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Probing Carotenoids in the Gall Wasp *Aulacidea hieracii in Vivo* †

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Abstract: Gall wasp *Aulacidea hieracii* (Hymenoptera: Cynipidae) produces galls on a hawkweed *Hieracium x robustum* and feeds on gall tissues during summer. We employed *in vivo* resonance Raman spectroscopy to assess changes in carotenoids in living juvenile forms of this insect during its development. We revealed that the carotenoid composition in the feeding summer gall wasp larvae differs from that in the gall tissues. Non-feeding winter larvae contain more carotenoids than summer forms, despite the absence of feeding. Moreover, the carotenoid composition of winter larvae differs from that of summer larvae. We address the question, whether *A. hieracii* can synthesize carotenoids.

Keywords: Gall wasp; Cynipinae; Raman scattering; Carotenoids

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1. Introduction

Carotenoids are multifunctional molecules essential for the prosperous existence of animals. Carotenoids enter the animal body with food [1]. Insects are not the exception: most insects obtain carotenoids from plants and transfer them into the trophic chain to predators or parasitoids. Few insects are able to synthesize carotenoids *de novo* [2,3] or modify them resulting in the new types of carotenoids [4]. Thus, insects seem to be a promising source of easily digestible carotenoids for the food and drug industry.

Gall wasp *Aulacidea hieracii* L., 1758 (Hymenoptera: Cynipidae) produces gall on a hawkweed *Hieracium x robustum* Fr. s. L., 1848 (Asteraceae), and feeds on gall tissues during summer, obtaining all the nutrients from gall tissues in the absence of other sources [5–7]. This insect is a prey of many predators (birds or other insects) and parasitoids, playing an important ecological role. The peculiarity of the gall wasp is the ability to winter inside the gall in the form of non-feeding, but mobile larvae [5,8]. Since carotenoids may play a role in diapause [9], we investigated the carotenoid content and composition in the gall wasp larvae in summer and winter using Raman spectroscopy – a noninvasive technique allowing to obtain information about molecular properties in living organisms and tissues [10]. Such an approach did not interfere with insect development, because insects remain intact.

2. Methods

Galls on a hawkweed were collected in the end of August (summer larvae) or January/February (winter larvae) in the fields and woods of the Saratov region, Russia. Galls were transferred to the laboratory in thermo containers, where they were carefully dissected layer by layer. Insect larvae were removed with tweezers and placed onto the slide to record Raman spectra with Renishaw inVia Raman microspectrometer (UK) with a 532 nm laser at a power of no more than 0.3 mW per point. The measurement parameters were the same for all studied objects. 5-6 spectra were collected from several spots of the larva. The baseline of each spectrum was subtracted in the Fityk program (fityk.nieto.pl). All insects remain alive after the measurements and were placed into Petri dishes for further development and identification. Plants were identified by Dr. M. V. Lavrentyev (Saratov State University), insects were identified by Dr. V.E. Gokhman (Moscow State University).

3. Results

We recorded Raman spectra of the living gall wasp *A. hieracii* larvae at different stages of development and hawkweed *Hieracium x robustum* gall tissues with 532 nm laser irradiation (Figure 1). Each peak in the Raman spectrum corresponds to the vibration of a certain molecular group. The applied laser provides resonance Raman conditions for carotenoid molecules [11], so they contribute mainly to the spectra of insect larvae. This results in 3 most intense peaks with maximum positions around: 1004, 1156, 1523 cm^{-1}, corresponding to vibrations of $–CH_3$, C–C, and C=C bonds, respectively [12,13]. Raman peak assignment is presented in Table 1.

