UNIVERSITY of WASHINGTON TACOMA

# *The ecology and origins of Diplolepis rosae in Washington*

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## Natural History of *Diplolepis rosae*

*Diplolepis rosae* is a gall wasp indigenous to Europe and was accidently introduced to the United States through its host plant the dogwood rose.

The female wasp induces the creation of galls on the host plant by injecting some eggs into a leaf bud. The larvae feeding on the leaf bud triggers the plant to create the gall.

The larvae overwinter in the gall and emerge in as an adult in the spring. *Diplolepis rosae* is able to reproduce parthenogenetically (without males).

### Parasitoid Wasps

*Torymous bedeguaris*

*Orthopelma brevicorne*

*Pteromalus albipennis*

*Pteromalus puparum*

*D. rosae* galls are susceptible to attacks from the parasitoids. Parasitoids are wasps that inject their eggs directly into the *Diplolepis* larvae. There is a slight positive correlation between gall size with smaller galls being more susceptible to parasitism (Laszlo et. al 2014)

*D. Rosae* galls are also susceptible to predation by invertebrates, particularly birds. Previous research has shown that larger galls are more obvious to birds and face more predation (Lazlo et. al 2014).

A common way researchers determine the identity of closely related species is by sequencing the cytochrome c oxidase subunit 1 (COI gene). This is done by performing PCR. The standard PCR setup consist of four steps:

1) Add required reagents and master mix to PCR tubes

2) Mix and centrifuge

3) Run tubes through thermocycler

4) Run amplified DNA through Agarose Electrophoresis to determine if the correct gene is amplified.

Correct: Fluorescent line in gel

Incorrect: no lines


## Rose Gall Ecology

**Methods:**

Galls were collected from a residential area (January 2020), Soos Creek trails (June 2019, November 2020) and Green River and allowed to over winter in laboratory settings. Gall size and number of open chambers was recorded. After the wasps emerged in the spring the species and total number of emerged wasps were recorded.

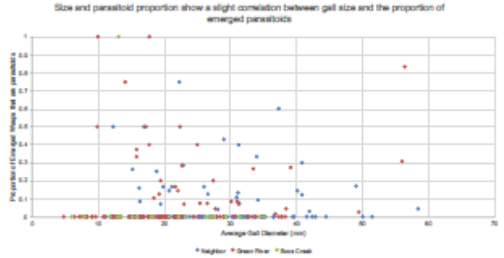



Figure 1: There is a trend that as the size of the gall increased the lower the proportion of the parasitoids that emerged was in most cases.

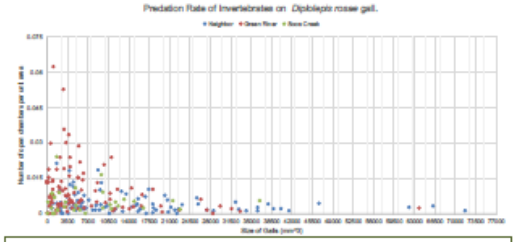
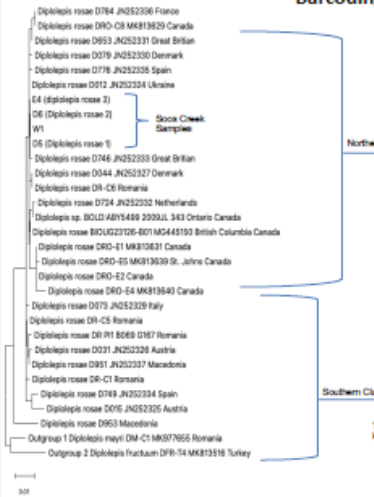



Figure 2: Graph of predation by birds. The surface area of each gall was calculated. Overall there seems to be a negative correlation between gall size and the proportion of open chambers per unit area

| | Site A (Neighbor's Shrub) | Site R (Green River) | Site C (Soos Creek) |
|---|---|---|---|
| No. of galls sampled | 101 | 103 | 34 |
| Diplolepis rosae | 864 | 372 | 60 |
| No. of total parasitoids and inquilines | 97 | 66 | 23 |
| Orthopelma spp. | 70 | 10 | 9 |
| Torymous bedeguaris | 16 | 34 | 12 |
| Pteromalus albipennis | 3 | 9 | 1 |
| Pteromalus puparum | 4 | 11 | 1 |
| Unknown | 4 | 1 | 0 |
| Total wasps | 961 | 377 | 155 |
| Average Gall volume (mm^3) | 15309.5 | 7300.8 | 7367.3 |
| Parasitism rate | 10.1% | 11.3% | 25.0% |

**Table 1:** Number of emerged gall inducers and parasitoid specimens in the observational study. Neighbor site generally had larger galls and experienced a slightly lower parasitism rate.

## Barcoding Wasps




**Genetic**: DNA extractions and PCR amplification were only conducted on samples collected in 2021 (Soos Creek) because of COVID restrictions. The gene of interest for barcoding is the cytochrome c oxidase subunit I which is useful for species identification. DNA was sent out for sequencing and the resulting data was compared against sequences from other studies (Zhang et. al 2019) and (DNA barcodes) on National Center for Biotechnical Information in order to determine the closest ancestral relative and to help build a phylogenetic tree.

*Orthopelma brevicorne*

*Orthopelma mediator*

The key differences being length of ovipositor, number of antenna segments, hairs on the legs, and DNA sequences

| Collected Sample | NCBI Samples | Percent identity to NCBI samples | Genetic distance |
|---|---|---|---|
| W3 Orthopelma brevicorne | Ichneumonidae_sp._BOLD:AAM7350_BIOUG05176-A11_Alberta_Canada | 99.34 | 0.007025901 |
| W3 Orthopelma brevicorne | Orthopelma_mediator_OT38_Denmark | 92.07 | 0.065329496 |
| E2 Torymous bedeguaris | Canada relative 2 | 100 | 0.000000000 |
| E2 Torymous bedeguaris | Torymus_chrysochlorus_10BBCHY-0410_ontario_canada | 90.60 | 0.114542134 |

One advantage of using the COI gene for DNA barcoding is that we are able to identify similar looking species. Before we sequenced our sample DNA I was convinced that we had Orthopelma mediator because of the characteristics of the wasp. After sequencing we discovered we actually had Orthopelma brevicorne which is in the same family and has similar features.

Table 1: Samples collected from Soos Creek were compared to samples from other studies with sequences on NCBI database . The Percent Identity describes how similar the collected samples were to the samples on the data base. The outgroups were included to show that different species even from the same family can have big genetic differences.

## Results

- Rough phylogenetic tree of *Diplolepis*
- Parasitism rate decreases slightly as galls get larger (Figure 1 & Table 1)
- The site with the largest galls (Neighbor) had smallest parasitism rate (Table 1)
- Based on data collected there does not seem to be more rates of bird predation for larger galls however we are not able to account for missing gall volume
- Collected wasp samples had high percent identity when compared to other studies (Canadian Field Journal)

## Future Work:

- In future work samples should be taken from more than one location and more wasps sampled
- Sequence samples based on ITS2 (Internal Transcribed Spacer 2) as well as COI
- Add sample sequences to GenBanks currently there are only European and Canadian sequences recorded

## REFERENCES

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