

Probing carotenoids in the gall wasp *Aulacidea hieracii* in vivo

Evelina I. Nikelshparg^{1,*}, Daniil N. Bratashov², Matvey I. Nikelshparg³, and Vasily V. Anikin⁴

¹ Department of Biophysics, Faculty of Biology, Lomonosov Moscow State University, Leninskie gory, 1-12, 119234, Moscow, Russia; evelinanikel@gmail.com

² Education and Research Institute of Nanostructures and Biosystems, Saratov State University named after Chernyshevsky, Astrakhanskaya street 83, 410012, Saratov, Russia; dn2010@gmail.com

³ MAOU "Gymnasium 3", Bolshaya Kazachya street 121, 410012, Saratov, Russia; matveynikel@yandex.ru

⁴ Department of Animal Morphology and Ecology, Faculty of Biology, Saratov State University named after Chernyshevsky, Astrakhanskaya street 83, 410012, Saratov, Russia; anikinvasiliiv@mail.ru

*Correspondence: evelinanikel@gmail.com;

Presented at the 1st International Electronic Conference on Entomology 01-15 July 2021

Introduction

Carotenoids are multifunctional molecules essential for the prosperous existence of animals. Carotenoids enter the insect body with food. Only few exceptions exist – aphids and gall midges can synthesize carotenoids *de novo*. 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 obtaining all the nutrients from it (Figures 1-2).

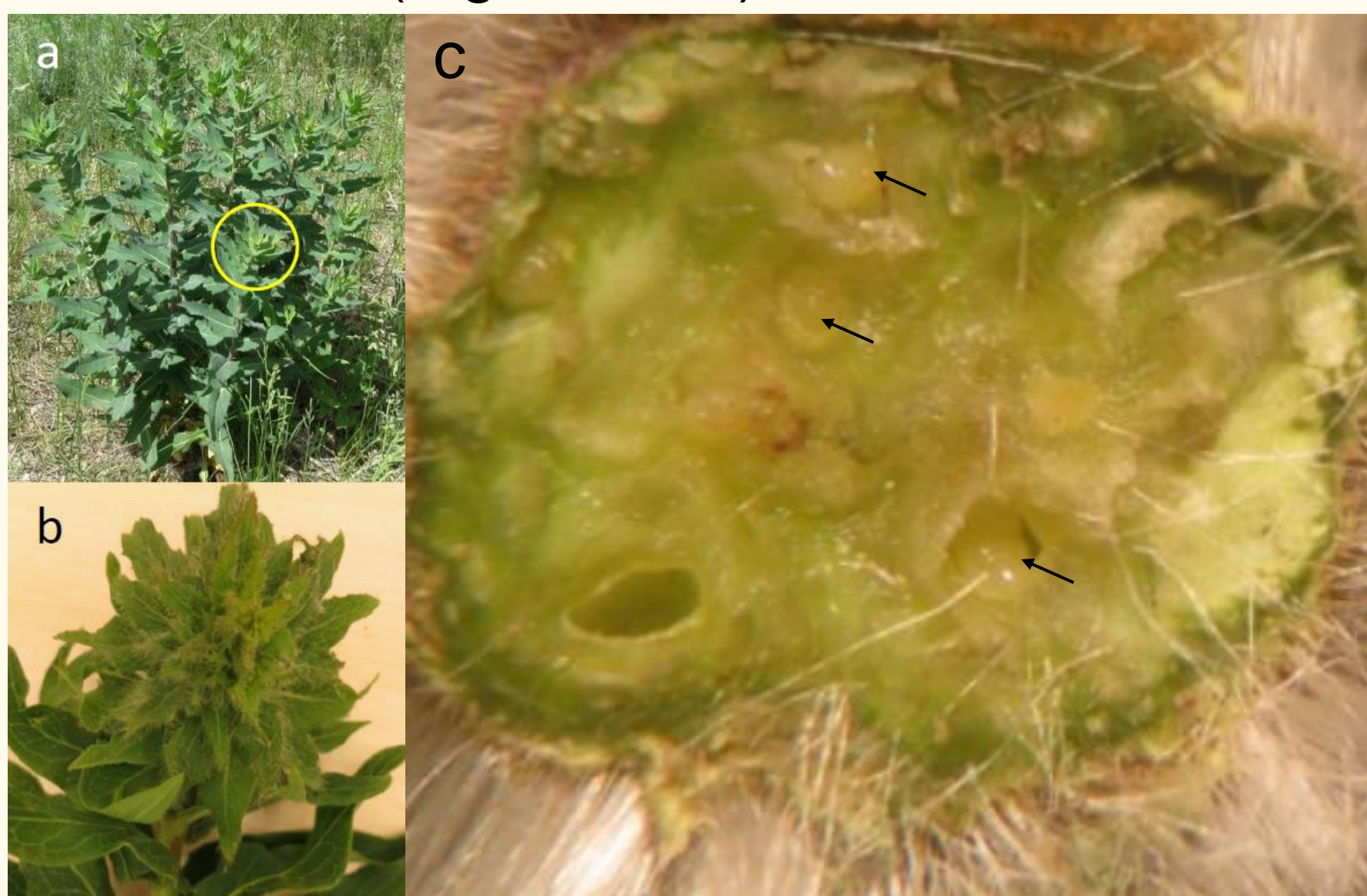


Figure 1. Gall of a hawkweed in nature (a); in laboratory (b); section of a fresh summer gall with insect larvae shown with arrows (c).