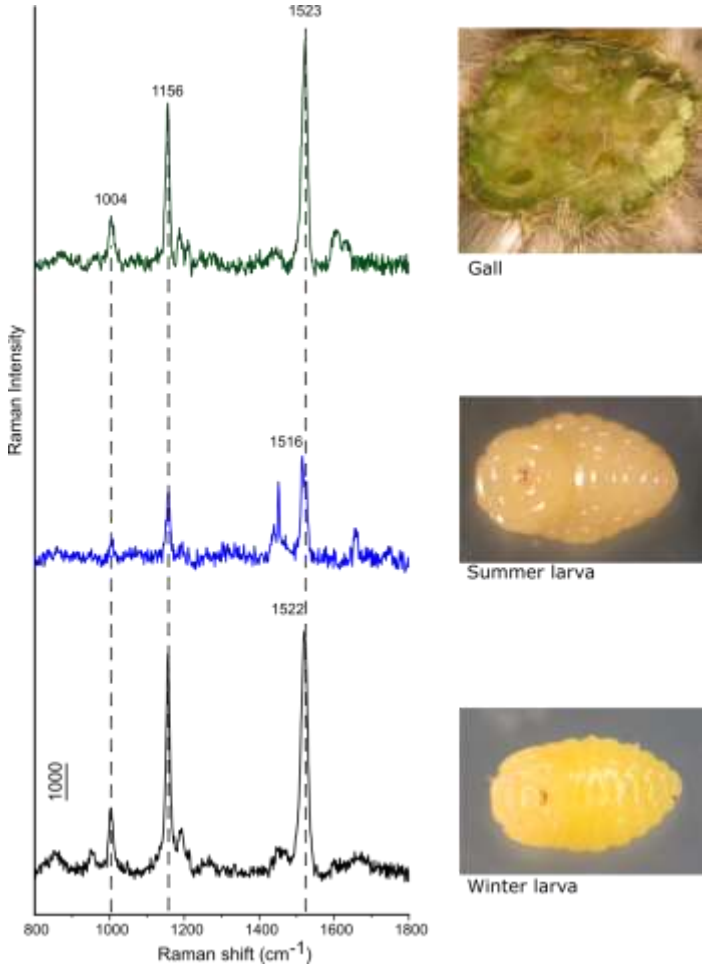

Figure 1. Typical Raman spectra (on the left) and photographs (on the right) of gall tissues, and gall wasp *A. hieracii* summer and winter larvae.

The intensity of Raman peaks correlates with the concentration of carotenoids [14] Thus, higher intensity of the spectra of the winter gall wasp larvae indicates the increase in carotenoid content in winter forms in comparison to summer forms. This result was unexpected since gall wasp larvae stop feeding in August, defecate, and enter diapause. So, the winter non-feeding forms of this insect cannot sequester carotenoids from the plant or elsewhere.

Table 1. Raman peak positions and assignment [1].

	-CH$_3$	**C–C**	**C=C**
Gall tissues	1004±0.1	1156±0.1	1523±0.3
Summer larvae	1005±0.5	1157±0.3	1516±0.3
Winter larvae	1004±0.3	1156±0.2	1522±0.2

[1] According to [11-16].

Then we analyzed the position of the peak corresponding to C=C bond vibrations in the Raman spectra of gall tissues and gall wasp summer and winter larvae (Table 1). The positions of Raman peaks characterize the structure and conformation of molecules of interest. The position of peak characteristic to C=C bonds in the spectra of carotenoids depends on the length of the carotenoid molecule. The bigger the value of the Raman shift, the shorter the isoprenoid chain in the carotenoid molecule [15,16]. It can be noticed that the Raman peak corresponding to C=C bond vibrations shifted to the high-frequency region in winter forms of insects. That means that winter forms of insects contain shorter carotenoids than summer forms. Moreover, the position of this peak in gall tissues and summer larvae differs dramatically, indicating the different composition of carotenoids in summer larvae and gall tissues it is feeding on.

Thus, the question arises, what is the source of carotenoids in gall wasp larvae? Can the gall be the only source of carotenoids for this insect?

4. Discussion

Most insects obtain carotenoids from plants [1]. In this case, the composition of carotenoids in plants and insects is the same [17,18]. However, some researchers noticed the discrepancy in composition and content of carotenoids in plants and insects feeding on these plants, explaining it by selective accumulation of carotenoids, modification of carotenoids by insect enzymes, or interaction of carotenoids with carotenoid-binding proteins [19,20]. These explanations may be relevant in case of the summer gall wasp larvae, but not of the winter gall wasp larvae. The discovered increase in carotenoid content and change in composition in non-feeding *A. hieracii* winter larvae lead to the suggestion that gall wasp *A. hieracii* may be able to synthesize carotenoids *de novo* as aphids and gall midges do [2,3]. This suggestion requires further investigations employing other methods such as HPLC, mass spectrometry, bioinformatics, etc.

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