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Using Light Brown Apple Moth, *Epiphyas postvittana*, for testing environmental DNA detection of insect pests from diverse plant species

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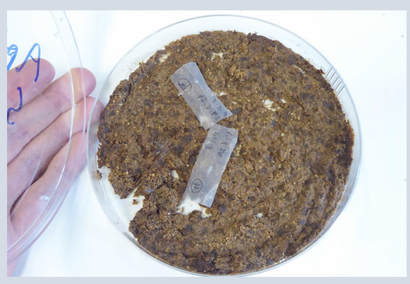
Summary

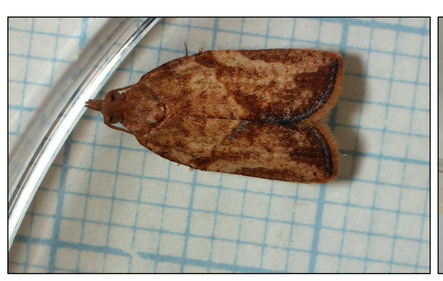
Environmental DNA (eDNA) can be used to detect invasive alien species and plant pests and has been proposed as a useful tool for biosecurity inspections of horticultural products. In this study I am testing eDNA approaches for detecting the Light Brown Apple moth, *Epiphyas postvittana*, on foliage. This is a non-native species in the UK, native to Australia.

This species is polyphagous and can be reared easily. This makes it an ideal organism to use to test eDNA sampling approaches on a wide variety of plants in replicated experiments. By rearing the species it is possible to carry out controlled eDNA experiments comparing sampling approaches from a range of types of plants with a consistent method. This will help optimise eDNA sampling for similar pests and identify opportunities to apply eDNA sampling to different pest introduction pathways.

Laboratory rearing of *E.postvittana*

A laboratory stock of *E.postvittana* was established by breeding from wild-caught pupae and setting up a culture on an artificial diet based on animal feed, water and other dry ingredients, reared in controlled conditions environmental conditions to ensure a plentiful supply of caterpillars for experiments. This stock could be used for a wide range of plant health experiments.





Detection/ diagnosis method

Barr et al published a probe-based real-time PCR assay for diagnosis of *E.postvittana* (Barr et al 2011). In the existing publication this assay was validated for diagnosis of samples in North America. Further testing will be carried out to:

- Ensure no false positives are generated by British tortricidae
- Test whether the assay is sensitive enough for use in environmental DNA in the form of water samples and extracts from foliage with feeding signs

(Barr, N. B., et al. "A multiplex real-time polymerase chain reaction assay to diagnose Epiphyas postvittana (Lepidoptera: Tortricidae)." Journal of economic entomology 104.5 (2011): 1706-1719.)

Planned herbivory detection experiments

Brief outline:

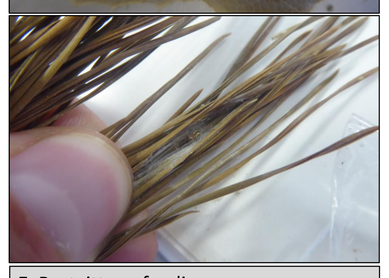
1) Set up laborotory stock and rearing method
2) Transfer larvae to plants for experiments
3) Test assay by Barr et al for sensitivity to environmental DNA and compare sensitivity on different plants

E.postvittana is capable of feeding on hundreds of diverse plant genera globally and surviving a wide range of environmental conditions. This flexibility will be harnessed in these experiments, to conduct experiments on a wide range of unrelated and dissimilar plants and compare eDNA sensitivity.

For example sensitivity of methods could be compared between:

- Woody plants and herbaceous plants
- Broad leaved trees and conifers
- Plants grown in different temperatures, greenhouses compared to outdoors

The adaptability of this species and efficient rearing method opens many opportunities for other experiments.



E. Postvittana feeding on Chrysanthemum (top), Mahonia (middle) and Scot's pine (bottom)

Objectives

- **Compare of eDNA detection methods** for conifers, broadleaved woody plants and herbaceous plants, starting with plants of importance to horticultural trade and biosecurity.
- **Design a potential model system** with a laboratory culture and environmental DNA method for experiments on detection of herbivorous insect pests
- **Highlight opportunities** for effective eDNA survey strategies
- **Identify challenges** to eDNA application can highlighted

Applications

- **Surveillance** for insect pests and invasive insect herbivores
- Targeting further **environmental DNA research**
- Research on other **insect detection methods**

References and further information: Singh, Pritam. "Aseptic rearing of Epiphyas postvittana (Lepidoptera: Tortricidae) on a meridic diet." New Zealand journal of zoology 1.1 (1974): 111-117.. Danthanarayana, W. "The bionomics, distribution and host range of the light brown apple moth, Epiphyas postvittana (Walk.)(Tortricidae)." Australian journal of zoology 23.3 (1975): 419-437.