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Study of insect metamorphosis by means of Raman spectroscopy

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Abstract

Raman spectroscopy is a unique non-invasive method for biological research. The method provides detailed information about the molecular composition of living organisms without their destruction. We were the first to harness Raman spectroscopy for studying such complex and obscure stages of insects as diapause and metamorphosis. Insects *Aulacidea hieracii* transform through several stages during their development: egg, larva, pupa, adult insect (imago). The most interesting and the understudied stages of any insect are larva and pupa. In the larval stage insect sometimes enters diapause – a sort of a “sleep” that enables them to sustain unfavorable environmental conditions in a certain period of time [1,7]. After passing through diapause, the organism "wakes up" and metamorphosis begins - the transformation into a pupa, and then into an adult insect. The Raman spectroscopy for the first time allowed us to visualize the molecular processes occurring inside insects. We revealed information about the presence, composition, concentration and movement of such vital molecules as carotenoids inside insects without harm to them. Every single experimental animal survived and developed into healthy adults. The obtained data are of significant scientific interest in the topics of aging and life extension, as well as the application of the Raman method in studies of living organisms.

Introduction

The gall-forming insect *Aulacidea hieracii* forms a gall on a plant *Hieracium x robustum*. A gall is a tumor on a plant. Inside the gall, a gall-forming larva develops, which stops feeding and hibernates for 8 months without leaving the gall (Fig.1).
The larva becomes outwardly motionless, but what happens inside it?

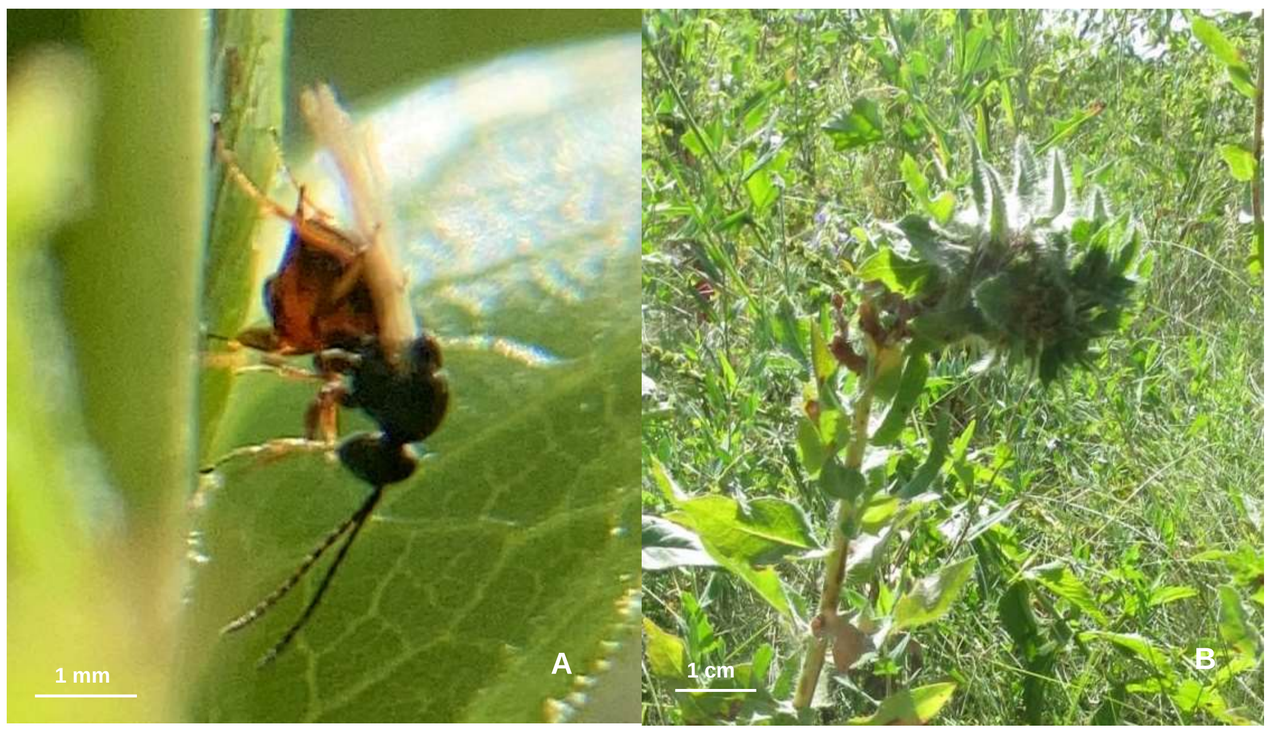

Fig. 1 *A* – Imago of *A. hieracii, B* – Gall formed by *A. hieracii.*

Research Aim

To non-invasively study the molecular composition of larvae and pupae of the gall-former *A. hieracii* by means of Raman spectroscopy.

Fig. 4 Diapause starts when it gets cold. The *A. hieracii* larva is motionless in winter and does not change morphologically. It is hard to imagine that there may take place active molecular processes inside such a larva.

Fig. 5 The metamorphosis begins after the diapause – the pupa is being formed. During the metamorphosis the body shape is changing, the pigmenting begins, first, the eyes are colored in red. It is considered that particularly carotenoid pigments are responsible for the coloring.

