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Non-invasive methods to determine biodiversity using plant gall volume and insect feces

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INTRODUCTION & AIM

The vast majority of methods for insect biodiversity assessment are invasive and require insect specimen collection and fixation. Specifically, current methods are unsuitable for studying insect communities that reside in plant galls, which serve as intricate habitats for various organisms, including hosts, predators, parasitoids, and inquilines. In this research, we propose new non-invasive methods for the quantitative and qualitative determination of insect biodiversity in plant galls. A gall is a mini ecosystem consisting of gall-formers, their parasitoids, predators, and inquilines. The main challenge in the determination of insect species in a gall is the need to dissect a gall, destroying a hidden ecosystem. The development of non-invasive methods will allow for the study of endangered insect species inside galls.

AIM: to develop noninvasive method to predict the number of insects and determine insect species in plant galls.

METHOD

The study was conducted from 2020 to 2023 in the forest-steppe zone of the Saratov region, Russia. To perform the regression equation, we used the linear regression formula: Y=a*x+b, where Y is the dependent variable (number of insects), X is the independent variable (gall volume), and a and b are the regression coefficients. We collected 20 galls in the steppe in February (Fig. 1). For each gall, we measured the length and radius in the laboratory using calipers. We calculated the volume of each gall as the volume of a cylinder (in accordance with the natural shape of the gall). Each gall was placed in a separate Petri dish. After all the insects emerged, they were identified at the species level and recorded in a table, which was used for regression analysis. This analysis was carried out in the GRAFPAD PRISM 8 program. The emerged insects were subsequently released into the wild.

To study insect excrement, we focused on the transition of the insect from the larval stage to the pupa stage (Anikin et al 2018; Gokhman & Nikelshparg 2021). During this period, the larvae free their intestines from excrement for the first and only time, then turn into pupae and then molt into an imago. We opened the wintering galls in February, extracted the diapausing insect larvae, and placed them in Petri dishes, one larva per dish. The larvae were 1–3 mm in size, so observations were carried out using a binocular microscope Mikromed MC-2-Zoom. Photos were taken every day with a Canon S100 camera. After the emergence of the imagoes, the species was identified, and the following parameters were recorded in the database: insect species, the appearance of excrement, color, size, consistency, and other features. The color of the excrement was determined using the HEX system in the open-access program https://gradients.app/ru. Luster - the optical characteristic of excrement was assessed as matte or glossy. Plasticity was determined by pressing the rounded head of an entomological pin onto a segment of excrement. If the segment was crumpled, it was considered soft, if it was crumbled – it was classified as fragile. A total of 330 insects were examined.

Fig. 1 – Gall on the hawkweed *Hieracium x robustum* in nature.

RESULTS & DISCUSSION

Counting insects in galls

As a result of the study, 405 insects emerged from the galls. Of these, 298 were gall formers and 107 were parasitoids. We compiled regression equations that can be used to determine the number of gall formers and parasitoids in a gall on *Hieracium robustum* Fr. (Fig. 2 B) in the field, as well as their sum, by measuring only the diameter and length of the gall using a caliper (Fig.2 A, C).

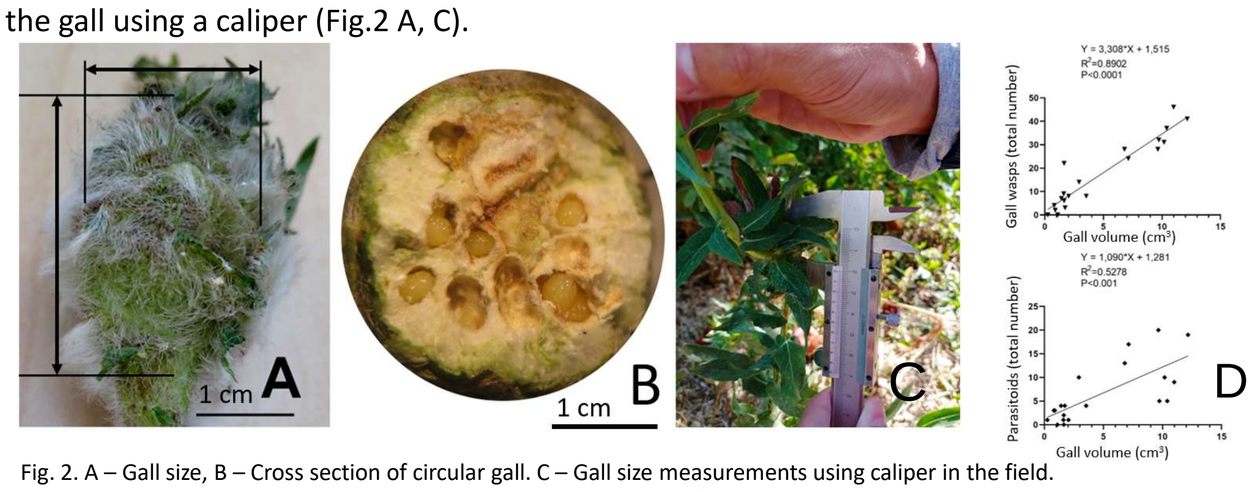

Fig. 2. A – Gall size, B – Cross section of circular gall. C – Gall size measurements using caliper in the field. D – The dependence of the number of emerged gall wasps *A. hieracii* on gall volume, dependence of the number of all emerged parasitoids on gall volume.

Excrements as species identifier

We found that the excrement of all studied insects is species-specific (Fig. 3). Feces excreted during the transition from the larval to the pupal stage has a segmental structure, while the imago has liquid excrements. For species identification, we propose four criteria: the number of segments of feces, the color and luster of the segments, the shape of the segments, and the plasticity of the segments.

	SPECIES	LARVA	EXCREMENTS	IMAGO	NUMBER OF SEGMENTS	AVERAGE COLOR LUSTER	SHAPE	PLASTICITY
1	***Aulacidea hieracii*** **(Bouché, 1834) Cynipidae)**				60	#E5AA3B MATTE	OVAL ELONGATED	FRAGILE
2	***Exeristes roborator*** **Fabricius, 1793 (Ichneumonidae)**				8	#6D6768 GLOSSY	OVAL	SOFT
3	***Ormyrus discolor*** **Zerova, 2005 (Ormyridae)**				15	#F0BB8B MATTE	CIRCULAR	SOFT
4	***Eurytoma hybrida*** **Zerova, 1978 (Eurytomidae)**				15	#696159 GLOSSY	OVAL, CIRCULAR	SOFT
5	***Eurytoma cynipsea*** **(Boheman, 1836) (Eurytomidae)**				45	#625F5B MATTE	OVAL ELONGATED	FRAGILE

Fig. 3. 1 – gall-former, 2, 3 – parasitoids, 4, 5 – related *Eurytomidae* species.

CONCLUSION

Using regression analysis, we established a precise correlation between the volume of *Hieracium robustum* Fr. galls and the number of its' inhabitants, as well as the ratio of hosts to parasitoids. Moreover, we found that each of the five insect species in these galls has a unique morphological pattern of the juvenile feces. Therefore, the species composition can be determined using these morphological observations. Importantly, this method is non-invasive, as the feces observation inside the galls is conducted after the emergence of gall inhabitants into their natural environment and seasonal plant death. Our research can pave the way for new methods in biodiversity measurements, specifically in plant galls.

FUTURE WORK / REFERENCES

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