

Hurdle Model by Site: UBCO

```
#Load in necessary libraries  
  
## Load in necessary libraries  
library(readxl)  
  
## Warning: package 'readxl' was built under R version 4.3.3  
  
library(lme4)  
  
## Warning: package 'lme4' was built under R version 4.3.3  
  
## Loading required package: Matrix  
  
## Warning: package 'Matrix' was built under R version 4.3.3  
  
library(DAAG)  
  
## Warning: package 'DAAG' was built under R version 4.3.3  
  
library(ggplot2)  
  
## Warning: package 'ggplot2' was built under R version 4.3.3  
  
library(dplyr)  
  
## Warning: package 'dplyr' was built under R version 4.3.3  
  
##  
## Attaching package: 'dplyr'  
  
## The following objects are masked from 'package:stats':  
##  
##     filter, lag  
  
## The following objects are masked from 'package:base':  
##  
##     intersect, setdiff, setequal, union
```

```
library(MuMIn)

## Warning: package 'MuMIn' was built under R version 4.3.3

library(car)

## Warning: package 'car' was built under R version 4.3.3

## Loading required package: carData

## Warning: package 'carData' was built under R version 4.3.3

## 
## Attaching package: 'car'

## The following object is masked from 'package:dplyr':
##       recode

## The following object is masked from 'package:DAAG':
##       vif

library(viridis)

## Warning: package 'viridis' was built under R version 4.3.3

## Loading required package: viridisLite

library(DHARMa)

## Warning: package 'DHARMa' was built under R version 4.3.3

## This is DHARMa 0.4.7. For overview type '?DHARMa'. For recent changes, type news(package = 'DHARMa')

library(tidyr)

## Warning: package 'tidyr' was built under R version 4.3.3

## 
## Attaching package: 'tidyr'

## The following objects are masked from 'package:Matrix':
##       expand, pack, unpack
```

```

#Import and Clean Data
data <- read_excel("~/UBCO/Invasion publication/Invasion_publication/Invasion_197198_ID_FINAL.xlsx")

#Remove missing values (SampleID24, 2017)
#Convert NAs to proper data type
data[data == "NA"] <- NA
#Remove nas
data <- drop_na(data)

#Set seed
set.seed(123)

#Review data structure
summary(data)

```

```

##      Year       Plot      SampleID      Plant
##  Min.   :2014   Length:285      Min.   : 1.00  Min.   :1.000
##  1st Qu.:2014   Class  :character  1st Qu.: 6.00  1st Qu.:1.000
##  Median :2015   Mode   :character  Median :12.00  Median :3.000
##  Mean   :2015                    Mean   :12.38  Mean   :3.232
##  3rd Qu.:2016                    3rd Qu.:18.00  3rd Qu.:4.000
##  Max.   :2017                    Max.   :24.00  Max.   :5.000
##      Distance      Copies_ul
##  Length:285      Length:285
##  Class  :character  Class  :character
##  Mode   :character  Mode   :character
##
##
```

```
head(data)
```

```

## # A tibble: 6 x 6
##   Year Plot      SampleID Plant Distance Copies_ul
##   <dbl> <chr>     <dbl> <dbl> <chr>    <chr>
## 1 2014 Aberdeen     1     1 10      2.1
## 2 2014 Aberdeen     1     1 25      0.08
## 3 2014 Aberdeen     1     1 50      0
## 4 2014 Tutt         2     1 10      0.26
## 5 2014 Tutt         2     1 25      5.5
## 6 2014 Tutt         2     1 50     21.8

```

```
tail(data)
```

```

## # A tibble: 6 x 6
##   Year Plot      SampleID Plant Distance Copies_ul
##   <dbl> <chr>     <dbl> <dbl> <chr>    <chr>
## 1 2017 KM 2        22     5 10      0
## 2 2017 KM 2        22     5 25      0
## 3 2017 KM 2        22     5 50      0
## 4 2017 KM 4        23     5 10      1
## 5 2017 KM 4        23     5 25      0.25
## 6 2017 KM 4        23     5 50      0.23

```

```

#Convert copies/uL to numeric type
data$Copies_ul <- as.numeric(data$Copies_ul)

#Pool distances per plant (one observation/plant)
data <- data %>%
  group_by(SampleID, Year, Plant, Plot) %>%
  summarize(Copies_ul = sum(Copies_ul, na.rm = TRUE), .groups = "drop")
summary(data)

