DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES

# THE EFFECT OF DIET BREADTH ON BIOPESTICIDE SUSCEPTIBILITY

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*Cover photo of two Bemisia tabaci, source: wikimedia.org*

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## Abstract

Pesticide resistance has evolved in insects for as long as the use of insecticides, and has caused us to continually develop new ones. An alternative to synthetic pesticides is biopesticides, which can consist of either living organisms or substances from organisms. Biopesticides containing living organisms can include bacteria, virus, fungi, as well as predators and parasites. They can be preferable to synthetics since they usually are less dangerous to humans, disintegrate faster and tend to be more targeted to specific pest species. Pest species ability to develop resistance to a synthetic insecticide seems to vary depending on the number of plant families the species feed on. The reason for this can be that polyphagous insects are better adapted to handling secondary metabolites from more or different plant species than do specialist insects. In this meta-analysis, I investigate whether a similar relationship exists among living biopesticides. The underlying hypothesis is that since plants have microorganisms in and on them, an insect feeding on several plants can have a history of being exposed to more diverse microbial communities compared to insects feeding on just one plant species throughout its life. I used a Bayesian multilevel regression model to answer the question: Are polyphagous pests less susceptible to novel pathogens and living biopesticides than specialist pests? The result showed no evidence for polyphagous pests to be more resilient against novel biopesticides. However, the posterior distribution is not mean centred, which can indicate a small effect of polyphagia on mortality. Further and more detailed studies of this question are strongly needed.

## Sammanfattning på Svenska

Insekter har utvecklat resistens mot insektspesticider lika länge som de har utsatts för det, vilket har resulterat i att människan har hela tiden behövt utveckla nya syntetiska insektsmedel. Biopesticider har kommit som ett alternativ till de syntetiska insektsmedelen, som antingen kan bestå av levande organismer som bakterier eller mikrosvampar eller ämnen från levande organimser. Fördelarna är att de kan vara mindre farliga för människor, bryts ner snabbare av naturen och kan vara artspecifika (och skadar då inte pollinerare och andra insekter). En tidigare meta-analys visar på att antalet växtfamiljer en insekt äter av påverkar dess utveckling av resistans. Anledningen tros vara att generalister är mer predisponerade att hantera olika växters sekundära metaboliter än specialister. Målet med den här studien är att se ett liknande samband finns gällande biopesticider. Det är rimligt att anta detta då de flesta växter har mikrober som lever i eller på dem och insekternas immunsystem måste kunna hantera dessa. Så om växternas mikrobiom skilje sig bör generalister kunna hantera levande biopesticider bättre än specialister. För att testa detta använde jag en bayesiansk multilevel regression. Resultatet visade ingen tydlig effekt av antalet växtfamiljet en insekt äter av. Fortsatta studier av ämnet kan ge ett mer precist result, vilket hade varit intressant att se.

# 1. Introduction

## 1.1 Insecticide resistance

The first insecticide was developed in 1939; six years later, the first case of insecticide resistance was reported, and since then the number of insect species with developed resistance would increase to over 500 at the end to the twentieth century; the development of resistance to insecticides and the development of new insecticides have since then become an arms race. (Denholm et al., 2002). An alternative to synthetic pesticides is biopesticide, and few insects' pest have developed resistance to them as of now (Mangan et al., 2023). To avoid large spreads of biopesticide resistance we need to understand as much as possible about the evolution of insecticide resistance and susceptibility to insecticides. The goal is to have sustainable agriculture with insecticides that are not harmful to the environment nor the farmers and in addition keep insects from getting resistant to the biopesticide, and then biopesticides won't lose their effectiveness.

## 1.2 Resistance to pesticide in polyphagous insects

Hardy et al. (2018) wanted to understand what drives the rate of insecticide resistance evolution and if it is affected by plant host use. The pre-adaptation hypothesis suggests that the insects physiological systems to handle secondary metabolites in plants are recruited also for synthetic pesticides. Therefore, since different plant families produce different secondary metabolites, polyphagous insects may have evolved tolerance to a wider variety of compounds than specialist insects. The hypothesis was supported by a comparative analysis that found a positive relationship between synthetic pesticide resistance and diet breadth, the latter estimated as the number of host plant families an insect species feeds from (Hardy et al., 2018). Here I ask if there is a similar relationship with living microbial biopesticides. One reason for this is that plants have micro-organisms that live both on the plants as well as inside them (Vandenkoornhuyse et al., 2015). When insects feed on plants, they also encounter the microorganisms, and it is possible polyphagous insects therefore have immune systems that are better adapted to handle a wider variety of pathogens, and therefore have stronger resistance to pathogenic biopesticides.

## 1.3 Plant microbiomes

The microbes can live both in and on the plant tissue and they can be bacteria, fungi or viruses. Microbes living inside the plants are called endophytes while microbes living outside of the plant are typically called epiphytes; however, microbes developed inside the plant can, even when they live on the outside of the plant be classified as an endophyte. Some microbes can be pathogens that cause harm to the plant, while others come with benefits. Microbes can help the plant collect nutrients or make the plant more tolerant to abiotic and biotic stress. The class of interaction, mutualistic or not, is not set, but can shift on a continuous scale (Partida-Martinez & Heil, 2011).