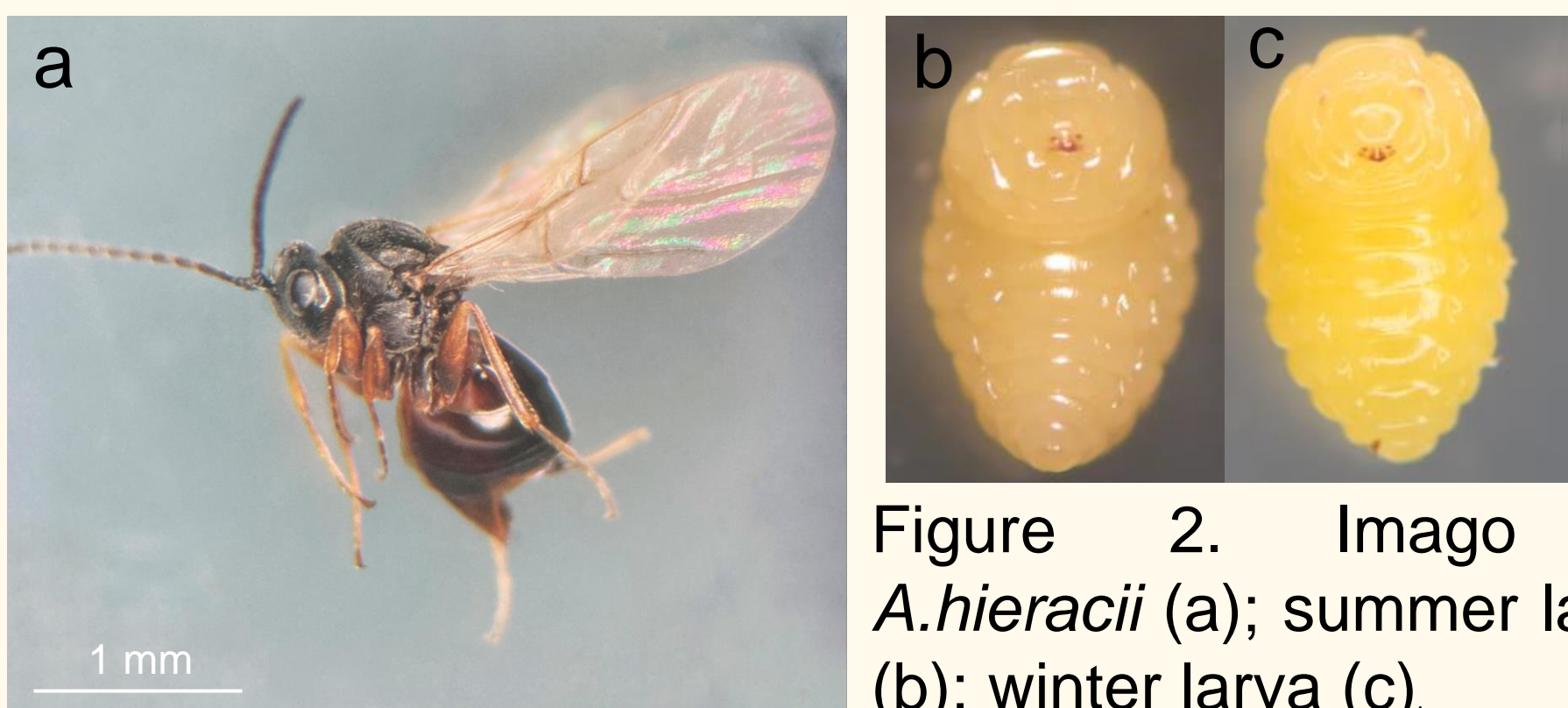


Figure 2. Imago of *A. hieracii* (a); summer larva (b); winter larva (c).

Methods

We investigated the carotenoid content and composition in the gall wasp larvae in summer and winter using Raman spectroscopy – a noninvasive technique allowing studying molecular properties of living organisms and tissues. Such an approach did not interfere with insect development, because insects remain intact. We used Renishaw inVia Raman microspectrometer (UK) with a 532 nm laser, which provides resonance Raman conditions for carotenoid molecules.