```

```

##      SampleID       Year      Plant      Plot
##  Min.   : 1.00   Min.   :2014   Min.   :1.000  Length:95
##  1st Qu.: 6.50   1st Qu.:2014   1st Qu.:2.000  Class  :character
##  Median :12.00   Median :2015   Median :3.000  Mode   :character
##  Mean   :12.38   Mean   :2015   Mean   :3.232
##  3rd Qu.:18.00   3rd Qu.:2016   3rd Qu.:4.000
##  Max.   :24.00   Max.   :2017   Max.   :5.000
##      Copies_ul
##  Min.   : 0.000
##  1st Qu.: 0.000
##  Median : 0.280
##  Mean   : 5.641
##  3rd Qu.: 2.740
##  Max.   :142.900

```

```

#Create Predictor Labels
#Plant 3 and 5 = inoculated (1), plant 1 and 4 = uninoculated (0)
#Plants 1 and 3 = achillea; 4 and 5, = artemesia
data <- data %>%
  mutate(
    hostID = ifelse(Plant > 3, "Artemesia", "Achillea"),
    inoculation = case_when(
      Plant %in% c(1,4) ~ 0L,
      Plant %in% c(3,5) ~ 1L,
      TRUE ~ NA_integer_
    ),
    Site = case_when(
      Plot %in% c("Aberdeen", "Quail", "Tutt") ~ "UBCO",
      Plot %in% c("KM 2", "KM 4", "KM 5") ~ "Knox",
      TRUE ~ NA_character_
    )
  )
head(data)

```

```

## # A tibble: 6 x 8
##   SampleID Year Plant Plot     Copies_ul hostID  inoculation Site
##       <dbl> <dbl> <dbl> <chr>      <dbl> <chr>      <int> <chr>
## 1       1   2014     1 Aberdeen     2.18 Achillea      0 UBCO
## 2       1   2015     1 Aberdeen     2.8  Achillea      0 UBCO
## 3       1   2016     1 Aberdeen     1.08 Achillea      0 UBCO
## 4       1   2017     1 Aberdeen     3.49 Achillea      0 UBCO
## 5       2   2014     1 Tutt        27.6 Achillea      0 UBCO
## 6       2   2015     1 Tutt        4.86 Achillea      0 UBCO