## 1.4 Biopesticide

Biopesticides are as previously stated an alternative to synthetic insecticides, they can be preferable since they are often less dangerous to humans, disintegrate faster and more targeted to specific pest species (Hezakiel et al., 2023). There are several types of biopesticides, e.g. virus, bacteria, fungi, and nematodes (Mangan et al., 2023), these are living biopesticides and are in focus in this study. Other types of biopesticide are molecules derived from plants or incorporated plant protectants, were the plant genome have received protein producing genes and then create their own biopesticide (Mangan et al., 2023). Since the non-living biopesticide has no connection to plant microbiomes they will not be included in the study. Other types of biological control also exist, like parasitoids and predators, and while they are living organisms, they also have no connection to plant microbiomes and will also be excluded. In addition, when used for pest management, parasitoids and predators are meant to be a continuous control of pest insects by reproducing to create a population on the fields (Gerling et al., 2001). Whereas such continuous presence may lead to coevolution with the target pest, microbial biopesticides are usually designed to disintegrate relatively fast (Hynes et al., 2011), thus preventing coevolution with the pest insects. Conversely, pests may evolve resistance also to biopesticides§, even if this is likely constrained by the more genes involved in resistance to living organisms as opposed to chemicals. Moreover, different types of biopesticide also have different risks of causing resistance evolution in pest insects; lower risk in nematodes and fungi, higher in bacteria and viruses (Mangan et al., 2023). We are still in the beginning of the 'biopesticide era', and as the use of biopesticide increases with roughly ten percent each year globally (Kumar & Singh, 2015), it is critical to find means to prevent resistance evolution from undermining this important development. Given the great diversity of biopesticides, from virus to nematodes, it is hard to make generalized predictions about how insects will react to them (Mangan et al., 2023).

### 1.5 Aim

Since polyphagous insect species have historically been in contact with a large number of different microbes, I predict that they are better predisposed to handle novel microbial biopesticides (with which they have not coevolved). The goal is to further understand susceptibility to biopesticides and specifically if a wide diet breadth can give insect species an advantage when treated with biopesticide. This is important because with the rise of biopesticides we need to keep insects from evolving resistance to be able to maintain sustainable crop protection. Therefore, I want to answer the question: Are polyphagous pests less susceptible to novel pathogens and living biopesticides than specialist pests?

## 2. Method

### 2.1 Meta-analysis

Meta-analyses are systematic reviews that synthesize previous studies to estimate the strength, generality and uncertainty in phenomena across multiple scientific studies or answer a new question (Gurevitch et al., 2018). When reporting meta-analysis there are systematic ways do this in, and using these methods increases the reporting quality and trustworthiness of the study; I have followed the PRISMA EcoEvo guidelines; PRISMA is an abbreviation and stands for *preferred reporting items for systematic reviews and meta-analyses* (O'Dea et al., 2021). Since there are a plethora of papers looking at effectiveness of different species and strains of microbes as biopesticide and these papers test on several different pest species, therefore, I thought this information could answer my research question when synthesized.

### 2.2 Publication bias and heterogeneity

Usually in meta-analysis you test for publication bias with Egger's regression and a funnel plot; however, Egger's regression is made for frequentist models and does not work with Bayesian or brms models. Therefore, I used a funnel plot to visually assess the risk of publication bias (Lin & Chu, 2018).

How much the effect sizes in the different studies differ is called 'between-study heterogeneity' and was estimated using the $I^2$ statistic, where a value of 25% is considered low, 50% moderate and 75% substantial heterogeneity, above which it is inappropriate to do a meta-analysis (Harrer et al., 2021).

## 2.3 Restrictions

Since this study can be seen as a continuation of the Hardy et al. (2018) study, but looking at biopesticides, I am able to use their synthesized data on the number of plant host families a species feed from. I restricted the species to only use species with a known diet breadth in Hardy et al.'s (2018) data set. In light of time constraints imposed by my thesis schedule, I further restricted the scope of the synthesis by examining the group with the greatest variation in diet breadth, the Hemiptera.

## 2.4 Eligibility criteria

In order for a study to be included, it had to be an experimental study with living microbes tested on hemipterans and the result given in mortality percentage or proportions with a no treatment control. The hemipteran species needed to be in the Hardy et al. (2018) dataset with data of host count families. Studies needed to have uncorrected mortality data or corrected mortality data with the control so it can be converted back, otherwise it was excluded. Review articles were also excluded.

## 2.5 Finding studies

The platform I used for searching papers was Web of Science (Clarivate™ (Web of Science™). © Clarivate 202.) with its database *Core collection*. Web of science's *All databases* did not have other relevant articles compared to the *Core collection*, therefore, to save time, it was used to collect articles. All articles were downloaded on April the 14th 2024, to then screened over the next weeks. The searched term used were the following:

> TS=((bio$control OR bio$management OR bio$insecticide OR bio$pesticide OR "biological control" OR microb* OR bio$larvicide* OR pathogen* OR virus OR viral OR bacter* OR fung* OR parasit*)
> AND (mort* OR surv* OR LC50 OR LC9*)
> AND (insect* OR pest* OR Larva* OR instar* OR pupa*) AND (resist* OR immunity OR susceptibility)
> AND (insert genus name) NOT "host immunity" NOT "host resistance" NOT "plant immunity" NOT vecto* NOT parasitoid).

The search term was altered from the search term from a similar metalysis to fit my question (Mostafanezhad et al., in prep).

