# Pseudomonas Epiphytic Growth and Virulence Analysis

2024 Summer

## Contents



## Load Libraries

```
pacman::p_load(ggplot2, readxl, ggbeeswarm, dplyr, tidyverse, devtools, cowplot,
    knitr, survival, here, tibble, lubridate, formatR, gridExtra, ggsurvfit, gtsummary,
    tidycmprsk, install = FALSE)

# Load strain colors green = syringae, blue = fluorescencs, broum = parallactic
strain_colors <- c(`194` = "sienna", `200` = "dodgerblue", `204` = "dodgerblue2",
    `205` = "dodgerblue3", `215` = "springgreen3", `216` = "dodgerblue4", `220` = "sienna4",
    `221` = "deepskyblue", `227` = "deepskyblue2", `228` = "deepskyblue3", B728a = "springgreen4",
    Cit7 = "springgreen2", Control = "black", pisi = "springgreen1")
```

```
pseud_epi_growth_2024summer_R <- read_excel("~/Desktop/Cornell/Hendry Lab/pseud-epi-growth/pseud_epi_gr

aphid_virulence_data <- read_csv("~/Desktop/Cornell/Hendry Lab/pseud-epi-growth/others_data/virulence_n
```

```
## Rows: 4973 Columns: 6
## -- Column specification --------------------------------------------------------
## Delimiter: ","
## chr (3): date, treatment, replicate
## dbl (3): individual, censored, time
##
## i Use `spec()` to retrieve the full column specification for this data.
## i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

## Goals

- Create Kaplan-Meier curve for Pseud. virulence data
- Use stats (Wilcox?) to determine statistical significance of each strain
- Compare virulence data with epiphitic growth ability

## Virulence Analysis

Note: *In order to help me with this analysis, I am using the following sites - Survival Analysis in R and Hazard Ratio: Interpretation & Definition.*

### Subset Data

```
cit7_data <- subset(aphid_virulence_data, treatment == "Cit7")
```

### Caclulcate survival probabilities for each strain and create dataframe

```
# Fit the survival model
km_fit <- survfit(Surv(time, censored) ~ treatment, data = aphid_virulence_data)

# Extract survival probabilities at specific time points
time_points <- c(24, 48, 72)
km_summary <- summary(km_fit, times = time_points)

# Initialize empty lists to store the results
times_list <- list()
treatment_list <- list()
surv_prob_list <- list()

# Loop over each treatment group and extract survival probabilities at
# specified time points
for (i in 1:length(km_fit$strata)) {
    treatment_name <- names(km_fit$strata)[i]
    for (t in time_points) {
        idx <- which(km_summary$time == t & km_summary$strata == treatment_name)
        if (length(idx) > 0) {
            times_list <- c(times_list, t)
            treatment_list <- c(treatment_list, treatment_name)
```

```
            surv_prob_list <- c(surv_prob_list, km_summary$surv[idx])
        } else {
            times_list <- c(times_list, t)
            treatment_list <- c(treatment_list, treatment_name)
            surv_prob_list <- c(surv_prob_list, NA)
        }
    }
}

# Create the data frame
surv_probs <- data.frame(time = unlist(times_list), treatment = unlist(treatment_list),
    surv_prob = unlist(surv_prob_list))

