LIVE FAST DIE YOUNG

Investigating nematode feeding behaviour using Demeter

DEMETER 1.1

Introduction

Plant parasitic nematodes parasitise every major crop[1], resulting in the destruction of 8.8-14.6% of global agricultural produce[2]. No perfect control measure exists; combining approaches to balance effectiveness, cost, regulatory compliance, and development time is the best way to control nematode damage. Exploring new routes for control can enrich this combination. A more complete understanding of host-pathogen interactions enables the development of more effective means of control.

Observation

Saswata Dey (PhD, Dpt. Plant Sciences) developed betalain expressing A. thaliana lines with a pink appearance and noticed that nematodes can take up this substance and break it down over time (Fig. 1). At any given time, some nematodes are pink, and some are not, and over time some nematodes appear to alternate between pink and white repeatedly.

Biological material

Heterodera schachtii (the beet cyst nematode) and *Arabidopsis thaliana* have a well-documented life cycle and genome and are easily cultured in the lab. Here, a genetic mutation (RUBY-10-01) causes A. thaliana to express betalain protein in its roots, giving a strong pink colour.

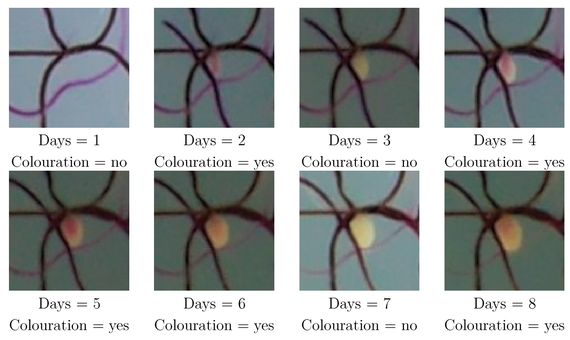

Figure 1: Observed pink colour acquisition and loss for H. schachtii over 8 days on A. thaliana RUBY-08-03.

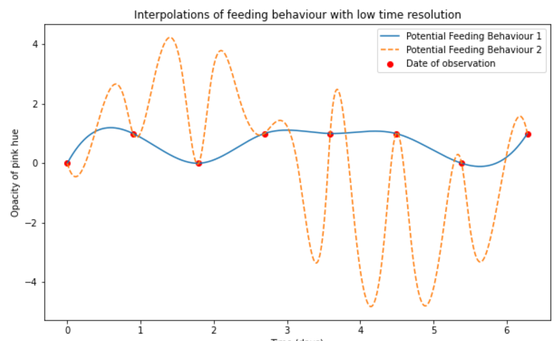

Figure 2: Variable extrapolations of feeding cycles using low time-resolution data

Hypothesis

Using pinkness as an indicator for feeding, tracking the occurrence of feeding cycles over nematode lifespan may demonstrate **higher frequency feeders reach maturity and moult faster** than their peers.

Engineering Need

Low time-resolution images cause aliasing of feeding cycle signal (Fig. 2). Existing equipment can only image 1/day. A high time-resolution device (image 1/hour) must automate this task. The device must not interfere with existing axenic culture methods. The output images must be of high quality to maximise data extraction in post processing. Digitisation of the data allows future analysis with more highly developed tools. In the meantime, a semi-automated image processing pipeline is developed to track nematode coloration in HSV colour space. See Figs. 3 and 4 for fabrication and logic diagrams.

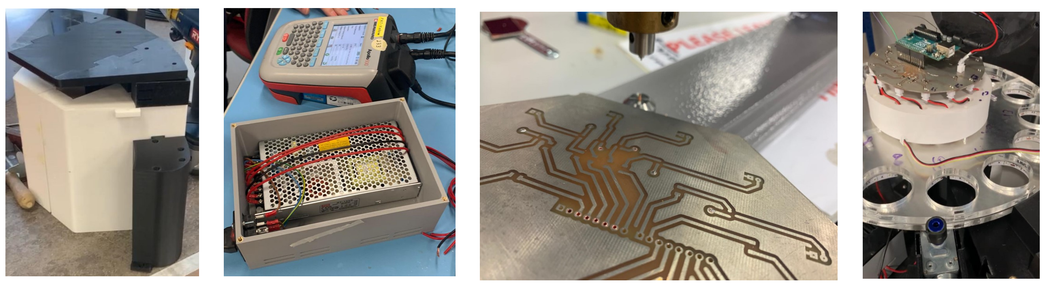

Figure 3: Fabrication methods – 3D printing, PAT tested power supply, PCB manufacture, I2C interface.