## 2.6 Data collection process and data items

I used the webpage rayyan.ai (Ouzzani et al., 2016) to screen abstracts and Zotero for screening full-text and recorded including or excluding decision with motivation for exclusion**.** For each study, I extracted the relative susceptibility of the focal species using estimates of mortality when exposed to the biopesticide or fungal strain compared to a control treatment in which insects were not exposed. These data were either extracted from tables or graphs, in the latter case using WebPlotDigitizer® (Clarivate™). Data for moderating variables, pest species, instar stage, biopesticide agent, biopesticide dose, dose unit, days post inoculation, and the sample size (number of insect individuals who make up the mean mortality percentage) were also collected, from either tables/graphs or text. Host count was taken from Hardy et al.'s (2018) published data. If small amounts of data were missing or if I was unsure of the details, I contacted the authors for extra information or clarification.

If the article had an experiment where instar levels were mixed, the middle instar level was used. My reasoning was that life stage was to be modelled as a continuous fixed effect, and the middle value for a group of instars might be close to the mean and better estimate the effect of instar across studies.

Meta-regressions typically weigh estimates according to their precision using the sample size to calculate uncertainty, I reasoned that it is better to overestimate the standard error than underestimate it. Therefore, if the estimated mortality rates for treatment and control groups had different sample sizes the lowest one was used for all of them. If the sample size was given as a span, the middle number was used for all of them, again to act as a mean value.

Several studies report the mortality for both treatment and control at several time intervals following inoculation, in which case I chose the day where the graph starts to level out; this was most often the last day. If there were several concentrations for the same microbe strain and or if there were several strains of the same microbe species then all were added, to get as much data as possible.

### 2.7 Effect size measure

In this case, all effect sizes are already in mortality percentage, but since I need to take the control mortality proportion into account, I transformed my data to log risk ratios (logRR), calculated by dividing the probability of the treatment with the probability of the control. Log risk ratio are similar to log odds ratios, but if the probability of the treatment and the control is over 0.1, log risk ratio is a better alternative (Bakbergenuly et al., 2019). The effect size log risk ratio was calculated using the r package *metafor* (Viechtbauer, W, 2010) and the function escalc; *metafor* also calculates a variance for each individual effect size, with which I calculated a standard error to account for sensitivity of each effect size in the model (Viechtbauer, W, 2010).

### 2.8 Meta-analytic model description

R and R Studio (R Core Team (2023)) was used for statistical analysis and to create tables and graphs. I used a brm multilevel model with the package *brms*, Bayesian Regression Models using Stan (Bürkner, 2017). The r package *ape* (Paradis E & Schliep, 2019) was used in handling the phylogenetic data along with creating the covariance matrix. The first model was fit using both fixed and random effects, the fixed effects, also called predictors, consisted of host count, days post inoculation, life stage as well as the natural logarithm of the dose in conidia or spores per milliliter. Then the random effects consisted of study ID, biopesticide or fungus/bacterial species and the covariance matrix over the species I had in my dataset. The second model had the same factors as the first model, but the fixed effects were mean-centered and scaled to allow for comparisons.

## 4. Result

### 4.1 Data extraction

When deciding on the final search terms, I had 763 papers (figure 1), of which 164 were duplicates and were removed at the first stage. I screened 599 abstracts, and out of those, 368 were excluded and 130 proceeded to the full text screening. In the full text screening 97 papers were excluded. The different reasons for exclusion were that 1) the papers did not report treatment mortality and control but used another format like LC50 (lethal concentration that kill 50% of the population) or controlled mortality, 2) the species in the study was not hemipteran or not a species with a known host count in Hardy et al. (2018), 3) multiple insect species were treated together, 4) the study did not use living microbes. The final number of papers that were extracted was 32. It was very common for the articles to report corrected mortality, following Abbott’s formula (Abbott., 1925) to correct for the control. Unless they also reported the control mortality, I was unable to back-calculate the actual mortality, and this made me unable to use the article and it was excluded under reason 1.