```

```

tail(data)

## # A tibble: 6 x 8
##   SampleID Year Plant Plot Copies_ul hostID    inoculation Site
##   <dbl> <dbl> <dbl> <chr>     <dbl> <chr>      <int> <chr>
## 1       23  2015     5 KM 4       0  Artemesia        1 Knox
## 2       23  2016     5 KM 4       0  Artemesia        1 Knox
## 3       23  2017     5 KM 4      1.48 Artemesia        1 Knox
## 4       24  2014     5 KM 5     11.8 Artemesia        1 Knox
## 5       24  2015     5 KM 5      3.5  Artemesia        1 Knox
## 6       24  2016     5 KM 5       0  Artemesia        1 Knox

#Filter to site (UBCO)
SITE <- "UBCO"
data <- data %>% filter(Site == SITE)

#Ensure all predictors and response are the correct type. Baseline year 2014 for
#intercept estimate
data <- data %>% mutate(
  hostID = factor(hostID),
  Site = factor(Site),
  Plot = factor(Plot),
  inoculation = factor(inoculation),
  Copies_ul = as.numeric(Copies_ul),
  Year = as.integer(Year),
  Year_centered = Year - 2014
)

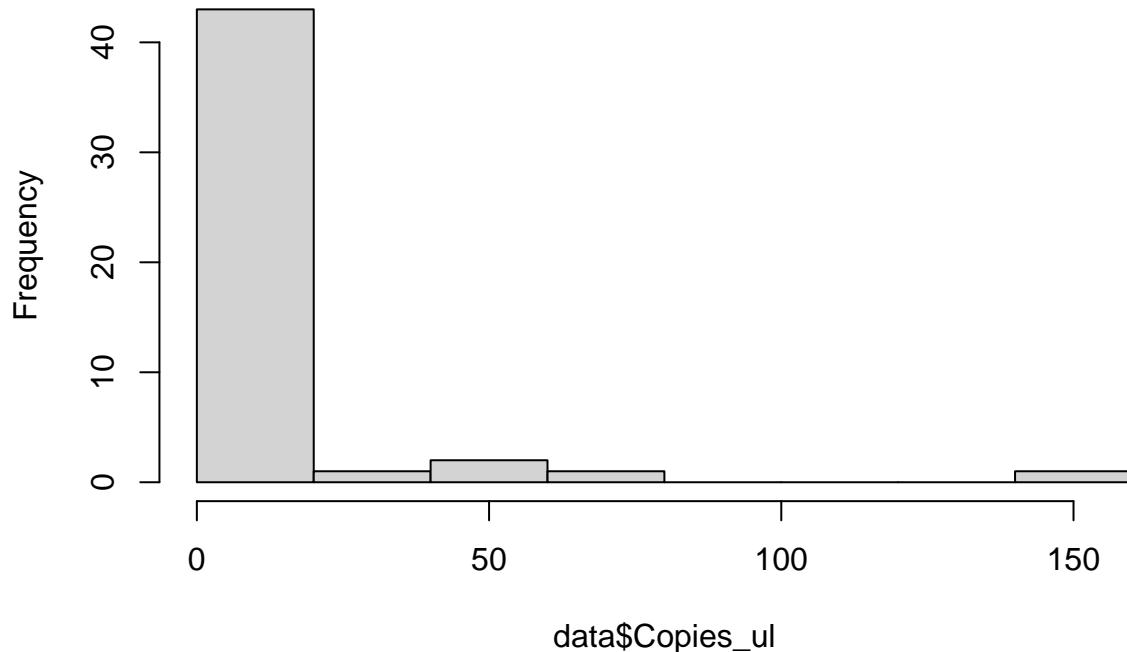
#Check zero inflation
data <- data %>% mutate(Presence = if_else(Copies_ul > 0, 1, 0))
prop_zero <- mean(data$Presence == 0)
print(prop_zero) #14%

## [1] 0.1458333

hist(data$Copies_ul) #zero inflation

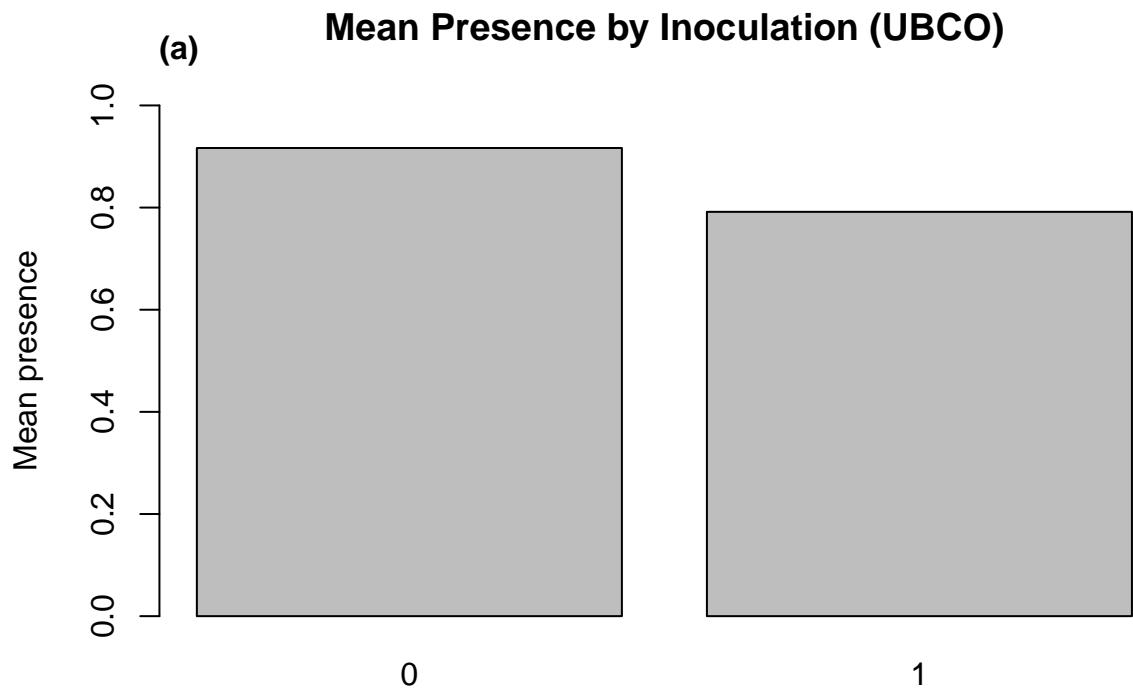
```