Results and Discussion

Raman spectra of insects contain 3 main peaks characteristic to C-CH₃, C-C, C=C group vibrations (Figure 3a). The higher the intensity of the Raman peaks, the larger the concentration of carotenoids. We revealed that:

- I. The winter larvae contain more carotenoids than summer larvae, despite the absence of feeding at this stage (Figure 3a).
- II. The Raman peak corresponding to C=C bond vibrations in summer larvae shifted to the low-frequency region in comparison to winter forms and gall tissues (Figure 3b-c). That means that summer larvae contain longer carotenoids than winter forms and gall tissues.

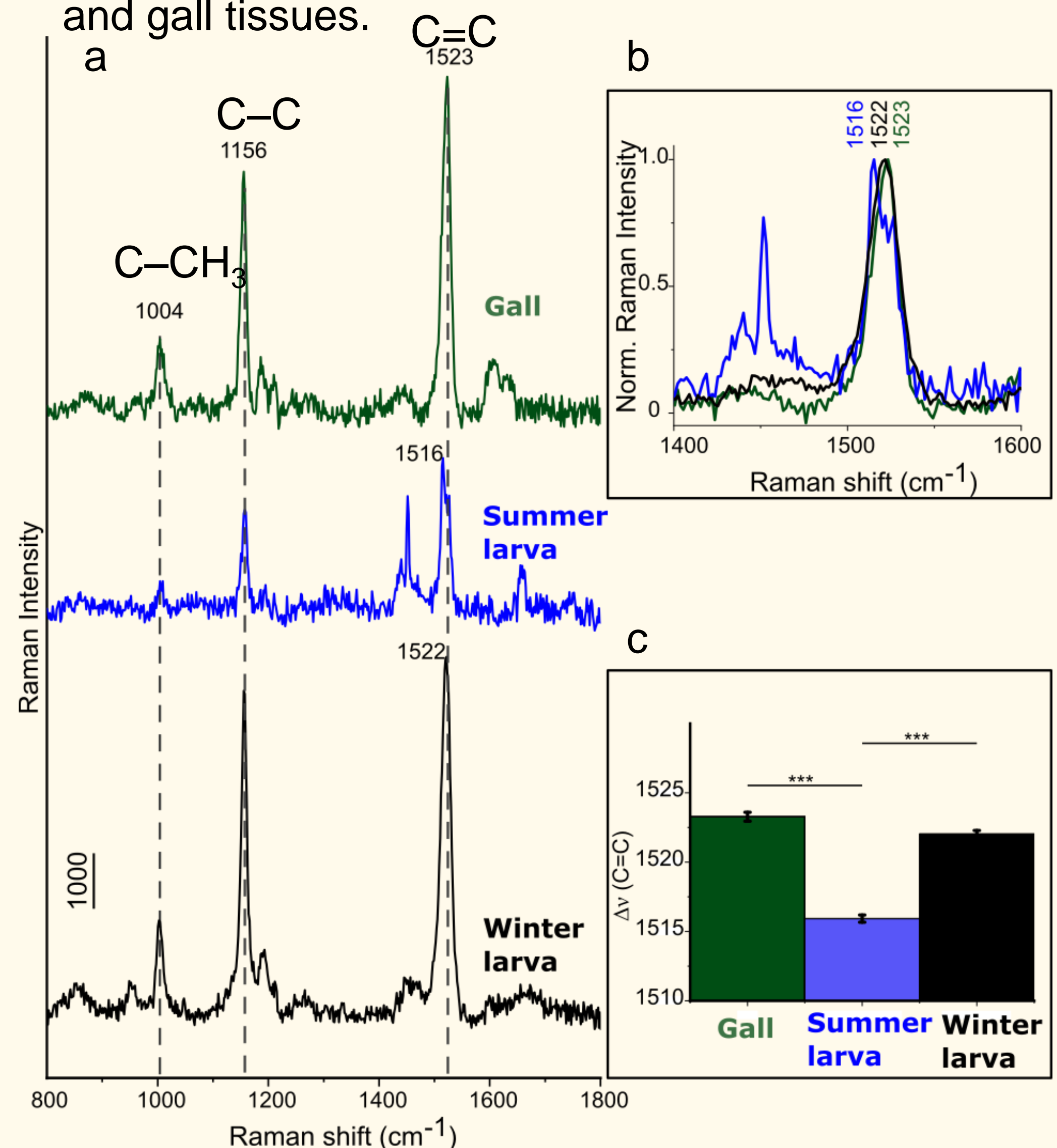


Figure 3. Raman spectra of gall tissues; summer larva and winter larva of the gall wasp (a). Scale – 1000 arbitr. units. Normalized Raman spectra in the region of a peak characteristic to C=C bonds (b). The position ($\Delta\nu$) of this peak (c). *** - $p < 0.001$ (ANOVA).

*These findings lead to the question, can the gall wasp larvae *A. hieracii* synthesize carotenoids de novo?*