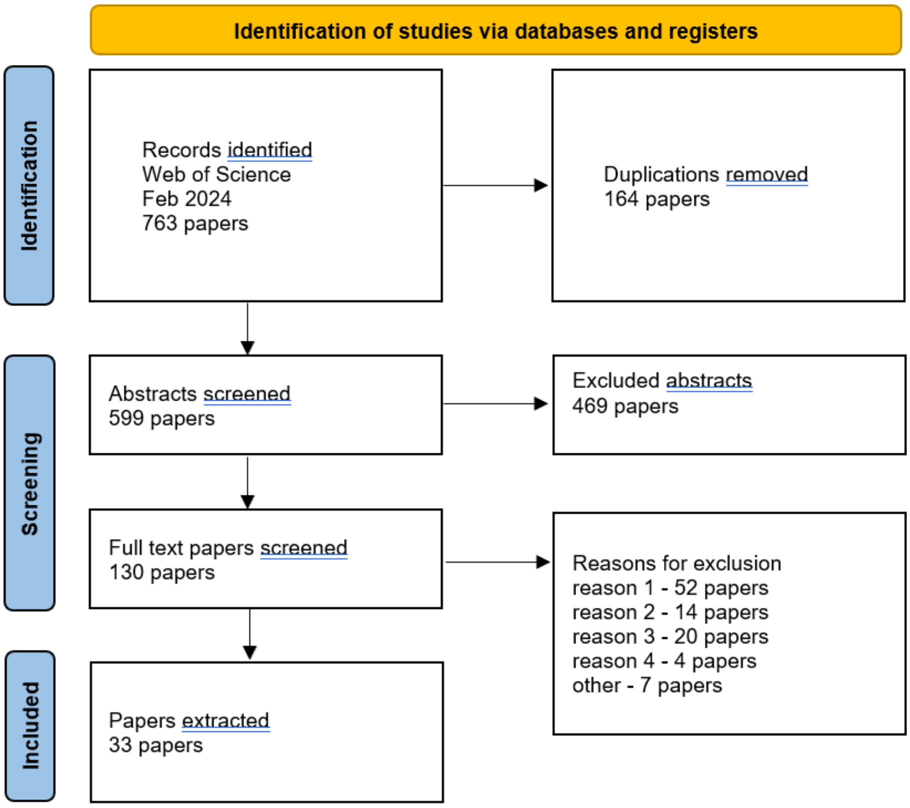


Figure 1: Prisma plot (O'Dea et al., (2021) visualizing the numbers of papers excluded or included in each step of the screening process. Reasons for exclusion were: 1) no reported mortality, 2) not hemipteran species or not a species from Hardy et al.'s (2018) data set, 3) did not use live microbes as biopesticide 4) did not separate insect species in tests. Other reasons were, the paper was not in English (2 papers), review articles or comments (3 papers), no sample size (1 paper), only the seed was treated with microorganisms (1 paper).

## 4.2 Publication bias and heterogeneity.

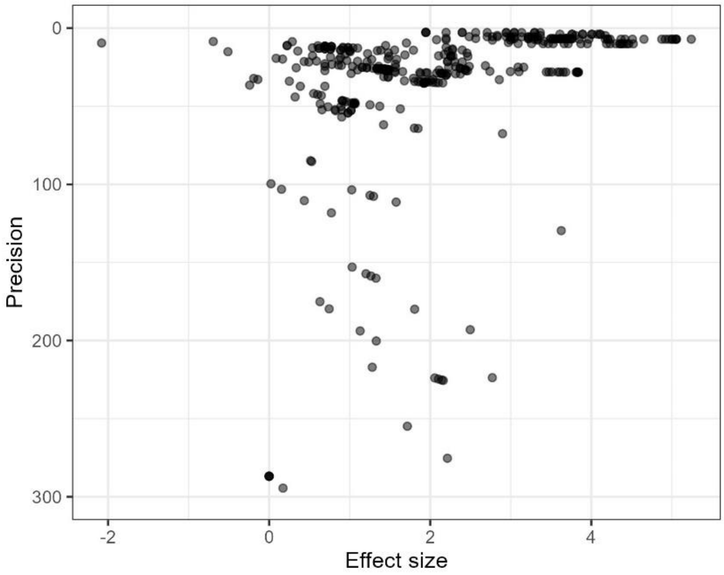


Figure 2: Funnel plot showing effect size (log risk ratio) on the x-axis and precision (1/standard error) on the y axis. Each dot represents one effect size. Asymmetry in the funnel plot indicates missing data.

As you can see in figure 2, there is a no evidence for publication bias. There is one small hole where the effect size is just over 2 and the precision is between 100 and 200. This could indicate that there is missing data but since there clearly are results with effect size around 2 and with medium high precision, it is unlikely that there are studies there, that did not get published and therefore caused a publication bias.

The calculated heterogeneity was 37% for the null model, which consisted of the response variable as well as the random effects, study ID, insect species and biopesticide. The null model ran for 20000 iterations, with 10000 of them being warm-ups, to get r-hat values below 1.02. Since the heterogeneity is above zero, the use of fixed effects is justified. In addition, the heterogeneity was not high enough for a meta-analysis to be inappropriate. Meaning the articles were not too different from one another to be able to synthesis their results.

## 4.3 Model construction and model fitting

In the end I had 33 studies with 18 different species, all within the order Hemiptera. The effect sizes, which consisted of log risk ratio of treatment mortality divided by control mortality, were clustered by study ID. My model also includes the fixed effects of days post treatment, larval instar, biopesticide dose and the random effects microbe biopesticide species as well as the insect species and phylogeny. A common way for the papers to measure the concentration was conidia per ml, which simplifies comparing the effect sizes between studies. Six studies reported spores/area or in a weight/weight concentration and they got excluded by the model and therefore only 27 studies with 14 insect species were included in the model. The final model, with both fixed and random effects, ran with 40000 iterations, 20000 of them being warmups; all R-hat values were 1.01 or lower which shows that the chains mixed well, and the result of the model can be interpreted.