# Replace 'treatment=' with an empty string
surv_probs$treatment <- gsub("treatment=", "", surv_probs$treatment)

# Print the data frame
print(surv_probs)
```

```
##    time treatment  surv_prob
## 1    24       194 0.77891156
## 2    48       194 0.18707483
## 3    72       194 0.12244898
## 4    24       200 0.53159851
## 5    48       200 0.05576208
## 6    72       200 0.02230483
## 7    24       204 0.54929577
## 8    48       204 0.08450704
## 9    72       204 0.05281690
## 10   24       205 0.56949153
## 11   48       205 0.11525424
## 12   72       205 0.07118644
## 13   24       215 0.79513889
## 14   48       215 0.44791667
## 15   72       215 0.22222222
## 16   24       216 0.56250000
## 17   48       216 0.27430556
## 18   72       216 0.15625000
## 19   24       220 0.35135135
## 20   48       220 0.19256757
## 21   72       220 0.17229730
## 22   24       221 0.65543071
## 23   48       221 0.21722846
## 24   72       221 0.09363296
## 25   24       227 0.40000000
## 26   48       227 0.22033898
## 27   72       227 0.16949153
## 28   24       228 0.65671642
## 29   48       228 0.15298507
## 30   72       228 0.10447761
## 31   24     B728a 0.68041237
## 32   48     B728a 0.40549828
## 33   72     B728a 0.10996564
```

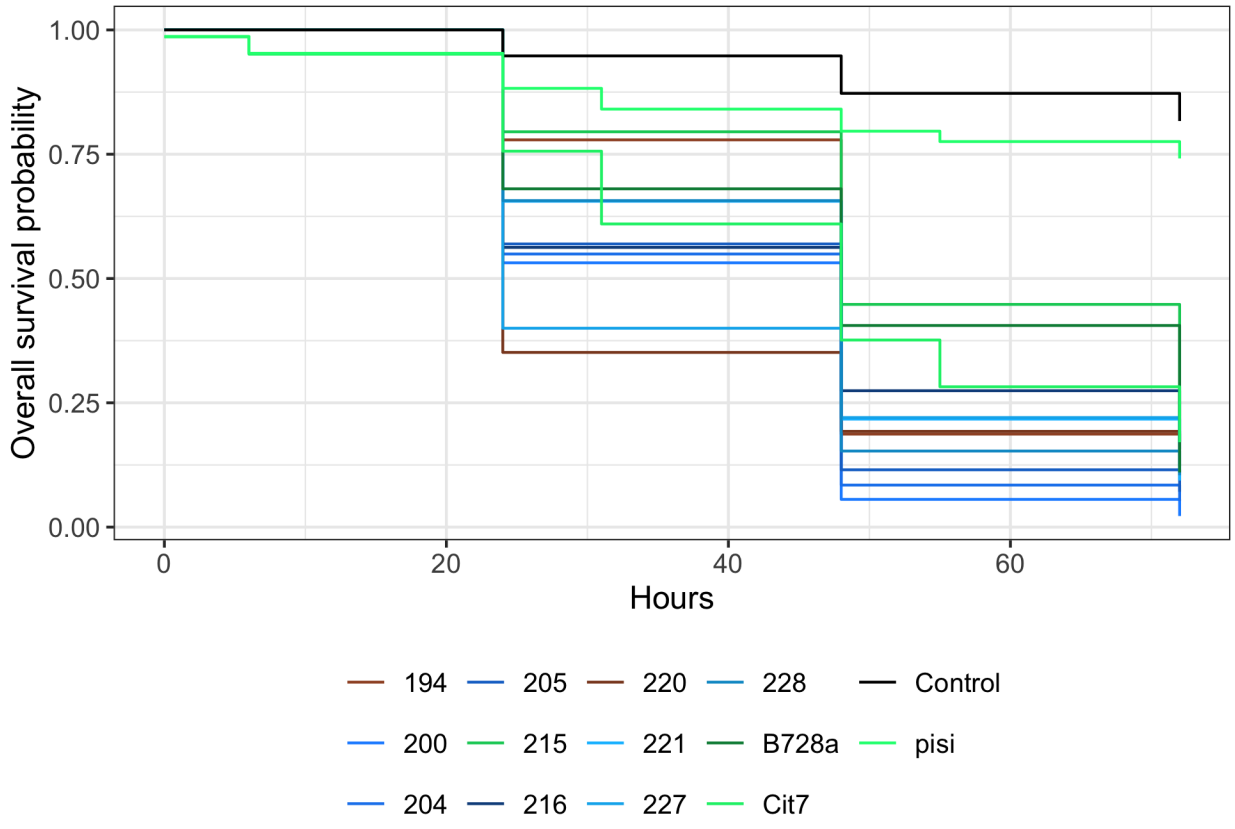
```
## 34   24      Cit7 0.75609756
## 35   48      Cit7 0.37630662
## 36   72      Cit7 0.17073171
## 37   24   Control 0.94777397
## 38   48   Control 0.87243151
## 39   72   Control 0.81678082
## 40   24      pisi 0.88250653
## 41   48      pisi 0.79634465
## 42   72      pisi 0.74151436
```

