

Havi Fecundity Analysis

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Load Libraries

```
pacman::p_load(ggplot2, readxl, ggbeeswarm, readr, dplyr, formatR, tidyr, tidyverse,
  devtools, cowplot, knitr, emmeans, ggpmisc, lme4, lmerTest, RColorBrewer, viridis,
  install = FALSE)
```

Load Data

```
aphid_count <- read_excel("data/fecundity_assay_count.xlsx") %>%
  filter(alate_alive_r1 != "control") %>%
  filter(block != 1) %>%
  mutate(nymphs_r1 = as.numeric(nymphs_r1), nymphs_r2 = as.numeric(nymphs_r2))
```

```
## Warning: There were 2 warnings in `mutate()`.
## The first warning was:
## i In argument: `nymphs_r1 = as.numeric(nymphs_r1)`.
## Caused by warning:
## ! NAs introduced by coercion
## i Run `dplyr::last_dplyr_warnings()` to see the 1 remaining warning.
```

```

# filter by block
aphid_count_block2 <- aphid_count %>%
  filter(block == 2)
aphid_count_block3 <- aphid_count %>%
  filter(block == 3)

# === Pivot to long format and remove alates with 0 babies ===
aphid_long <- aphid_count %>%
  pivot_longer(cols = c(starts_with("nymphs_r"), starts_with("alate_alive_r")),
    names_to = c(".value", "round"), names_pattern = "(.*)_r(\\d)") %>%
  filter(nymphs > 0) %>%
  filter(block != 4)