### 4.4 Posterior distributions

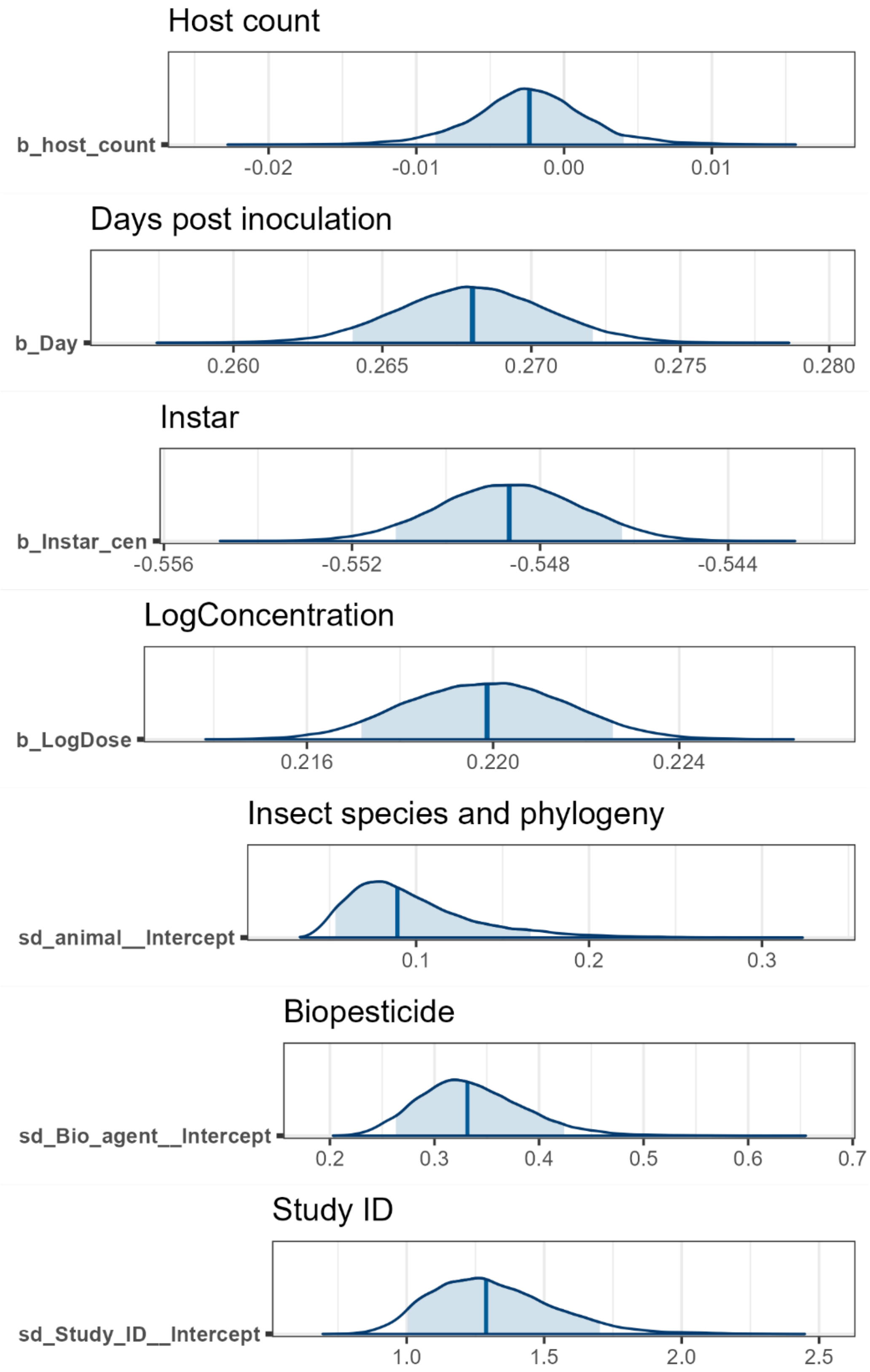


Figure 3: Shows the posterior distributions of the other effects in the model. Host count, days post inoculation, instar and concentration are fixed effects and therefore have a directional effect, where negative effect decreases mortality risk ratio, while positive values increase mortality risk ratio. Insect species, biopesticide and study ID are random effects and have no directional effects. The dark blue line shows median value, and the light blue part shows 89% highest density interval.

The posterior distribution, shown in figure 3, shows the probabilities of each factor's effect on mortality. Even if the distribution for host count overlap with zero effect, it is notable that most of it lies on the negative size, this means there is more evidence of the predicted effect than not. The effect shown is the effect on one added plant family in the host count of an insect species. The effect of days post inoculation and concentration are both positive, meaning an increase in days post inoculation or an increase in concentration will increase the insect's mortality risk. The negative effect of Instar level therefore means that as the insects age the mortality risk decreases. The effect of the random factors, insect species (with accounting for relatedness), biopesticide and study ID have no direction, but all of them have an effect since the posterior distributions does not include zero even though they are of different magnitudes.

