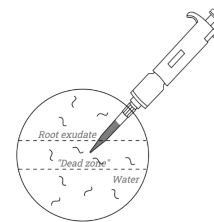


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Chemosensory assay

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Protocol status: Other

The protocol is developed based on literature, but has not been tested yet.

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Abstract

Neuropeptide-like proteins (NLPs) are a subclass of neuropeptides utilized by the nervous system of the nematodes. Altering the levels of the NLPs affects the behaviour of the neuronal networks in the nematode leading to a changed behavioural output. With this experiment, the behaviour of the *Globodera pallida* towards the root exudate of the potato plant is studied in a simple agar plate experiment. This chemosensory experiment is retrieved from the paper of Warnock and colleagues from 2017.


CITATION

Neil D. Warnock, Leonie Wilson, Cheryl Patten, Colin C. Fleming, Aaron G. Maule, Johnathan J. Dalzell (2017). Nematode neuropeptides as transgenic nematocides. PLoS Pathogens.

LINK

[10.1371/journal.ppat.1006237](https://doi.org/10.1371/journal.ppat.1006237)









Globodera Pallida Maintenance

- 1 **The nematode *Globodera pallida* is currently on the quarantine list of the EU and experimental work with this nematode is only allowed in a biosafety level 2 lab (BSL-2).**
- 1.1 *Globodera pallida* will be grown on a potato plant cultivar. The soil surrounding the potato roots will be collected and made moisture free by drying for seven days, sieve washed to collect the nematode cysts.
- 1.2 The cysts will be incubated in the potato root exudate in  17 °C in the dark till the nematode juveniles hatch. The freshly hatched infective juveniles (J2) are to be used for the chemosensory assay.

NLP production

- 2 The neuropeptide-like proteins (NLPs) for this experiment will be ordered online. As starting point, the NLP14A will be purchased. If non-effective, other NLPs will be assessed.



Preparation chemosensory assay

- 3 **The chemosensory assay towards the root exudate will be conducted on agar plates. This experiment will allows us to study the effect of the NLP on the behaviour of the nematode towards the potato root exudate.**
- 3.1 By pouring  500 mL of ddH₂O through the soil three times, root exudate will be collected from 10 potato plants, aged 3 to 6 weeks in  1 L pots. Exudate from each plant will be combined, filter sterilised and stored at  4 °C for not more than a month.
- 3.2 Root exudate agar plugs will be made by melting 1.25 % agar in ddH₂O, cooling to  50 °C before mixing with 4 parts of root exudate. The agar will then be solidified at  Room temperature .
- 3.3 A 60 mm petri dish is to be divided into two section, a negative and a positive side, with a 5 mm "dead zone" on either side. The petridish will be filled with  15 mL of 0.25 % weight/volume agar and then solidified.  3 mL of 0.25 % w/v agar slurried in spring water at  7 (prepared previously by stirring with a magnetic stirrer for several hours

to obtain a smooth consistency) will be added to the petri dish and spread evenly over the surface. The attractant, root exudate and the control (water), 0.25 % agar plugs are to be embedded in the agar slurry, on either side of the assay area.

Chemosensory assesment

4 By inoculating the nematodes in the NLPs and placing them in the "deadzone" of the agar plates, we are able to assess whether the nematodes show repellent behaviour towards the potato root exudate located at one side of the petri dish.

4.1 Before the start of the chemosensory experiment, the *Globodera pallida* nematodes are to be incubated for  24:00:00 in  200 µL for each peptide in a 24 well plate at a determined concentration.

4.2 100 *Globodera pallida* J2 juveniles will be added by pipette to the centre of the plate. The nematodes who will move out of the "dead zone" after 3 hours will be counted and their location scored. The distribution of the nematodes will be used to create a chemotaxis index for each plate. This experiment will be conducted 10 times.

Expected result

The nematodes are expected to show altered behaviour towards the potato root exudate due to the effect of the NLPs. Whereas some NLPs will cause a more attractant behaviour towards the root exudate, others will cause a repellent effect.

Citations

Neil D. Warnock, Leonie Wilson, Cheryl Patten, Colin C. Fleming, Aaron G. Maule, Johnathan J. Dalzell. Nematode neuropeptides as transgenic nematocides

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