```

Survival

Survival Calculations

```

# === Survival summary by block and round ===
survival_summary <- aphid_long %>%
  group_by(treatment, block, round) %>% # Group data by treatment condition, experimental block, and round
  summarise(
    # Count how many alates were alive in each group (handling NAs just in case)
    alive_n = sum(alate_alive == "Yes", na.rm = TRUE),
    # Count how many alates were dead in each group
    dead_n = sum(alate_alive == "No", na.rm = TRUE),
    # Compute total number of alates observed (alive + dead)
    total = alive_n + dead_n,
    # Calculate percent survival per block per round (0-100%)
    survival_pct = 100 * alive_n / total,
    # Drop grouping structure after summarising (avoids nested data frames in later steps)
    .groups = "drop"
  )

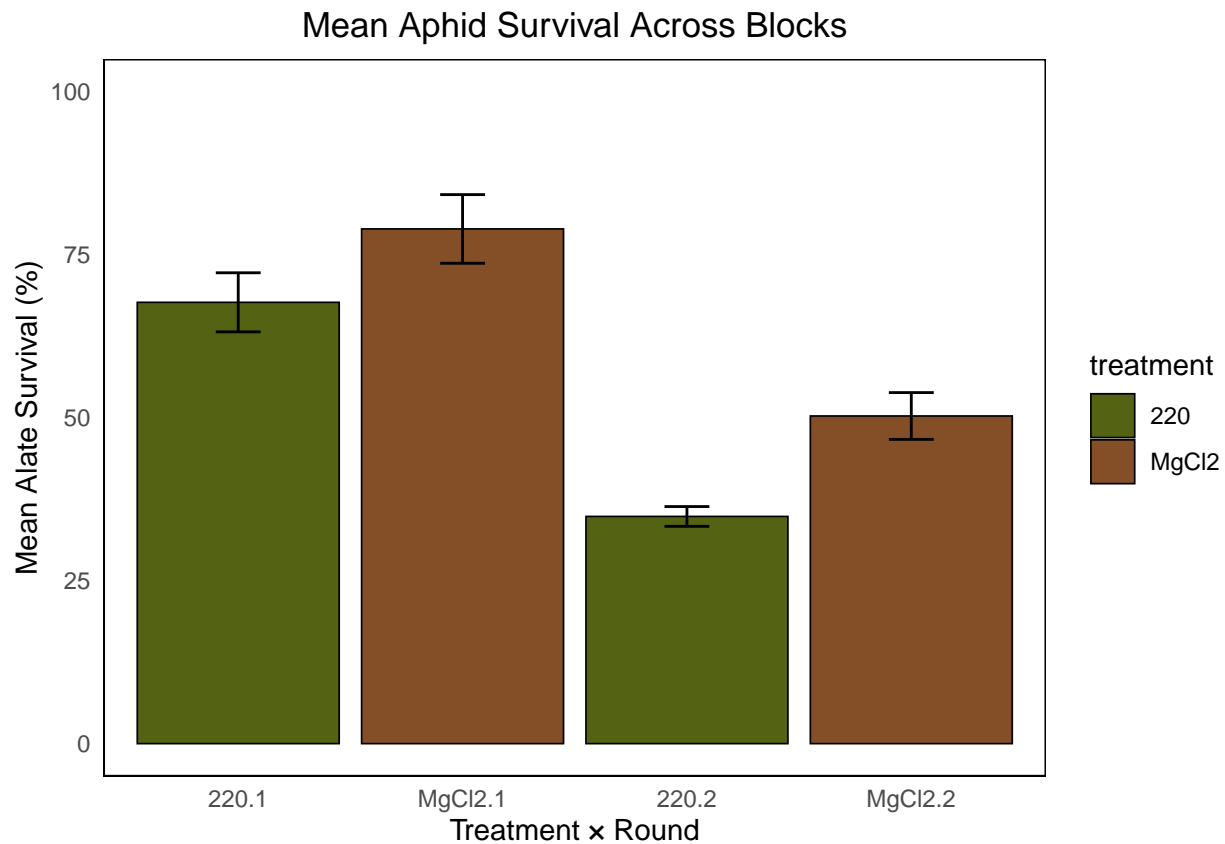
# === Average survival across blocks ===
survival_summary_avg <- survival_summary %>%
  group_by(treatment, round) %>% # Now group just by treatment and round (collapsed across blocks)
  summarise(
    # Compute the mean survival percentage across all blocks in each treatment/round
    mean_survival = mean(survival_pct, na.rm = TRUE),
    # Calculate standard error: standard deviation divided by square root of the number of blocks
    se_survival = sd(survival_pct, na.rm = TRUE) / sqrt(n()),
    # Drop grouping again for a clean summary table
    .groups = "drop"
  )

```

Survival Plots

```
# === All Blocks ===
ggplot(survival_summary_avg, aes(x = interaction(treatment, round), y = mean_survival,
  fill = treatment)) + geom_col(color = "black", size = 0.3, position = position_dodge(width = 0.9)) +
  geom_errorbar(aes(ymin = mean_survival - se_survival, ymax = mean_survival +
    se_survival), width = 0.2, color = "black", position = position_dodge(width = 0.9)) +
  labs(x = "Treatment × Round", y = "Mean Alate Survival (%)", title = "Mean Aphid Survival Across Blocks",
  scale_fill_manual(values = c("#546214", "#844e27")) + ylim(0, 100) + theme_minimal() +
  theme(legend.position = "right", panel.background = element_rect(fill = "white"),
    panel.grid = element_blank(), plot.title = element_text(hjust = 0.5))
```

```
## Warning: Using `size` aesthetic for lines was deprecated in ggplot2 3.4.0.
## i Please use `linewidth` instead.
## This warning is displayed once every 8 hours.
## Call `lifecycle::last_lifecycle_warnings()` to see where this warning was
## generated.
```



