

## Electrical Penetration Graph Recording (Whitefly)

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**[Abstract]** Electrical penetration graph (EPG) was first developed by Mclean and Kinsey (1964) and later modified by Tjallingii (1978 and 1985). The EPG system turns a phloem-sucking insects and its host plant into part of an electrical circuit that is completed when the insect's mouthparts penetrate the plant. The electrical signal is amplified by an amplifier and digitized by a converter. Fluctuations in voltage and electrical resistance are recorded and can be matched to specific feeding events. Current protocol is an efficient use of the EPG system to record whitefly feeding behavior on plants.

### Materials and Reagents

1. Plant (*e.g.*, 2-3 true leaf stage)
2. Newly-emerged (*e.g.*, 2-5 d old) female whiteflies

### Equipment

1. Ice-bag or box
2. Glass dish (Diameter = 6 cm)
3. Gold wire (Diameter = 12.5  $\mu$ m, <http://www.epgsystems.eu/products.php>)
4. Silver glue (Vial of 2ml glue, <http://www.epgsystems.eu/products.php>)
5. Faraday cage (self-made cage with 10 mesh wire net, length = 60 cm, width = 60 cm, height = 70 cm)
6. Insect-proof cage (self-made cage with 80 mesh nylon screening, length = 40 cm, width = 40 cm, height = 60 cm)
7. Transparent tubes (self-made tube with bottle, 3 cm long, inner diameter = 5 mm)
8. Small ice-chilled glass dish
9. Giga-8 DC-EPG system with  $10^9$  Ohm input resistance (Wageningen University, <http://www.epgsystems.eu/products.php>)
10. DI710-UL analogue-to-digital converter (DATAQ Instruments, <http://www.epgsystems.eu/products.php>)

11. Computer
12. Dissecting microscope

### **Software**

1. PROBE 3.4 software (Wageningen University, advanced version can be downloaded from <http://www.epgsystems.eu/downloads.php>)

### **Procedure**

1. The age of newly-emerged whiteflies should be estimated precisely. All adults are used within 2-5 d of their emergence.
  - a. They are obtained by picking a plant leaf infested with fourth-instar nymphs, removing any adults, and putting the infested leaf on an uninfested plant (of the same species) in an insect-proof cage.
  - b. After 24 h, remove the nymph-infested leaf and any excess nymphs; the only whiteflies remaining in the cage are adults that have emerged in the past 24 h. After 48 h, 2-3 d-old whitefly adults can be randomly picked for use in the experiment.
  - c. After 120 h, any remaining whiteflies are discarded.
2. Only female whiteflies are used because sexes might differ in their probing behavior. Adults are sexed by using transparent bottle tubes (3 cm long, inner diameter = 5 mm) to pick up individual whiteflies, with one whitefly per tube. Then a dissecting microscope is used to examine each whitefly in its tube in order to determine its sex.
3. A newly-emerged (2-5 d) female whitefly is immobilized in a small ice-chilled glass dish which is put on an ice bag or ice box beforehand.
4. Attach a 1.5 cm x 12.5  $\mu$ m gold wire to the whitefly's dorsum using a droplet of water-based silver glue (Figure 1).
5. The wired whitefly is then connected to the input of the Giga-8 probe and place on the lower surface of the bottom leaf of the appropriate host plant (Figure 1).
6. Each replicate whitefly-plant-probe combination is placed into an electrically-grounded Faraday cage to shield the setup from electrical noise.
7. Six to eight hours should be recorded according to the respective experiment objectives.
8. EPG signals are digitized with a DI710-UL analogue-to-digital converter and acquired with PROBE 3.4 software.
9. All experiments are carried out at  $26 \pm 2$  °C, 70% relative humidity, and under artificial light (1,500 lux) with a 14:10 h L: D regime.

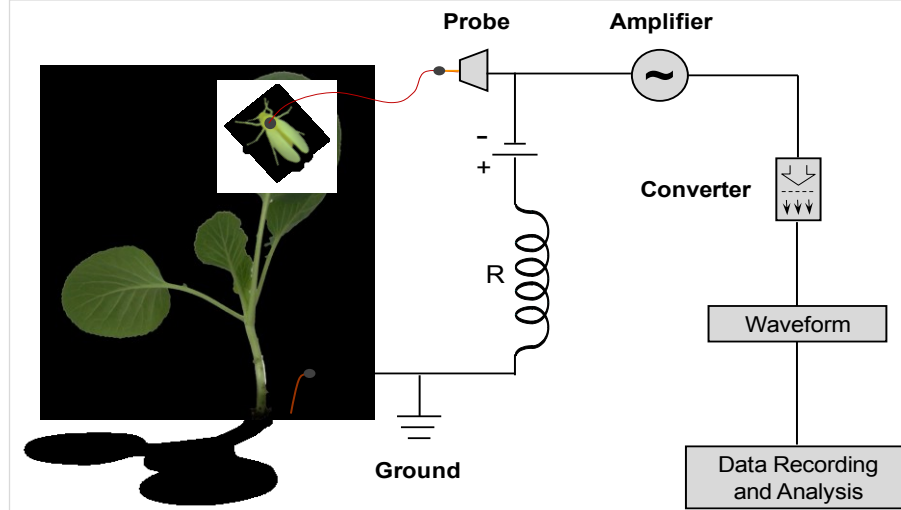


Figure 1. Schematic diagram of DC-EPG, especially for the whitefly-plant-probe combination when attaching the gold wire with silver glue (Liu *et al.*, 2013).

### Acknowledgments

This protocol is adapted from Liu *et al.* (2013).

### References

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