```
# If you need to save it to a file, you can use the following command
# write.csv(surv_probs, 'survival_probabilities.csv', row.names = FALSE)
```

## Make Kaplan-Meier Plot

### Cohort Survival Curve

```
survfit2(Surv(time, censored) ~ treatment, data = aphid_virulence_data) %>%
    ggsurvfit() + labs(x = "Hours", y = "Overall survival probability") + scale_color_manual(values = s
```



# Epiphytic Growth Analysis

## Calculate mean/variance epiphytic growth ability

```
# Replace NA with a lower value or remove them for visualization For this
# example, I'll remove rows with NA in CFU_per_10_leafdiscs
cleaned_data <- pseud_epi_growth_2024summer_R %>%
    filter(!is.na(CFU_per_10_leafdiscs))

# Convert CFU_per_10_leafdiscs to numeric, handling scientific notation
cleaned_data$CFU_per_10_leafdiscs <- as.numeric(gsub("<", "", cleaned_data$CFU_per_10_leafdiscs))
```

```
## Warning: NAs introduced by coercion
```

```
# Calculate mean and variance for each strain
strain_stats <- cleaned_data %>%
    group_by(strain) %>%
    summarise(mean_CFU = mean(CFU_per_10_leafdiscs, na.rm = TRUE), sd_CFU = sd(CFU_per_10_leafdiscs,
        na.rm = TRUE))

# Print the calculated statistics
print(strain_stats)
```

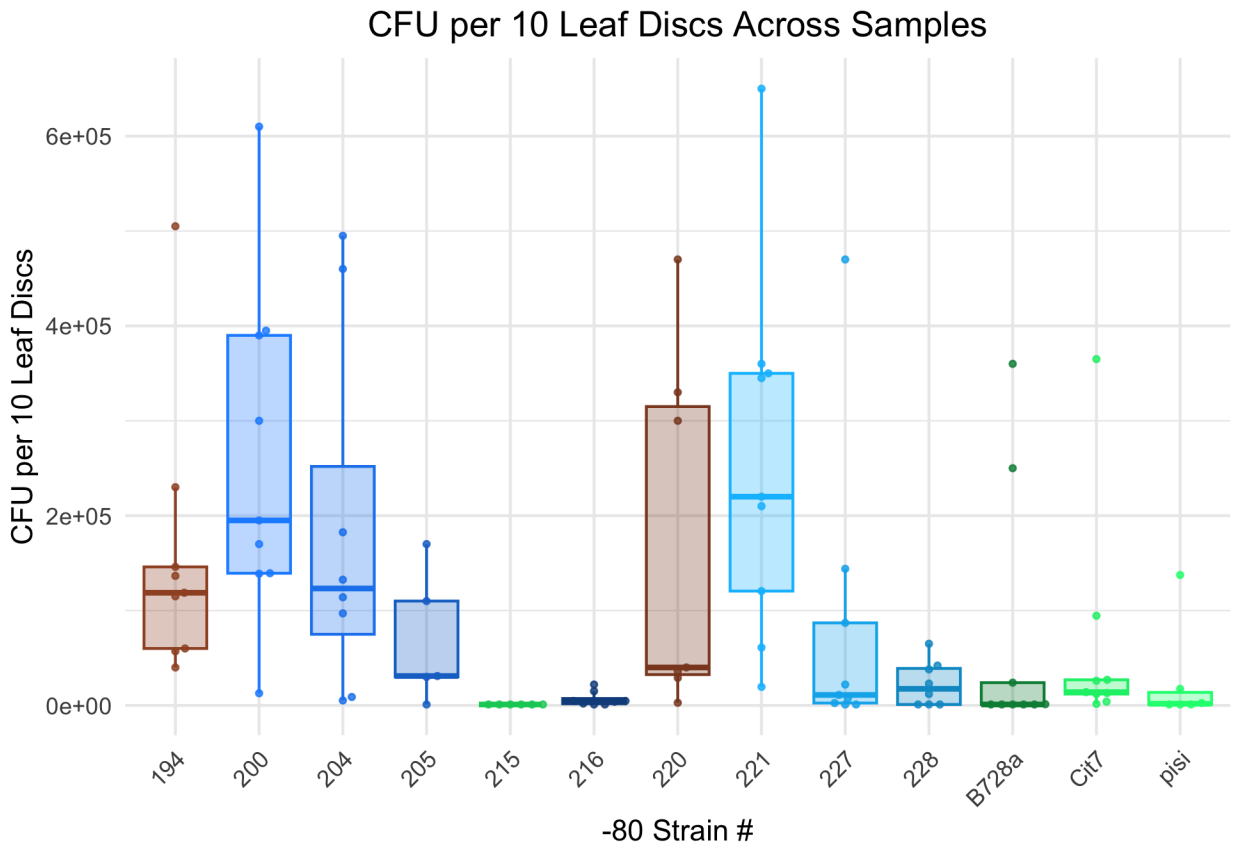
```
## # A tibble: 13 x 3
##    strain mean_CFU  sd_CFU
##    <chr>     <dbl>   <dbl>
##  1 194     156472. 142871.
##  2 200     261214. 180932.
##  3 204     186888. 189203.
##  4 205      68400   69766.
##  5 215       1000       0
##  6 216       6788.   7614.
##  7 220     172543. 189362.
##  8 221     259556. 193140.
##  9 227      82872. 153323.
## 10 228      22875   23673.
## 11 B728a    71144. 135614.
## 12 Cit7     61906. 117046.
## 13 pisi     26758.  54637.
```

## Plot Epiphytic Growth

