Are females attracted by wound volatiles to cane splits?

Early stages in the development of a plant derived attractant for raspberry cane midge.

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Background

The raspberry cane midge, *Resseliella theobaldi,* is an important pest of red raspberry (*Rubus idaeus*) in the UK and much of north and central Europe. The adult midge lays eggs in the splits of young canes, either caused by natural splitting or by mechanical injury. The larvae feed on the pith causing lesions which allow entry of a range of diseases such as cane blight fungus and midge blight.

There has already been a great deal of research into the development of female sex pheromone traps for local monitoring of male midges. However, advancement of this monitoring and mass trapping technology to aid the control of midge numbers requires an attractant to lure the females as they emerge from the soil in early spring (first generation) and during the second generation (coinciding with fruit harvest and the main time of fungal colonisation).

A system is being developed for entrainment of volatiles from localised regions of raspberry stems (immature plants) and canes (mature plants).

Methods

Entrainment of volatiles

Firstly, copper framed enclosures covered in plastic (PET) film (Figure 1), are positioned on the plant to isolate a region of air above the plant stem. Once positioned on the plants the enclosures were partially sealed. The enclosed space was flushed out with filtered air at about 200 ml/minute for 10 minute (Figure 2). The airline is removed and the enclosures sealed. Volatiles present in the vicinity of the stem are entrained using a solid phase microextraction (SPME) fibre which is inserted into the enclosure and exposed to the air. A fibre holder needle was pushed though a small perforation in the film and the fibre was exposed as close to and parallel with the stem or wound as possible. Entrainment times could be variable, but were initially set to 3 hours. Entrainments were carried out in pairs, one being an unwounded control plant and the other having a 2-3 cm manually created wound (Figure 3). On completion of entrainment, the SPME fibre was withdrawn into its protective sheath, and the fibre holder assembly was removed and fitted to the autosampler of the GC-MS system.

Figure 1 Sampling Enclosure - Copper support frames with PET film attached

Figure 2 Copper support frame/ film close around plant stem and flushed out with filtered air.

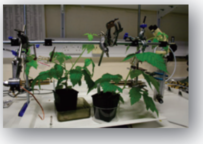


Figure 3 Sampling of a pair of plants, unwounded on left hand side, wounded on right hand side

Chemical analysis of entrained volatiles

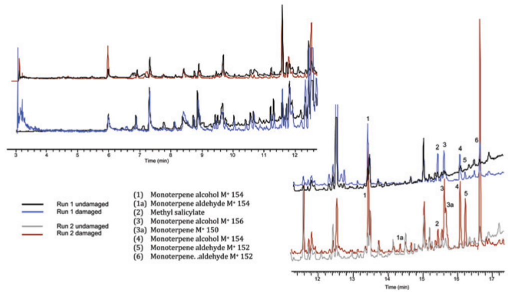
Separation of volatiles was effected on a DB 1701 GC column (30m x 0.32mm x 1.0 µm) using helium carrier gas at a flow rate of 1.2 ml/min. The GC -MS consisted of a ThermoFinnigan Tempus Time-of-flight (TOF) system operating at a data acquisition rate of 3 spectra/second. Data was acquired using the Xcalibur software package. Samples were desorbed for 2 minutes into a PTV injector assembly at 200 °C, operating in splitless mode.

Results and Discussion

The results from two replicate analyses of wounded and undamaged plants are shown in Figure 4. The profile of volatile compounds emitted from artificially wounded canes entrained on the SPME fibre are broadly similar for all four plants over the initial 13 minutes of the chromatographic separation.

There are, however, clear differences between the wounded and undamaged plants over the period 13-17 minutes in the chromatogram. The main difference is that there is an increase in the abundance of a suite of monoterpenes and also in methyl salicylate in the volatiles entrained from wounded plants, in comparison with unwounded controls. These terpenes appear to constitute a group of structurally related components, many of which are known to have behavioural effects on insects and plants (e.g. attract natural enemies) and/or are produced in response to insect herbivory.

Figure 4 Total ion chromatogram (TIC) traces of volatile compounds entrained from damaged and undamaged plants using SPME fibres.



Future research

Once the active attractant compounds are identified using GC-EAG, we intend to study the potential of enhancing the efficacy of cane midge sex pheromone traps (attracting males) by also deploying host-derived plant volatiles (attracting females). The overall aim is to develop IPDM tools for raspberry growers, who now need pest control strategies requiring minimal pesticide inputs.

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