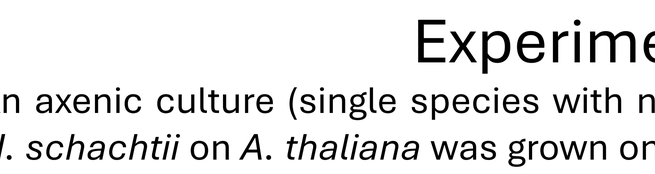

Figure 4: Logic flow diagrams for (left) normal operation and (right) index calibration

Experiment

An axenic culture (single species with no contaminating organism) of *H. schachtii* on *A. thaliana* was grown on water agar solution in 50 mm petri dishes. The dishes underwent imaging for 21 days to produce a dataset of over 50,000 images. Some images could not be used due to inappropriate quality; device improvements were considered.

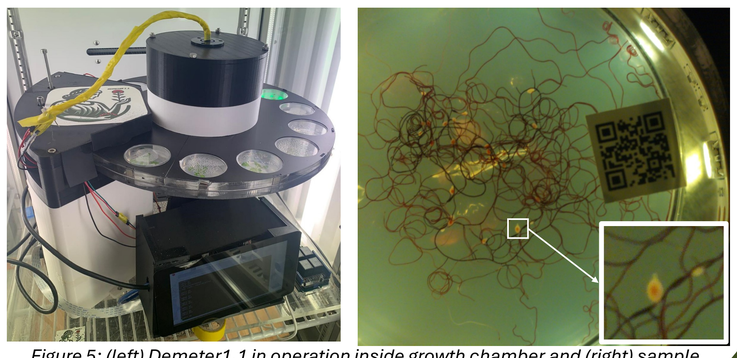

Figure 5: (left) Demeter1.1 in operation inside growth chamber and (right) sample image from experiment.

Results

Using the produced dataset and image processing pipeline, the changing pinkness of nematodes could be quantified over time (Fig. 6). However, no cyclical feeding behaviour was observed, with most nematodes either staying pink once acquired (41%), or never turning pink (45%).

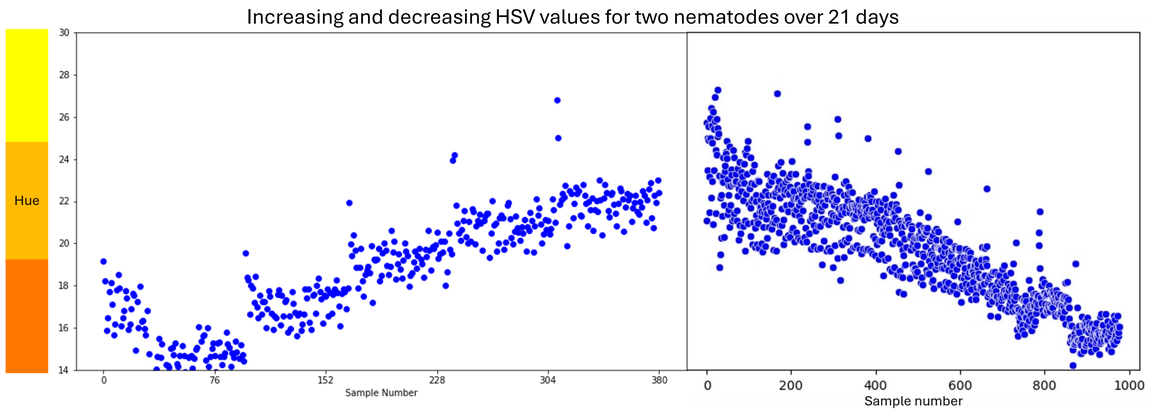

Figure 6: Increase and decrease HSV values for nematodes, showing decrease and increase pinkness respectively.

Discussion

The results demonstrate that the imaging device and processing pipeline have the capability of quantitatively measuring pinkness (in the form of HSV hue value) over time. However, an element of the experimental setup has altered the colouration behaviour seen in Saswata Dey's images, and no cycles of feeding could be observed. Possible culprits include the use of RUBY-10-01 as opposed to RUBY-08-03 (the latter has lower probability of silencing the betalain expression), or the impact of flashing lights that enable imaging during the plants' circadian sleep cycles.

It was noted that the disruption of nematode feeding cycles by the setup is worth investigating to uncover a possible control method in the future. Furthermore, some nematodes were observed to grow without ever acquiring pink colouration, also raising questions on feeding and metabolism.

Conclusion

This work has developed novel imaging technology for high time-resolution use in nematological study, and a method for quantitatively measuring nematode colour over time has been produced. No cyclical behaviour in feeding could be observed due to shortfalls in the experimental setup, and therefore a relation between feeding frequency and time to moulting could not be demonstrated.

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References:

[1] Nicol, J. M. et al. Current Nematode Threats to World Agriculture. Genomics and Molecular Genetics of Plant-Nematode Interactions 21–43 (2011) doi:10.1007/978-94-007-0434-3_2.

[2] Singh, S. K., Hodda, M. & Ash, G. J. Plant-parasitic nematodes of potential phytosanitary importance, their main hosts and reported yield losses. EPPO Bulletin 43, 334–374 (2013).