```
# Create the box plot
ggplot(data = cleaned_data, aes(x = strain, y = CFU_per_10_leafdiscs, color = strain,
    fill = strain)) + geom_boxplot(outlier.shape = NA, alpha = 0.3) + geom_beeswarm(stroke = 0.5,
    size = 0.8, alpha = 0.8) + labs(title = "CFU per 10 Leaf Discs Across Samples",
    x = "-80 Strain #", y = "CFU per 10 Leaf Discs") + theme_minimal() + scale_fill_manual(values = str
    scale_color_manual(values = strain_colors) + theme(plot.title = element_text(hjust = 0.5),
    axis.text.x = element_text(angle = 45, hjust = 1), legend.position = "none")
```

```
## Warning: Removed 12 rows containing non-finite outside the scale range
## (`stat_boxplot()`).
```

```
## Warning: Removed 12 rows containing missing values or values outside the scale range
## (`geom_point()`).
```




## Combine epiphytic and virulence data

```
# Assuming strain_stats has a column 'strain' and surv_probs has a column
# 'treatment' Rename columns if necessary to match the key for joining
strain_stats <- strain_stats %>%
    rename(treatment = strain)

# Combine strain_stats and surv_probs using left_join
epi_virulence_data <- left_join(strain_stats, surv_probs, by = "treatment")

# Print the combined data
print(epi_virulence_data)
```

```
## # A tibble: 39 x 5
##    treatment mean_CFU  sd_CFU  time surv_prob
##    <chr>        <dbl>   <dbl> <dbl>     <dbl>
```

```
##  1 194        156472. 142871.    24    0.779
##  2 194        156472. 142871.    48    0.187
##  3 194        156472. 142871.    72    0.122
##  4 200        261214. 180932.    24    0.532
##  5 200        261214. 180932.    48    0.0558
##  6 200        261214. 180932.    72    0.0223
##  7 204        186888. 189203.    24    0.549
##  8 204        186888. 189203.    48    0.0845
##  9 204        186888. 189203.    72    0.0528
## 10 205         68400   69766.    24    0.569
## # i 29 more rows
```