Histogram of data\$Copies_ul

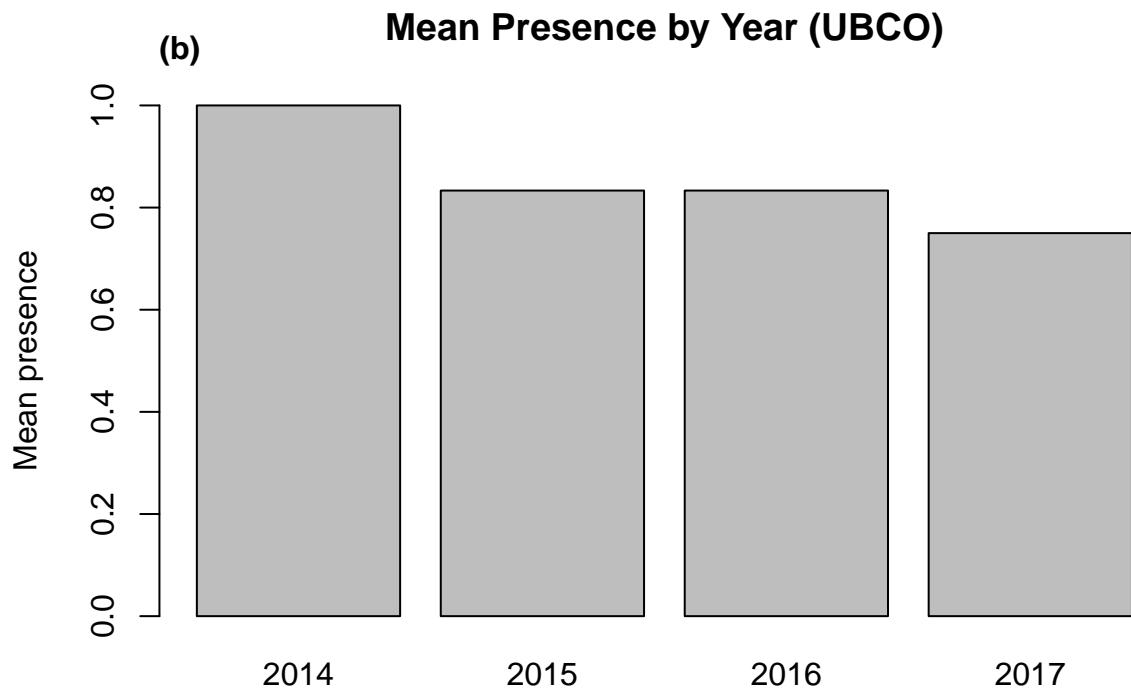


```
zero_df <- data %>% filter(Presence == 0)
View(zero_df)
SUBSET <- data %>% filter(Copies_ul > 0) %>% mutate(Year_centered = Year - 2014)

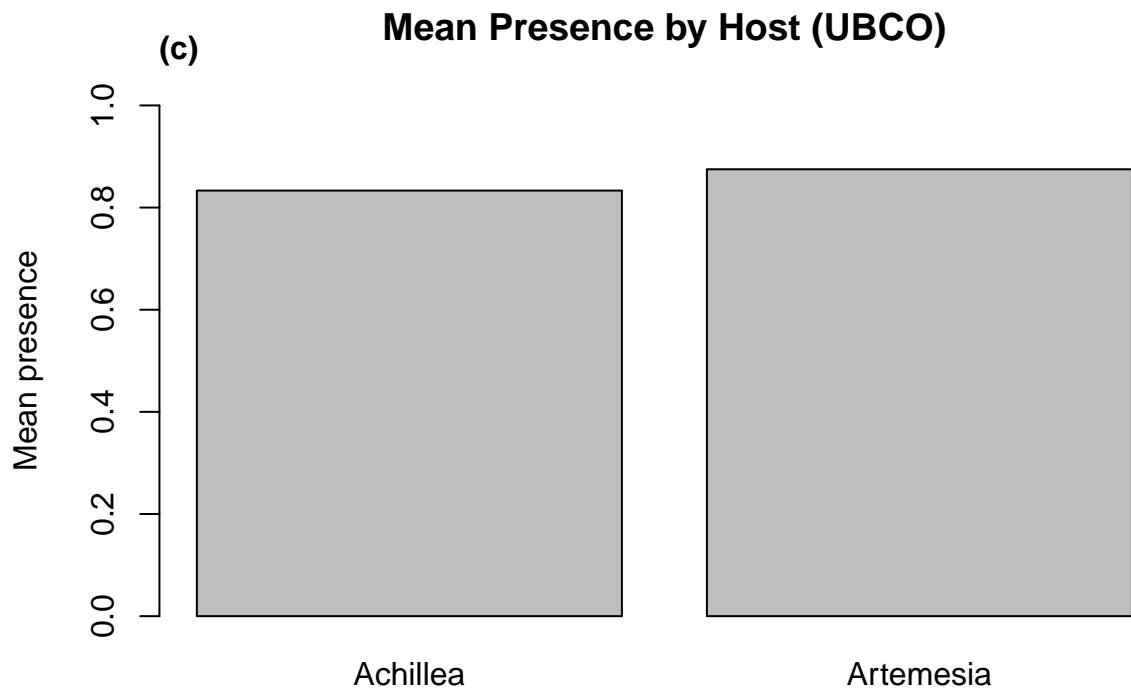
#Simple One-Way Visualizations
#Create the mean presence plots
#Inoculation
barplot(tapply(data$Presence, data$inoculation, mean, na.rm = TRUE),
        ylim = c(0, 1),
        ylab = "Mean presence",
        main = "Mean Presence by Inoculation (UBCO)")
mtext("(a)", side = 3, line = 1, adj = 0, font = 2) #Add label
```



```
#Year  
barplot(tapply(data$Presence, data$Year, mean, na.rm = TRUE),  
        ylim = c(0, 1),  
        ylab = "Mean presence",  
        main = "Mean Presence by Year (UBCO)")  
mtext("(b)", side = 3, line = 1, adj = 0, font = 2)
```



