Review on A. Mrázová PhD thesis: Interactions between plants, herbivorous insect and predators: mechanisms and ecological importance

This PhD thesis focuses on the importance of tri-trophic interactions, particularly, between plants and avian predators of herbivory insects, and on the mechanisms that regulate such communication. In a way, this thesis explores a novel research topic that can be considered part of two ongoing paradigm shifts. The first concerns the realisation that insectivorous birds do not exclusively use visual cues to find their prey. The second sees communications between plants and higher species stronger than previously believed.

Chapter I presents a synthesis of the evidence about the use of birds of herbivore-induced plant volatiles (HIPVs) and found contradictory results. Here, future research questions to be investigated are also suggested. Particularly, three questions are identified (i) whether birds can differentiate between small and large herbivore infestations, (ii) whether birds can distinguish the herbivore developmental stage, and (iii) how far away can birds detect volatile signals. Despite the candidate recommending these research questions, none of them was investigated during this PhD project. Out of curiosity, I wonder why?

In Chapter II, evidence is provided that the application of methyl jasmonates on *Quercus pyrenaica* causes a reaction in the tree which is comparable to that caused by herbivores. The attractiveness of these volatile compounds to insectivorous birds was measured by exposing artificial caterpillars made of plasticine for 14 days, but none of these caterpillars was attacked. The choice of artificial caterpillars and of exposing them for multiple days to answer the question tackled in this experiment surprised me. The attractiveness of HIPVs for birds may be innate but also learned from experience (there are studies supporting both ideas). I wonder if then birds could learn to associate these volatiles with the fake prey and avoid them. Wouldn't it be safer to repeat the experiments on several occasions rather than for two continuous weeks?

In Chapter III, in contrast with the results from the experiment in Chapter II, evidence is provided that the application of MeJa on *Ficus hahliana* trees doubles predation rates measured using artificial caterpillars. The authors also found that predation rates decrease along an altitudinal gradient (200-2700 m asl) and that ants are the main predators at lower altitudes, while birds are relatively more important at higher altitudes.

In Chapter IV, two *Quercus* species with different constitutive defences were compared assuming that one of them would react more intensively after the application of MeJa. The attractiveness to predators was measured using artificial caterpillars (and was found to be higher on treated trees than on untreated trees, with no differences between tree species). Simultaneously, insect communities were sampled using beating techniques, and it was expected that herbivore abundance would decrease in MeJa-treated saplings (because of higher predator abundances) and that predator abundance would increase because they would be attracted by the volatiles. This chapter, like the others, is very interesting and tackles several questions at the same time. While I believe this experiment has a great exploratory value, I have found the testability of some hypotheses problematic.

For example, p.111 reads "*Based on different constitutive performance of studied oak species, we assume that English oak invests into inducible defence and responds more intensively to induction by MeJA than sessile oak*"

What does "more intensively" mean? The reaction of the treated plant will be faster? A higher concentration of volatiles will be produced? Will the composition of these volatiles include one or more specific compounds? On p.124, in the results, it is mentioned that among the tens of compounds, the focus of the analysis was on terpenes. Is it OK to take such a decision after the results have been collected?

Another example at p.111 is the following: *We also assume that the mean body size of arthropod communities is affected by MeJA treatment. It is likely that body size of chewers will decrease as the leave tissue of induced trees is less palatable, than the leaf tissue of healthy trees, while the mean body size of other feeding guilds remains unchanged.* I wonder if it would not have been easier to test this hypothesis by feeding herbivore larvae with defence-induced and undefended leaves to assess their growth rate/body mass. The insects that were collected are not necessarily host-specialists, and their current body size may have resulted from feeding on other plants.

Interestingly, while predation on artificial caterpillars increased on MeJa-treated saplings, the treatment did not affect overall arthropod abundance. Only predatory arthropod abundance was affected ($p = 0.03$). I wonder how you explain this inconsistency, and if these results change if you exclude ants (that are not only predators). Maybe ants drive these results and are attracted by specific compounds?

There are also a few minor additional issues that might be useful to clarify during the presentation, even if not so important.

In this experiment, saplings were separated by 5 m. How was this distance chosen and did not you fear it was too short? In Chapters 2 and 6 trees (or clusters) were separated by 20 m.

It is not clear why you did not always select the model with the lowest AIC (what visible differences in the raw data are you referring to? p.117).