```r
# Subset data for three time points
subset_data_24 <- epi_virulence_data %>%
    filter(time == 24)
subset_data_48 <- epi_virulence_data %>%
    filter(time == 48)
subset_data_72 <- epi_virulence_data %>%
    filter(time == 72)

# Calculate correlation coefficient between survival probability and epiphytic
# growth ability
correlation_24 <- cor(subset_data_24$surv_prob, subset_data_24$mean_CFU)
correlation_48 <- cor(subset_data_48$surv_prob, subset_data_48$mean_CFU)
correlation_72 <- cor(subset_data_72$surv_prob, subset_data_72$mean_CFU)
```

## Plot it for different times

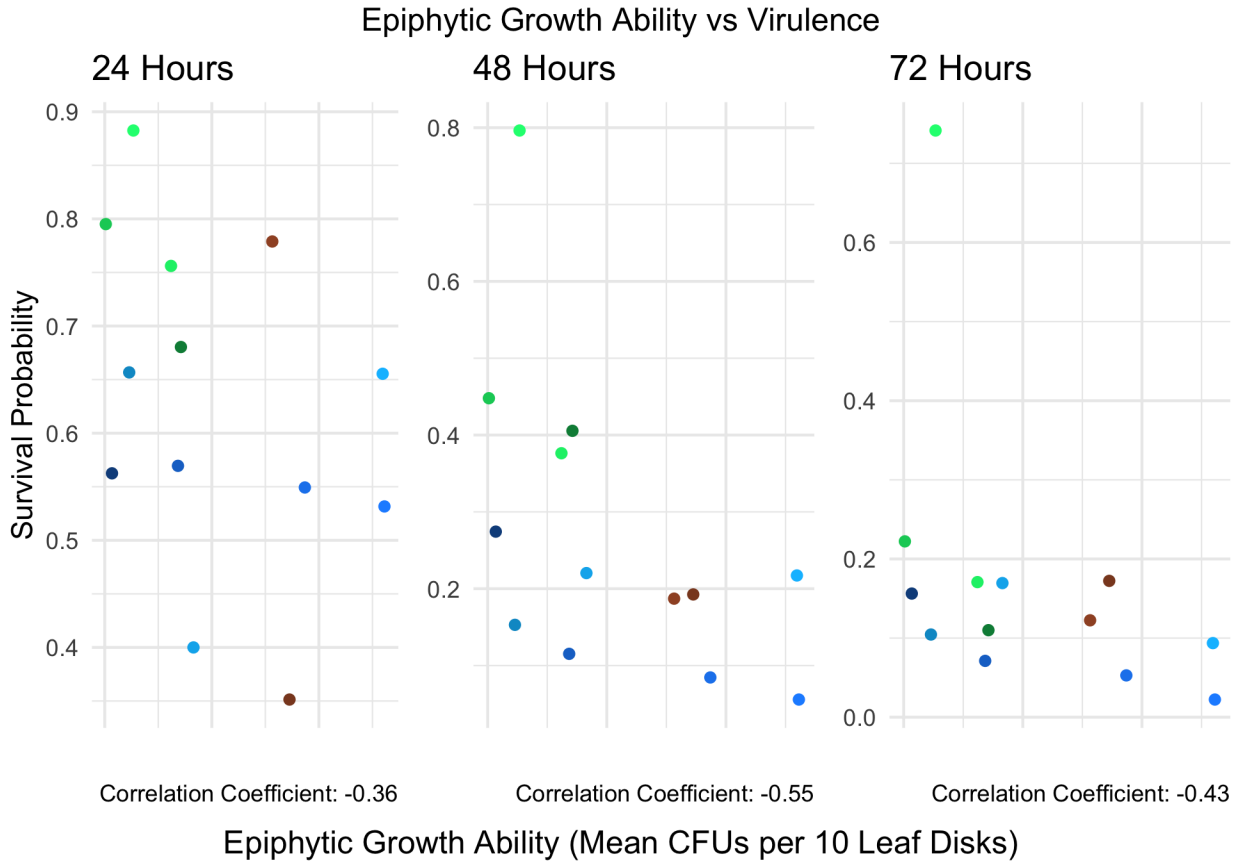
```r
# Create scatter plot for 24 hours
p_24 <- ggplot(subset_data_24, aes(x = mean_CFU, y = surv_prob, color = treatment)) +
  geom_point() +
  geom_smooth(method = "lm", se = FALSE) +
  labs(title = "24 Hours",
       x = "",  # Remove x-axis label
       y = "Survival Probability",
       caption = paste("Correlation Coefficient:", round(correlation_24, 2))) +
  scale_color_manual(values = strain_colors) +
  theme_minimal() +
  theme(legend.position = "none", axis.text.x = element_blank())  # Remove x-axis labels

