Do adult *Manduca sexta (L.)* display chemical signatures of their larval host plants?

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Introduction

Many herbivorous insects are polyphagous in nature and their dietary hosts typically contain secondary plant substances, often with toxic characters. Despite the presence of toxic or other unusual chemicals such as non-protein amino acids, some insects may thrive upon these plants. Prime examples of this include the monarch caterpillar (feeds upon milkweed), the tobacco budworm (feeds upon cotton and tobacco), and the tobacco hornworm (feeds upon solenaceous plants).

Chemical signatures are markers from the larval host plant detectable upon or in the cuticle or in other tissues of the adult insect. but the markers are dependent upon the larval diet of the insect. These chemical signatures may be used to determine the host plant upon which the adult insect fed as a larva. The tobacco budworm (TBW) was at the focus of one study that explored chemical signatures. In the case of the TBW cotitine was determined to be a chemical signature of tobacco in the adult while gossypol was found to be a marker for cotton.

While it has been shown that TBW displays chemical signatures, this phenomenon may or may not occur in other insects. To determine if other insects might exhibit dietary markers of the larval insect in the adult, the tobacco hornworm (THW) was studied and reared upon different diets. The THW is a polyphagous insect that often feeds on solenaceous plants containing toxic alkaloids.

Objective

To determine if host plant specific chemicals ingested by the larval stage of *Manduca sexta* are retained within the wing cuticle of the adult stage.

Materials and Methods

In the experiment over 200 THW specimens were reared upon three different diets. The control group was reared upon artificial diet purchased from BioServ. The second group was reared on the same artificial diet but which had been spiked with 10 ppm of a selected broad spectrum of chemicals found in nature. The spiked chemicals included nicotine, caffeine, sinigrin, feurulic acid, p-coumaric acid, phytic acid, α-cyclodextrin, and tridecane. Each of the chemicals were diluted to 10 ppm in the 2L of water used in diet preparation. The protocol for diet preparation is found on the BioServ website. The third group was reared upon a natural diet of tobacco.

The rearing process for artificial diet involved incubating the THW eggs upon sliced diet in petri dishes. About a week after hatching the second instar larvae were transferred to larger community containers containing block diet and allowed to feed and develop to pupation in these chambers. After a week in the pupal stage, the pupae were moved to large emergence chambers built out of PVC and fine mesh. The rearing process for the tobacco fed THW involved placing THW eggs upon fully grown tobacco plants in enclosures and allowing them to complete their natural life cycle.

Three days after the adults emerged they were frozen at -80˚C and stored until later analysis. For analysis one pair of wings were removed from the adult and placed into a micro-test tube filled with 500 mL of dichloromethane (DCM). The micro-test tube was then vortexed for approximately one minute. The DCM extract was filtered using a micropipette and glass wool, in order to remove residual wing scales.. The extract was then analyzed using gas chromatography mass spectrometry. Parameters for the g.c. mass spec involved holding the injection port at 250˚C. The oven was held at 40˚C for 7 minutes and then ramped to 250˚C at 5˚C per minute with a hold for 5 minutes at 250˚C.

Results

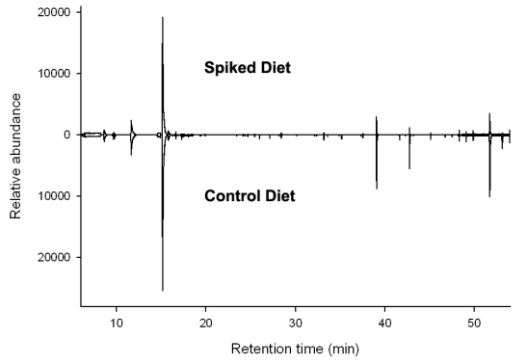

Fig 1: Gas chromatogram depicting the chemical content of a wing extract from an adult reared upon spiked diet being compared against wing extract from an adult reared upon the control diet.

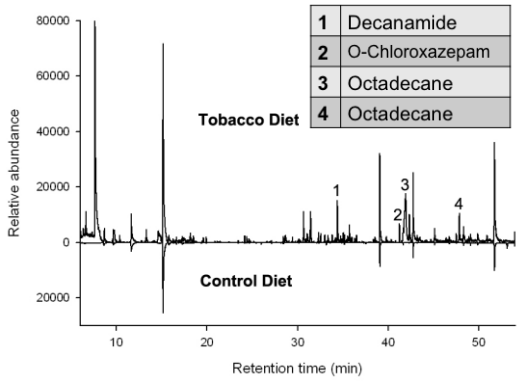

Fig 2: Gas chromatogram depicting the chemical compound content of a wing extract from an adult reared upon the natural tobacco diet compared against a wing extract from an adult reared upon the control diet. The table highlights the differences between the two extract samples.

Discussion

The wing extract from an adult reared upon the spiked diet and the wing extract from an adult reared upon the control diet showed no significant differences when compared via gas chromatography/mass spectroscopy and is depicted in **Fig 1**. The lack of differences between the two samples may be a result of using too low concentration of the spiked chemicals in the diet preparation. Also the spiked chemicals may not have been uniformly mixed during diet preparation. In order to adjust for this a higher concentration of the spiked chemicals will need to be used in future experiments.

The wing extract from an adult reared upon the natural tobacco diet and the wing extract from an adult reared upon the control diet showed several significant differences when compared via gas chromatography mass spectroscopy and is depicted in **Fig 2**. While none of the different compounds detected involved nicotine or any known metabolites various nitrogenous compounds were detected. The result provides evidence that *Manduca sexta* does display chemical signatures of host plant.

To explore the phenomenon of chemical signatures further the following approaches may be taken. Primarily the new study should introduce more host plants and analyze additional body parts of the adult insects. Furthermore, different insects should be introduced to the study, as well as finding examples in nature. Wild *M. sexta* can be collected and analyzed, as well as, bagworms, which stay on the same host throughout their life cycle and can be easily collected.

References

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