In Table 2 (p.119), why were some variables considered not relevant?

p.132 – I would suggest rephrasing the following sentence using "was smaller" instead of "decreased" because you have not tested the mechanism behind this relationship: *the mean body size decreased significantly in chewers after MeJA application…*

In Chapter V, in an aviary experiment setting, the attraction of two passerine bird species to chemical and visual signals of herbivore presence, visual-only, and chemical-only, were compared. The authors claimed that shrubs providing chemical + visual signals were preferred over the control, and that shrubs providing chemical-only signals were preferred over shrubs providing visual-only signals. The major problem with this chapter (which may just be a simple overlook) is that Figure 1 (p.170) does not provide consistent results with the text. In fact, from Figure 1 appears that birds spent more time searching for prey on control shrubs than in shrubs providing chemical and visual signals. This aspect should be clarified during the presentation, as the relevance of Experiment 2 also depends on the correctness of these results.

Assuming that the text is correct and not the figure, a question arises about the interpretation of the results. The attractiveness of treated shrubs is measured using two parameters, the number of visits to individual shrubs and the total time spent on them. *During Experiment 1, both bird species chose the shrubs providing none or both signals equally often (ANOVA $\chi 2 = 0.59$, $Df = 1$, $p > 0.05$).* (p.166) but spent more time on treated shrubs than on control shrubs. In the discussion, the authors concluded by saying that *We confirmed our hypothesis that naïve Great and Blue tits prefer to search food on shrubs that emit both*

chemical and visual signals rather than on control shrubs, which give no signal. I wonder if this conclusion is that unequivocal given that the number of visits was not significantly different.

Moreover, I fear that the results from the statistical analysis might have been misinterpreted and I have two recommendations. The first is to try removing Side from the model. The relevance of this factor was not explained in the introduction or M&M, and it comes as a surprise in the results. Why was it tested at all? This doubt returns in the discussion: *In the Experiment 2, Great tits arrived at chemical shrubs more often when placed on the left side of the aviary, while Blue tits preferred chemical signals when placed on the right side of the aviary* (p.174) and *...Given the random nature of these results, we do not consider them noteworthy* (p.175). The way I see it, there is no justification to test this factor in the model; removing it might also ease the interpretation of the results.

The second recommendation is to check again whether the treatment in Experiment 2 was indeed significant when the response parameter was the number of visits. The intercept of the summary model in Table 1 is not significant, and in a model where the response parameter was coded with cbind, this should indicate no significance for the treatment. For example:

```
myc <- sample(c(0,1), 250, replace = T, prob = c(.2,.8)) # high frequency of ones.
fac <- c(rep("A", 125), rep("B", 125))
dat <- data.frame(fac,myc)
dat$myd <- ifelse(dat$myc==0,1,0)
mtr <- glm(cbind(myc,myd) ~ 1,
   family = binomial,
   data = dat)
summary(mtr)

myc2 <- sample(c(0,1), 250, replace = T, prob = c(.5,.5)) # no sig. differences
fac2 <- c(rep("A", 125), rep("B", 125))
dat2 <- data.frame(fac2,myc2)
dat2$myd2 <- ifelse(dat2$myc2==0,1,0)
mtr2 <- glm(cbind(myc2,myd2) ~ 1,
       family = binomial,
       data = dat2)
summary(mtr2)
```

In Chapter VI, predator activity measured using artificial caterpillars was higher in potted *Quercus robur* and *Q. petraea* saplings sprayed with MeJa than in untreated ones, and that the chance that a caterpillar was attacked decreased with the distance from the focal treated tree when conspecific trees were used. I wonder whether results obtained with saplings can be representative of adult trees, and eventually what could be the biases. I also wonder if the candidate thinks that the results from this experiment suggest that conspecific trees benefit from being close (<2 m) to each other and to what extent.

Overall, this thesis advances our understanding of tri-trophic interactions and use of chemical cues for foraging by birds. Anna Mrazova`s PhD study was completed satisfactorily. Particularly, she co-authored three articles published in international peer-reviewed journals and three more are in preparation, she went abroad to Germany and Spain to learn new techniques and presented her work at conferences.

Since this is also a young field of research, it would be interesting to learn whether the candidate can summarise the most important conclusions from these experiments and is aware of other studies pointing

in the same directions, and what are her recommendations and suggested future directions for this field. For example, from the many lessons learned from these experiments and considering that there are still several unknowns (e.g., how long can these compounds remain stable in the atmosphere), do we understand better how bird foraging behaviour is shaped by these chemical volatiles?

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