# Create scatter plot for 48 hours
p_48 <- ggplot(subset_data_48, aes(x = mean_CFU, y = surv_prob, color = treatment)) +
  geom_point() +
  geom_smooth(method = "lm", se = FALSE) +
  labs(title = "48 Hours",
       x = "",  # Remove x-axis label
       y = "",  # Remove y-axis label
       caption = paste("Correlation Coefficient:", round(correlation_48, 2))) +
  scale_color_manual(values = strain_colors) +
  theme_minimal() +
  theme(legend.position = "none", axis.text.x = element_blank(), axis.title.y = element_blank())  # Rem
```

```r
# Create scatter plot for 72 hours
p_72 <- ggplot(subset_data_72, aes(x = mean_CFU, y = surv_prob, color = treatment)) +
  geom_point() +
  geom_smooth(method = "lm", se = FALSE) +
  labs(title = "72 Hours",
       x = "",  # Remove x-axis label
       y = "",  # Remove y-axis label
       caption = paste("Correlation Coefficient:", round(correlation_72, 2))) +
  scale_color_manual(values = strain_colors) +
  theme_minimal() +
  theme(legend.position = "none", axis.text.x = element_blank(), axis.title.y = element_blank())  # Rem

# Combine the plots
grid.arrange(p_24, p_48, p_72, nrow = 1,
             top = "Epiphytic Growth Ability vs Virulence",
             bottom = "Epiphytic Growth Ability (Mean CFUs per 10 Leaf Disks)")
```

```
## `geom_smooth()` using formula = 'y ~ x'
## `geom_smooth()` using formula = 'y ~ x'
## `geom_smooth()` using formula = 'y ~ x'
```



## Session Information