## 4.5 Forest plot

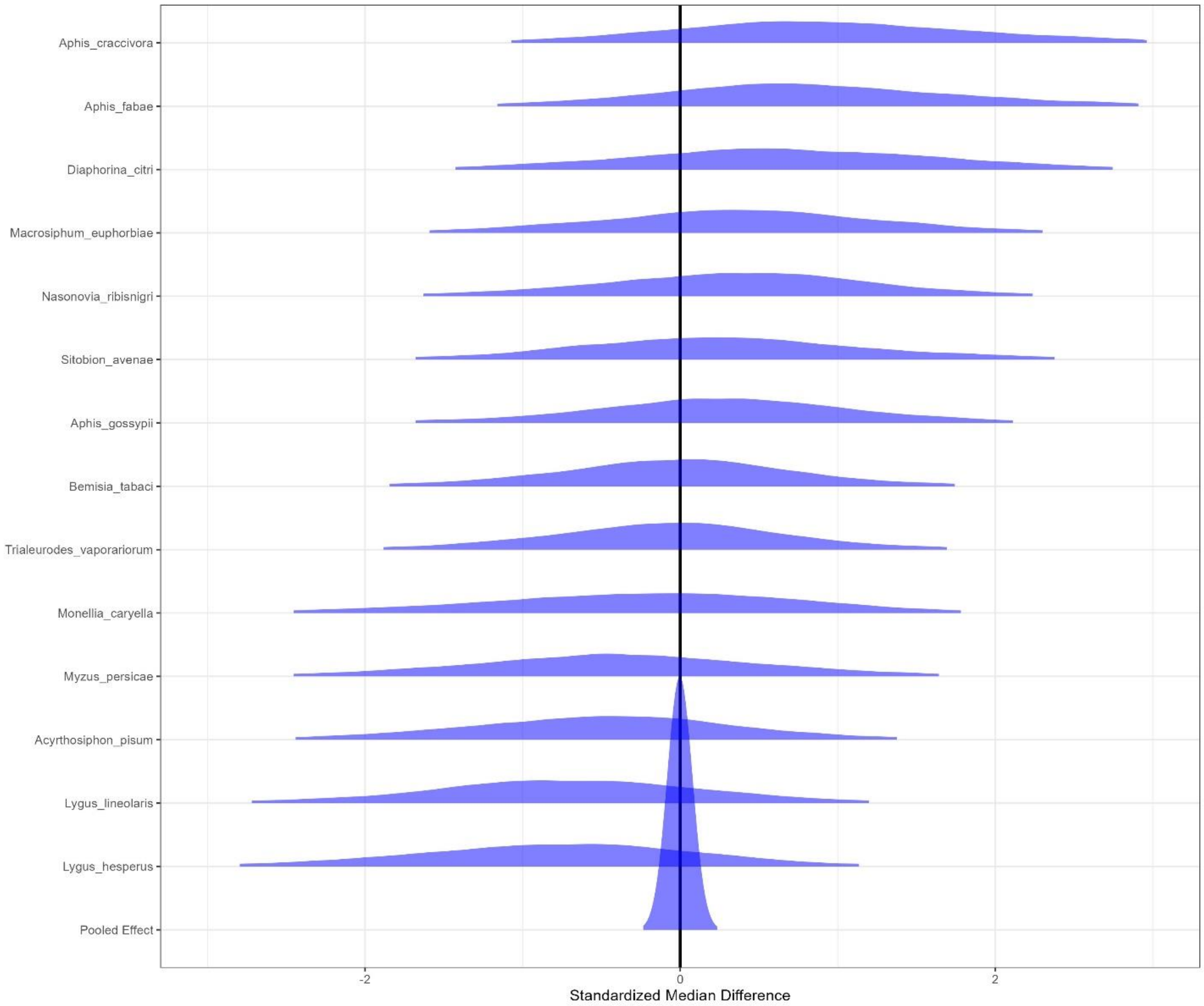
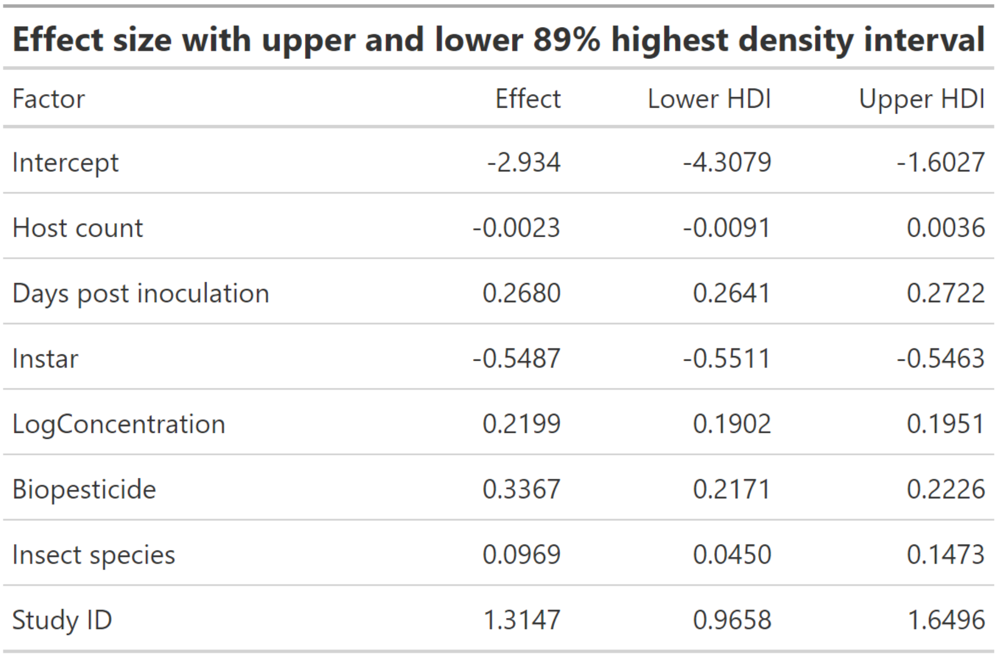


Figure 4: Forest plot showing the effect of host count stratified by insect species as well as the pooled effect of host count.

The effect of host count seems to vary a lot depending on the insect species as you can see in figure 4, and have much wider distributions compared to the pooled effect. The order of the species follows the order of the host counts effect and does not follow the number of plant host families the species feeds from.

## 4.6 Table

Table 1: Summary of the model which analyses host count effect on mortality as well as shows the effect of the moderating factors.