Fecundity

Fecundity Calculations

```

# === Step 1: Calculate fecundity summary by block and round ===
fecundity_by_block <- aphid_long %>%
  # Group data by treatment condition, block (replicate), and round (e.g. R1 vs R2)
  group_by(treatment, block, round) %>%
  # Summarise the fecundity statistics within each block × treatment × round group
  summarise(
    mean_fecundity = mean(nymphs, na.rm = TRUE), # Mean number of nymphs per alate
    se_nymphs = sd(nymphs, na.rm = TRUE) / sqrt(n()), # Standard error = SD / sqrt(n blocks)

    .groups = "drop" # Ungroup to simplify downstream summarization
  )

# === Step 2: Aggregate fecundity across blocks for each treatment and round ===
fecundity_by_treatment <- aphid_long %>%
  # Now group by treatment and round only (collapse across blocks)
  group_by(treatment, round) %>%
  # Summarise the average of block means and calculate standard error
  summarise(
    mean_nymphs = mean(nymphs, na.rm = TRUE), # Mean of block-level means
    se_nymphs = sd(nymphs, na.rm = TRUE) / sqrt(n()), # Standard error = SD / sqrt(n blocks)
    .groups = "drop" # Drop grouping for a flat summary table
  )

```

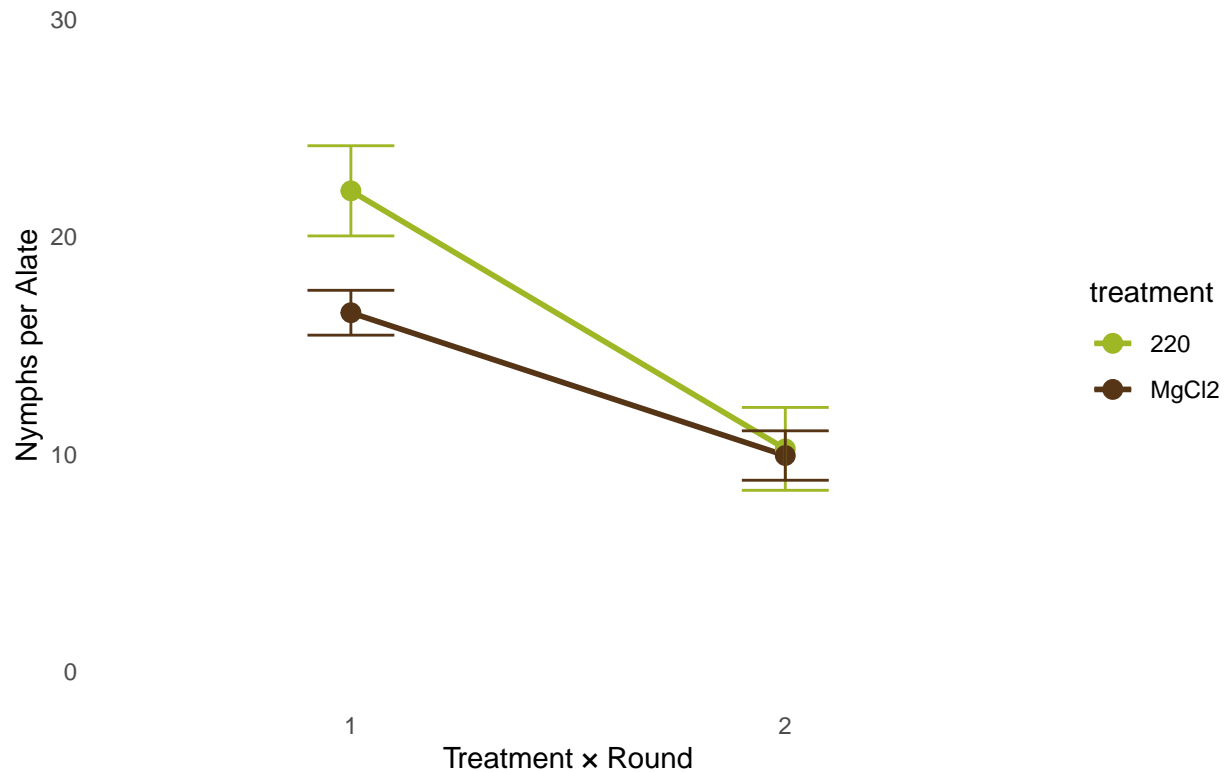
Fecundity Plots

```

# Line plot
ggplot(data = fecundity_by_treatment, aes(x = round, y = mean_nymphs, group = treatment,
  color = treatment)) + geom_line(size = 1) + geom_point(size = 3) + geom_errorbar(aes(ymin = mean_nymphs - se_nymphs,
  ymax = mean_nymphs + se_nymphs), width = 0.2) + labs(x = "Treatment × Round",
  y = "Nymphs per Alate", title = "Aphid Fecundity by Treatment and Round (Blocks 1-3)") +
  scale_color_manual(values = c("#9eb724", "#563517")) + ylim(0, 30) + theme_minimal() +
  theme(legend.position = "right", panel.grid = element_blank(), panel.grid.minor.x = element_blank())
  plot.title = element_text(hjust = 0.5))