```
devtools::session_info()
```

```
## - Session info ---------------------------------------------------------------
##  setting  value
##  version  R version 4.4.0 (2024-04-24)
##  os       macOS Ventura 13.4
##  system   x86_64, darwin20
##  ui       X11
##  language (EN)
##  collate  en_US.UTF-8
##  ctype    en_US.UTF-8
##  tz       America/New_York
##  date     2024-06-06
##  pandoc   3.1.11 @ /Applications/RStudio.app/Contents/Resources/app/quarto/bin/tools/x86_64/ (via rma
##
## - Packages -------------------------------------------------------------------
##  ! package       * version date (UTC) lib source
##  P backports       1.4.1   2021-12-13 [?] CRAN (R 4.4.0)
##  P beeswarm        0.4.0   2021-06-01 [?] CRAN (R 4.4.0)
##  P bit             4.0.5   2022-11-15 [?] CRAN (R 4.4.0)
##  P bit64           4.0.5   2020-08-30 [?] CRAN (R 4.4.0)
##  P broom           1.0.6   2024-05-17 [?] CRAN (R 4.4.0)
##  P broom.helpers   1.15.0  2024-04-05 [?] CRAN (R 4.4.0)
##  P cachem          1.0.8   2023-05-01 [?] CRAN (R 4.4.0)
##  P cellranger      1.1.0   2016-07-27 [?] CRAN (R 4.4.0)
##  P cli             3.6.2   2023-12-11 [?] CRAN (R 4.4.0)
##  P colorspace      2.1-0   2023-01-23 [?] CRAN (R 4.4.0)
##  P cowplot       * 1.1.3   2024-01-22 [?] CRAN (R 4.4.0)
##  P crayon          1.5.2   2022-09-29 [?] CRAN (R 4.4.0)
##  P devtools      * 2.4.5   2022-10-11 [?] RSPM
##  P digest          0.6.35  2024-03-11 [?] CRAN (R 4.4.0)
##  P dplyr         * 1.1.4   2023-11-17 [?] CRAN (R 4.4.0)
##  P ellipsis        0.3.2   2021-04-29 [?] RSPM
##  P evaluate        0.23    2023-11-01 [?] CRAN (R 4.4.0)
##  P fansi           1.0.6   2023-12-08 [?] CRAN (R 4.4.0)
##  P farver          2.1.2   2024-05-13 [?] CRAN (R 4.4.0)
##  P fastmap         1.1.1   2023-02-24 [?] CRAN (R 4.4.0)
##  P forcats       * 1.0.0   2023-01-29 [?] CRAN (R 4.4.0)
##  P formatR       * 1.14    2023-01-17 [?] RSPM
##  P fs              1.6.4   2024-04-25 [?] CRAN (R 4.4.0)
##  P generics        0.1.3   2022-07-05 [?] CRAN (R 4.4.0)
##  P ggbeeswarm    * 0.7.2   2023-04-29 [?] CRAN (R 4.4.0)
##  P ggplot2       * 3.5.1   2024-04-23 [?] CRAN (R 4.4.0)
##  P ggsurvfit     * 1.1.0   2024-05-08 [?] CRAN (R 4.4.0)
##  P glue            1.7.0   2024-01-09 [?] CRAN (R 4.4.0)
##  P gridExtra     * 2.3     2017-09-09 [?] RSPM
##  P gt              0.10.1  2024-01-17 [?] CRAN (R 4.4.0)
##  P gtable          0.3.5   2024-04-22 [?] CRAN (R 4.4.0)
##  P gtsummary     * 1.7.2   2023-07-15 [?] CRAN (R 4.4.0)
##  P here          * 1.0.1   2020-12-13 [?] CRAN (R 4.4.0)
```