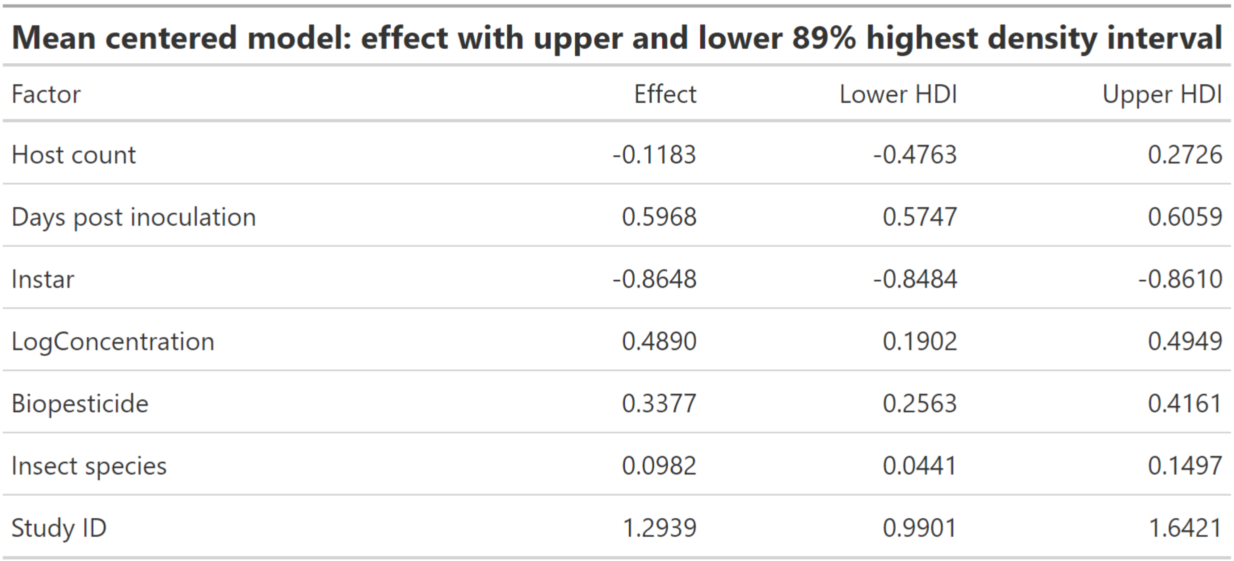
**Effect size with upper and lower 89% highest density interval**

| Factor | Effect | Lower HDI | Upper HDI |
|---|---:|---:|---:|
| Intercept | -2.934 | -4.3079 | -1.6027 |
| Host count | -0.0023 | -0.0091 | 0.0036 |
| Days post inoculation | 0.2680 | 0.2641 | 0.2722 |
| Instar | -0.5487 | -0.5511 | -0.5463 |
| LogConcentration | 0.2199 | 0.1902 | 0.1951 |
| Biopesticide | 0.3367 | 0.2171 | 0.2226 |
| Insect species | 0.0969 | 0.0450 | 0.1473 |
| Study ID | 1.3147 | 0.9658 | 1.6496 |

As shown in table 1, there is no clear effect of host count on mortality, even if the posterior distribution is centered on the negative side of zero. On another note, the moderating factors: days post inoculation, life stage and concentration affect all have an effect on mortality. The positive effect of concentration and days post inoculation means that as concentration or days post inoculation increases, the mortality of the insects increases. The negative effect of life stage means that as the life stage of the insect increases, the mortality decreases. The effects of the random factors, biopesticide, insect species and study ID are all evident though they are not directional. The intercept shows the overall effect size of the model.

## 4.7 Mean-centered model

Table 2: Summary of the model which analyses host count effect on mortality as well as shows the effect of the moderating factors in the model where the fixed effects were scaled.



**Mean centered model: effect with upper and lower 89% highest density interval**

| Factor | Effect | Lower HDI | Upper HDI |
|---|---:|---:|---:|
| Host count | -0.1183 | -0.4763 | 0.2726 |
| Days post inoculation | 0.5968 | 0.5747 | 0.6059 |
| Instar | -0.8648 | -0.8484 | -0.8610 |
| LogConcentration | 0.4890 | 0.1902 | 0.4949 |
| Biopesticide | 0.3377 | 0.2563 | 0.4161 |
| Insect species | 0.0982 | 0.0441 | 0.1497 |
| Study ID | 1.2939 | 0.9901 | 1.6421 |

In table 2, the fixed factors, host count, days post inoculation, instar and concentration are mean-centered and scaled. This means that the effect column shows the effect of one extra standard deviation of the factor and enables comparisons between the fixed factors. The graph shows that the effect of host count is in fact a lot smaller than the other fixed factors, as well as that instar level have the biggest effect on mortality the effect of instar is the largest.

# 5. Discussion

This meta-analysis further delved into any advantages insect pests may get from being polyphagous. The result of the study did not support my prediction of polyphagous pest being less susceptible to biopesticide and therefore having a lower mortality risk when being exposed to them, instead, the number of plant families an insect feeds from has no evident effect on the mortality risk.

## 5.1 Data extraction

As shown in the Prisma plot (Fig. 1), the most common reason for excluding a paper during the full-text screening was that the result was not reported in percent or proportion mortality. The alternative could be survival probability, LC50 or corrected mortality. Of these, corrected mortality was the most common one. Corrected mortality was always calculated with the Abbots formula (1925), and to be able to back transform the Abbots formula the control needed to be known. Therefore, there were several papers with not enough data to be included. One could of course contact the authors to ask for the control mortality or the raw data, but because of the time limits of the project I did not prioritize this. However, this is something that hopefully can be added in the future to gather more data. The difficulties I faced in extracting data for this synthesis also shows the importance of publishing data, especially if we want to be able to use meta-analyses to look at bigger questions than what one study can answer.

## 5.2 Heterogeneity

My null model had a heterogeneity of 37% which is considered low to moderate (Harrer et al ., 2021) and a meta-analysis is justiciable. While Hardy et al. (2018) did collect information from the literature, they did not do a meta regression but comparative models and therefore did not test for heterogeneity (Hardy et al., 2018) consequently, I cannot compare the heterogeneity of their model with mine.