```

Aphid Fecundity by Treatment and Round (Blocks 1–3)



```
# Plot: vertical boxplot with jitter
ggplot(aphid_long, aes(x = interaction(treatment, round), y = nymphs, fill = treatment)) +
  geom_boxplot(outlier.shape = NA, width = 0.5, alpha = 0.7, color = "black") +
  geom_jitter(width = 0.2, size = 2, alpha = 0.7, shape = 21, color = "black") +
  labs(title = "Aphid Fecundity by Treatment and Round (Blocks 1-3)", x = "Treatment x Round",
       y = "Nymphs per Alate") + scale_fill_manual(values = c("#546214", "#844e27")) +
  ylim(0, NA) + theme_minimal() + theme(legend.position = "none", panel.grid = element_blank(),
    plot.title = element_text(hjust = 0.5))
```

Aphid Fecundity by Treatment and Round (Blocks 1–3)



Statistics

alate death

```
# filter by round
surv_r1 <- survival_summary %>%
  filter(round == 1)
surv_r2 <- survival_summary %>%
  filter(round == 2)

# === Round 1 ===

ch_r1 <- glm(cbind(alive_n, dead_n) ~ treatment, family = binomial, data = surv_r1)

summary(ch_r1)
```

```
##
## Call:
## glm(formula = cbind(alive_n, dead_n) ~ treatment, family = binomial,
##      data = surv_r1)
##
## Coefficients:
##              Estimate Std. Error z value Pr(>|z|)
```

```
## (Intercept)      0.7340      0.3512    2.090    0.0366 *
## treatmentMgCl2   0.5878      0.5307    1.108    0.2681
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
## Null deviance: 2.23391 on 3 degrees of freedom
## Residual deviance: 0.98665 on 2 degrees of freedom
## AIC: 17.499
##
## Number of Fisher Scoring iterations: 4
```

```
# Pairwise comparisons (log-odds, back-transformed to probability)
emmeans(ch_r1, ~treatment, type = "response")
```

```
## treatment prob      SE df asymp.LCL asymp.UCL
## 220        0.676 0.0770 Inf      0.511      0.806
## MgCl2      0.789 0.0661 Inf      0.632      0.891
##
## Confidence level used: 0.95
## Intervals are back-transformed from the logit scale
```

```
# === Round 2 ===
```

```
ch_r2 <- glm(cbind(alive_n, dead_n) ~ treatment, family = binomial, data = surv_r2)
summary(ch_r2)
```

```
##
## Call:
## glm(formula = cbind(alive_n, dead_n) ~ treatment, family = binomial,
## data = surv_r2)
##
## Coefficients:
## Estimate Std. Error z value Pr(>|z|)
## (Intercept) -0.6061      0.5075  -1.194    0.232
## treatmentMgCl2  0.6061      0.6328   0.958    0.338
##
## (Dispersion parameter for binomial family taken to be 1)
##
## Null deviance: 1.09525 on 3 degrees of freedom
## Residual deviance: 0.15939 on 2 degrees of freedom
## AIC: 15.442
##
## Number of Fisher Scoring iterations: 3
```

```
emmeans(ch_r2, ~treatment, type = "response")
```

```
## treatment prob      SE df asymp.LCL asymp.UCL
## 220        0.353 0.1160 Inf      0.168      0.596
## MgCl2      0.500 0.0945 Inf      0.323      0.677
```

```
##
## Confidence level used: 0.95
## Intervals are back-transformed from the logit scale
```