Methods

For our aim, we have applied for the first time the method of Raman spectroscopy (RS). The main advantage of the Raman method is its non-invasiveness, since only light affects the object and nothing else, and, as the result, we get a large amount of information. Raman spectra were recorded using a Renishaw inVia Raman microspectrometer (UK) microscope with a 532nm laser.

Fig. 2 Raman spectroscopy allows to see different functional groups of molecules: C-C, C=C *и* C-CH3 bonds in carotenoids.

Fig. 3 Nikelshparg M. during workflow.

Results

1. As a result of the study, we found that carotenoids are present in the larvae, the composition of carotenoids changes during diapause.

2. After emerging from hibernation, the larva starts metamorphosis - the transformation into an adult insect - imago (Fig. 5).

For the first time, we were able to register carotenoids inside a living insect during metamorphosis. In *A. hieracii* pupae, the largest amount of carotenoids is concentrated not in the red eyes, as we expected, but in the legs and wings primordia (Fig. 6).

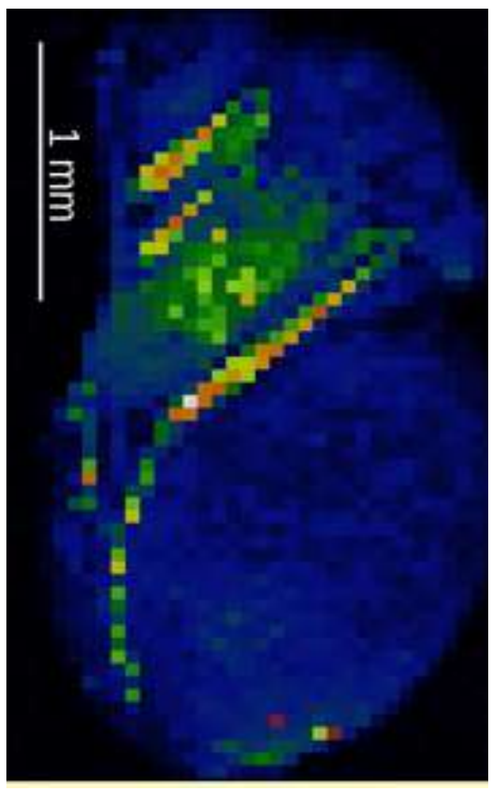

Fig. 6 Raman map of the pupa showed an extraordinary distribution of carotenoids. We have not found carotenoids in eyes, the biggest amount of carotenoids is concentrated in wings and legs.

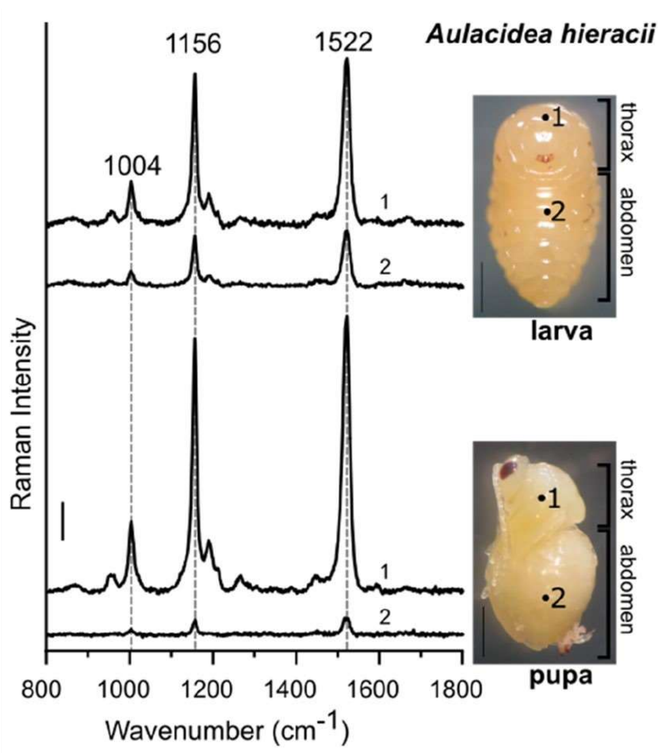

Fig. 7 Raman spectra from different areas (schematically shown as black dots) of the insect body (left). Vertical bar corresponds to 10,000 arbitrary units of Raman intensity. Optical images of larva and pupa of the gall wasp *A. hieracii* (right). Vertical bar corresponds to 0.5 mm.

Conclusions

For the first time, we noninvasively studied the molecular composition of *A. hieracii* insect larvae during their feeding and diapause, and *A. hieracii* pupae during metamorphosis with the help of Raman spectroscopy. The research showed that during the winter diapause, changes in such vital molecules as carotenoids occur, their synthesis takes place and their concentration increases. Thus, molecular processes continue inside the insect, despite the morphological constancy and the complete absence of motor activity. It is believed that insects are not able to synthesize carotenoids but receive them from food only. However, our studies have shown that the non-feeding larva acquires a different set of carotenoids: the isoprenoid chain becomes longer, and the amount of carotenoids increases. The difference can be explained by the synthesis of carotenoids by the insect itself. We have visualized for the first time the movement of carotenoids during insect metamorphosis. Our study leads to fundamental questions in insect molecular composition during metamorphosis. We consider the method of Raman spectroscopy to be promising in the non-invasive study of biological objects.

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