```
##  P hms             1.1.3   2023-03-21 [?] CRAN (R 4.4.0)
##  P htmltools       0.5.8.1 2024-04-04 [?] CRAN (R 4.4.0)
##  P htmlwidgets     1.6.4   2023-12-06 [?] CRAN (R 4.4.0)
##  P httpuv          1.6.15  2024-03-26 [?] RSPM
##  P knitr         * 1.46    2024-04-06 [?] CRAN (R 4.4.0)
##  P labeling        0.4.3   2023-08-29 [?] CRAN (R 4.4.0)
##  P later           1.3.2   2023-12-06 [?] RSPM
##  P lattice         0.22-6  2024-03-20 [?] CRAN (R 4.4.0)
##  P lifecycle       1.0.4   2023-11-07 [?] CRAN (R 4.4.0)
##  P lubridate     * 1.9.3   2023-09-27 [?] CRAN (R 4.4.0)
##  P magrittr        2.0.3   2022-03-30 [?] CRAN (R 4.4.0)
##  P Matrix          1.7-0   2024-03-22 [?] CRAN (R 4.4.0)
##  P memoise         2.0.1   2021-11-26 [?] CRAN (R 4.4.0)
##  P mime            0.12    2021-09-28 [?] CRAN (R 4.4.0)
##  P miniUI          0.1.1.1 2018-05-18 [?] RSPM
##  P munsell         0.5.1   2024-04-01 [?] CRAN (R 4.4.0)
##  P pacman          0.5.1   2019-03-11 [?] CRAN (R 4.4.0)
##  P pillar          1.9.0   2023-03-22 [?] CRAN (R 4.4.0)
##  P pkgbuild        1.4.4   2024-03-17 [?] RSPM
##  P pkgconfig       2.0.3   2019-09-22 [?] CRAN (R 4.4.0)
##  P pkgload         1.3.4   2024-01-16 [?] RSPM
##  P profvis         0.3.8   2023-05-02 [?] RSPM
##  P promises        1.3.0   2024-04-05 [?] RSPM
##  P purrr         * 1.0.2   2023-08-10 [?] CRAN (R 4.4.0)
##  P R6              2.5.1   2021-08-19 [?] CRAN (R 4.4.0)
##  P Rcpp            1.0.12  2024-01-09 [?] CRAN (R 4.4.0)
##  P readr         * 2.1.5   2024-01-10 [?] CRAN (R 4.4.0)
##  P readxl        * 1.4.3   2023-07-06 [?] CRAN (R 4.4.0)
##  P remotes         2.5.0   2024-03-17 [?] CRAN (R 4.4.0)
##    renv            1.0.7   2024-04-11 [1] CRAN (R 4.4.0)
##  P rlang           1.1.3   2024-01-10 [?] CRAN (R 4.4.0)
##  P rmarkdown       2.26    2024-03-05 [?] CRAN (R 4.4.0)
##  P rprojroot       2.0.4   2023-11-05 [?] CRAN (R 4.4.0)
##  P rstudioapi      0.16.0  2024-03-24 [?] CRAN (R 4.4.0)
##  P scales          1.3.0   2023-11-28 [?] CRAN (R 4.4.0)
##  P sessioninfo     1.2.2   2021-12-06 [?] RSPM
##  P shiny           1.8.1.1 2024-04-02 [?] RSPM
##  P stringi         1.8.3   2023-12-11 [?] CRAN (R 4.4.0)
##  P stringr       * 1.5.1   2023-11-14 [?] CRAN (R 4.4.0)
##  P survival      * 3.6-4   2024-04-24 [?] CRAN (R 4.4.0)
##  P tibble        * 3.2.1   2023-03-20 [?] CRAN (R 4.4.0)
##  P tidycmprsk    * 1.0.0   2023-10-30 [?] CRAN (R 4.4.0)
##  P tidyr         * 1.3.1   2024-01-24 [?] CRAN (R 4.4.0)
##  P tidyselect      1.2.1   2024-03-11 [?] CRAN (R 4.4.0)
##  P tidyverse     * 2.0.0   2023-02-22 [?] CRAN (R 4.4.0)
##  P timechange      0.3.0   2024-01-18 [?] CRAN (R 4.4.0)
##  P tzdb            0.4.0   2023-05-12 [?] CRAN (R 4.4.0)
##  P urlchecker      1.0.1   2021-11-30 [?] RSPM
##  P usethis       * 2.2.3   2024-02-19 [?] RSPM
##  P utf8            1.2.4   2023-10-22 [?] CRAN (R 4.4.0)
##  P vctrs           0.6.5   2023-12-01 [?] CRAN (R 4.4.0)
##  P vipor           0.4.7   2023-12-18 [?] CRAN (R 4.4.0)
##  P vroom           1.6.5   2023-12-05 [?] CRAN (R 4.4.0)
##  P withr           3.0.0   2024-01-16 [?] CRAN (R 4.4.0)
```

```
##  P xfun            0.43    2024-03-25 [?] CRAN (R 4.4.0)
##  P xml2            1.3.6   2023-12-04 [?] CRAN (R 4.4.0)
##  P xtable          1.8-4   2019-04-21 [?] RSPM
##  P yaml            2.3.8   2023-12-11 [?] CRAN (R 4.4.0)
##
##  [1] /Users/zahavahrojer/Desktop/Cornell/Hendry Lab/pseud-epi-growth/analysis/renv/library/macos/R-4
##  [2] /Users/zahavahrojer/Library/Caches/org.R-project.R/R/renv/sandbox/macos/R-4.4/x86_64-apple-darwi
##
##  P -- Loaded and on-disk path mismatch.
##
## ------------------------------------------------------------------------------
```