Stats on alate fecundity

```
# filter by round
aphid_count_r1_alive_only <- aphid_count %>%
  filter(block != 4, alate_alive_r1 == "Yes") # Only include live alates in R1
aphid_count_r2_alive_only <- aphid_count %>%
  filter(block != 4, alate_alive_r2 == "Yes") # Only include live alates in R2

# Exclude round 4
aphid_count_alive_and_dead <- aphid_count %>%
  filter(block != 4)

# Only include aphids that had kids
aphid_count_alive_and_dead_yes_nymphs_r1 <- aphid_count %>%
  filter(block != 4, nymphs_r1 != 0)
aphid_count_alive_and_dead_yes_nymphs_r2 <- aphid_count %>%
  filter(block != 4, nymphs_r2 != 0)

# === Round 1: Alive only ===

ch_fec_r1_alive <- lm(nymphs_r1 ~ treatment, data = aphid_count_r1_alive_only)

summary(ch_fec_r1_alive)
```

```
##
## Call:
## lm(formula = nymphs_r1 ~ treatment, data = aphid_count_r1_alive_only)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -21.0    -5.2    -1.2     4.4    29.0
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    24.000      1.835   13.080 < 2e-16 ***
## treatmentMgCl2  -6.800      2.484   -2.737  0.00842 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 9.174 on 53 degrees of freedom
## Multiple R-squared:  0.1238, Adjusted R-squared:  0.1073
## F-statistic: 7.492 on 1 and 53 DF,  p-value: 0.008418
```

```
emmeans(ch_fec_r1_alive, ~treatment)
```

```
## treatment emmean SE df lower.CL upper.CL
## 220          24.0 1.83 53      20.3      27.7
```



```
## MgCl2      17.2 1.67 53      13.8      20.6
##
## Confidence level used: 0.95
```

```
# === Round 1: Alive and dead ===
```

```
ch_fec_r1 <- lm(nymphs_r1 ~ treatment, data = aphid_count_alive_and_dead)
summary(ch_fec_r1)
```

```
##
## Call:
## lm(formula = nymphs_r1 ~ treatment, data = aphid_count_alive_and_dead)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -20.450  -5.869  -0.062   5.800  32.550
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    20.450      1.708   11.970 <2e-16 ***
## treatmentMgCl2  -4.775      2.416   -1.976  0.0517 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 10.81 on 78 degrees of freedom
## Multiple R-squared:  0.04769,    Adjusted R-squared:  0.03548
## F-statistic: 3.906 on 1 and 78 DF,  p-value: 0.05165
```

```
emmeans(ch_fec_r1, ~treatment)
```

```
## treatment emmean SE df lower.CL upper.CL
## 220         20.4 1.71 78      17.0      23.9
## MgCl2       15.7 1.71 78      12.3      19.1
##
## Confidence level used: 0.95
```

```
# === Round 1: Alive and dead, yes kids ===
```

```
ch_fec_r1 <- lm(nymphs_r1 ~ treatment, data = aphid_count_alive_and_dead_yes_nymphs_r1)
summary(ch_fec_r1)
```

```
##
## Call:
## lm(formula = nymphs_r1 ~ treatment, data = aphid_count_alive_and_dead_yes_nymphs_r1)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -20.108  -5.804  -0.500   4.892  30.892
##
## Coefficients:
```

```
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)    22.108      1.636   13.510  <2e-16 ***
## treatmentMgCl2 -5.608      2.299   -2.439   0.0171 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 9.954 on 73 degrees of freedom
## Multiple R-squared:  0.07537,    Adjusted R-squared:  0.0627
## F-statistic: 5.951 on 1 and 73 DF,  p-value: 0.01714
```

