of the soil samples.** *Several soil samples are collected from potato fields in the area of Groningen & Numansdorp, the Netherlands. The soil from Groningen is predominantly sand whereas the soil from Numansdorp is clay.*

1.1 After collection of the soil samples, they are air-dried and sieved through a **2 mm** sieve screen before being powdered with an electric mill. The powder obtained is sieved through a **100 µm** mesh screen. Before being pulverized and sieved (**100 µm**), the schists were manually broken with a hammer.

1.2 **30 g** of powdered substrate are suspended in a dioxanwater (1:1, v/v) solvent to make a slurry which is spread as a 0.7 mm thick layer on a 20 x 20 cm glass plate with the help of a thin-layer spreader. The plates are dried at Room temperature and stored until being used for chromatographic tests. When necassary, pyrolysed matrices were obtained after a 3-day period in an oven at **600 °C** .

2 **C14 Isotope labeling compounds.** *In order to detect the diffusion of the samples without affecting its structure, we are isotope labeling the compounds and, eventually visualizing them usign autoradiographic films. Three compounds are used: Glucose, glutamic acid & NLP14a.*

3 **Performing the thin layer chromatorgraphy.** *The samples are loaded on the soil plate and by the movement of the water the compound are horizontally transferred to the other end of the plate. See the graphical abstract for overview.*

3.1 Approximately 50 000 dpm of each ^{14}C-labelled compound is spotted with a micro-syringe at **2.5 cm** from the bottom edge of the soil plate. After depositing the spots (distance between two spots = **2.5 cm**), the plates are allowed to develop in a closed plastic chamber using distilled water as a solvent.

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3.2 A sheet of filter paper dipping into the developing solved fed solvent continously to the substrate at the base of the plate, thus leading to a relatively uniform flow. During the course of the experiment, the whole device is kept at a perfect horizontal position.

3.3 Solvent migration occured at a distance of **17.5 cm** from the baseline. The plates are then dried at room temperature. In total, the migration will last between 2.5 and 9 hours.

3.4 Autoradiographic films are applied to the dried plates for **72:00:00** . The distances covered by the products on the thin layer compared to that covered by water are measured on the radiochromatograms.

From the experiments we can determine the relative diffusion of the NLP from the potato plant. According to previous research, root exudate are measured up to **1.2 mm** away from the plant but the spread may be even further since organisms can "sense" the plant's exudate up to 10 cm away. Most likely, it is all dependent on the environmental conditions. By taking a compound from the plant's exudate that diffuses far away from the plant (Glucose) and one that stays close (Glutamic acid), we have two controls to compare the diffusion of our NLP to.

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