## 5.3 Model fitting

My model needed a relatively high number of iterations to effectively sample the data. With the use of priors, you set restrictions for the sampling of the data which results in the need of fewer iterations. My model contained default priors of the brms function, since setting priors is difficult and nonintuitive in the beginning, the use of chosen priors it is possible that the model would have needed fewer iterations to get good r-hat values (Bürkner, 2017).

## 5.4 The effects on mortality

I find no strong evidence that an evolutionary history of feeding from different plant families gives insect species an advantage in handling novel biopesticides. Not only does its posterior distribution include 0, the effect of host count is also relatively small compared to the moderating factors in the model as shown in the mean-centered and scaled model, which shows the effect of one extra standard deviation instead of one extra level of the factor in question. The effect is about one fourth of the effect of concentration and one eighth of the effect of instar. Which would indicate that there is no real advantage with being polyphagous. The forest plot further underlines this since the effect one extra host count on mortality does not follow the trend of increasing host count of the insect's species.

The mean-centered and scaled model also shows that the instar level has the biggest effect on the other fixed factors, and it is about twice as big as that of concentration. Therefore, it could be more effective to continuously treat younger individuals can be more effective than using higher concentrations less often. However, this relationship would need further studies to see if this is the case. The random factors also have evident effects on mortality, all in different magnitude. The effect of insect species is the smallest and close to zero, this indicates that biopesticide affects difference species the same. However, different microbial species have the biggest effect on mortality, indicating that different microbial species have differences in how effective they are at killing insects.

If there is no or very little effect of polyphagia then this is of benefit to us, considering then polyphagous insect pests that are resistant to several synthetic insecticides might not be as hard to control with biopesticides. One example is *Bemisia tabaci*, who is a pest worldwide and can cause farmers to lose half of their yield, not only by feeding on the plant but also by transmitting diseases between plants; *Bemisia tabaci*, are also resistant to almost every chemical that is used as synthetic pesticides (Basit, 2019). Another polyphagous pest, *Aphis glossypii*, have also evolved resistance to most of the synthetic pesticides on the market (Herron et al., 2001). To compare the host count of these species, *Bemisia tabaci* feeds from 67 plant families and *Aphis glossypii* feeds from 171 plant families (Hardy et al., 2018). If these polyphagous pests do not have an advantage in handling biopesticides, it might mean that there won't be similarly frequent instances of pesticide resistance evolution in these pests.

If there is an effect of diet breadth on mortality, it will probably be much smaller than the effect of host count on synthetic insecticide in Hardy et al.'s (2018) study. This is likely caused by the differences in the insect's metabolic systems compared to the immune system, this could be due to the fact that microbes can evolve faster that the secondary metabolites in plants, due to their much shorter generation time compared to plants (Biere & Tack, 2013). This is just speculations and further studies would be needed to see if this is the case

In Hardy et al.'s (2018) study, they did one comparative model over all insect's orders in their data set, Hemiptera, Lepidoptera, Coleoptera, Diptera, Thysanoptera, and Hymenoptera; as well as one comparative model only including Hemipteran species. The models' result did not show any big differences (Hardy et al., 2018). This could indicate that adding additional insect orders to this meta-analysis would not have a big effect on the outcome, in addition there is also a chance that other insect order also will not show an advantage in being polyphagous.

## 5.5 Intergraded pest management

Intergraded pest management, IPM, are long term plans made specifically for each field to lessen the extent of insecticide resistance evolution and create more sustainable farming; the goal is to lessen the need of pesticides though field diversification and only use specific pesticides to the species causing problems; the goal is not to kill all insects but to keep one species from taking over (Barzman et al., 2015). Pesticide rotation can also be used minimize resistance evolution by exposing the pest to several pesticides, at different times or different places resulting in the insects not always being exposed to the same insecticide (Mangan et al., 2023).

Insects will evolve resistance to biopesticide even if they are not better predisposed at handling them or not. Still, information regarding how insects react to biopesticide is essential in minimizing resistance evolution and therefore keeping the effectiveness of the biopesticide is important. When exposed pesticides, insect pest are exposed to selection pressure, which causes them to evolve resistance (Gassmann et al., 2009). Hardy et al. (2018) discusses in their paper that the reasons for differences in evolutionary rates are due to physiological systems and that these could make resistance evolution faster for the polyphagous insects, as if the foundation is already set (Hardy et al., 2018). Now if insect species, no matter their diet breadth, have similar sensitivity to biopesticide it could mean that they will have similar rate of resistance evolution, since no one have any predisposed physiological functions and the biopesticide seem to put a similar selection pressure on the different species. A similar evolution rate would simplify the pesticide rotation since a set time interval would work for most species, when using biopesticide.

A bigger study could find a more precise effect of host count effect on mortality. Due to the few articles that have studied insect resistance or resistance evolution in insects with biopesticide, is it harder to do a meta-analysis looking at resistance in relation to the host count of a species. It might be possible to do experiments looking at biopesticide resistance evolution. However, if the goal is to use the information to create effective integrated management plans, then other studies which look at, for example, resistance evolution during pesticide rotation might be easier and supply similar information.

### 5.6 Conclusion

Although we cannot rule out an advantage of being polyphagous when exposed to biopesticides, we can quite safely conclude that it should be substantially smaller than the advantage of polyphagia when exposed to traditional, synthetic pesticides. None the less, further studies are needed of this and other factors behind resistance to biopesticides. This study has started to explore the relationship between diet breadth and susceptibility to biopesticides, and the possible applications of this to agriculture.

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