```
emmeans(ch_fec_r1, ~treatment)
```

```
## treatment emmean SE df lower.CL upper.CL
## 220         22.1 1.64 73     18.8     25.4
## MgCl2       16.5 1.61 73     13.3     19.7
##
## Confidence level used: 0.95
```

```
# === Round 2: Alive only ===
```

```
ch_fec_r2_alive <- lm(nymphs_r2 ~ treatment, data = aphid_count_r2_alive_only)
summary(ch_fec_r2_alive)
```

```
##
## Call:
## lm(formula = nymphs_r2 ~ treatment, data = aphid_count_r2_alive_only)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -8.7857 -4.5060  0.2143  4.3333 12.2143
##
## Coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)    18.667      2.509    7.441 6.77e-07 ***
## treatmentMgCl2 -5.881      2.998   -1.961  0.0655 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 6.145 on 18 degrees of freedom
## Multiple R-squared:  0.1761, Adjusted R-squared:  0.1303
## F-statistic: 3.847 on 1 and 18 DF,  p-value: 0.0655
```

```
emmeans(ch_fec_r2_alive, ~treatment)
```

```
## treatment emmean SE df lower.CL upper.CL
## 220         18.7 2.51 18     13.40     23.9
## MgCl2       12.8 1.64 18      9.34     16.2
##
## Confidence level used: 0.95
```

```
# === Round 2: Alive and dead ===
```

```
ch_fec_r2 <- lm(nymphs_r2 ~ treatment, data = aphid_count_alive_and_dead)
```

```
summary(ch_fec_r2)
```

```
##
## Call:
## lm(formula = nymphs_r2 ~ treatment, data = aphid_count_alive_and_dead)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -9.267  -6.113  -1.960   4.733  20.040
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)      6.960      1.433   4.857 1.1e-05 ***
## treatmentMgCl2    2.307      1.940   1.189   0.24
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 7.165 on 53 degrees of freedom
## (25 observations deleted due to missingness)
## Multiple R-squared:  0.02597,    Adjusted R-squared:  0.007596
## F-statistic: 1.413 on 1 and 53 DF,  p-value: 0.2398
```

```
emmeans(ch_fec_r2, ~treatment)
```

```
## treatment emmean SE df lower.CL upper.CL
## 220          6.96 1.43 53     4.09     9.83
## MgCl2        9.27 1.31 53     6.64    11.89
##
## Confidence level used: 0.95
```

```
# === Round 2: Alive and dead, yes kids ===
```

```
ch_fec_r1 <- lm(nymphs_r2 ~ treatment, data = aphid_count_alive_and_dead_yes_nymphs_r2)
```

```
summary(ch_fec_r1)
```

```
##
## Call:
## lm(formula = nymphs_r2 ~ treatment, data = aphid_count_alive_and_dead_yes_nymphs_r2)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -9.235  -5.235  -1.235   4.071  16.765
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    10.2353     1.6403   6.240 1.64e-07 ***
## treatmentMgCl2  -0.3067     2.0795  -0.147   0.883
```

```
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 6.763 on 43 degrees of freedom
## Multiple R-squared:  0.0005057, Adjusted R-squared:  -0.02274
## F-statistic: 0.02176 on 1 and 43 DF,  p-value: 0.8834
```

```
emmeans(ch_fec_r1, ~treatment)
```

```
## treatment emmean SE df lower.CL upper.CL
## 220          10.24 1.64 43      6.93     13.5
## MgCl2         9.93 1.28 43      7.35     12.5
##
## Confidence level used: 0.95
```