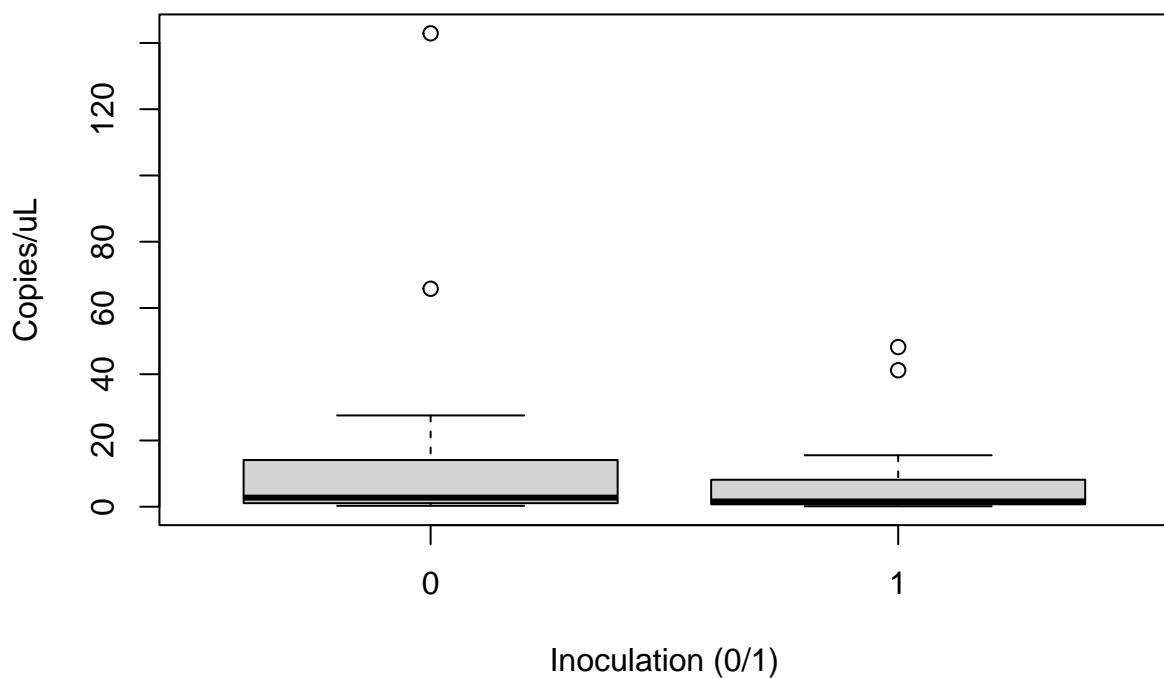
```
#hostID  
barplot(tapply(data$Presence, data$hostID, mean, na.rm = TRUE),  
       ylim = c(0, 1),  
       ylab = "Mean presence",  
       main = "Mean Presence by Host (UBCO)")  
mtext("(c)", side = 3, line = 1, adj = 0, font = 2)
```



```
#Median abundance
#Inoculation
boxplot(Copies_ul ~ inoculation, data = SUBSET,
        xlab = "Inoculation (0/1)",
        ylab = "Copies/uL",
        main = "Abundance by Inoculation")
mtext("(d)", side = 3, line = 1, adj = 0, font = 2)
```

(d)

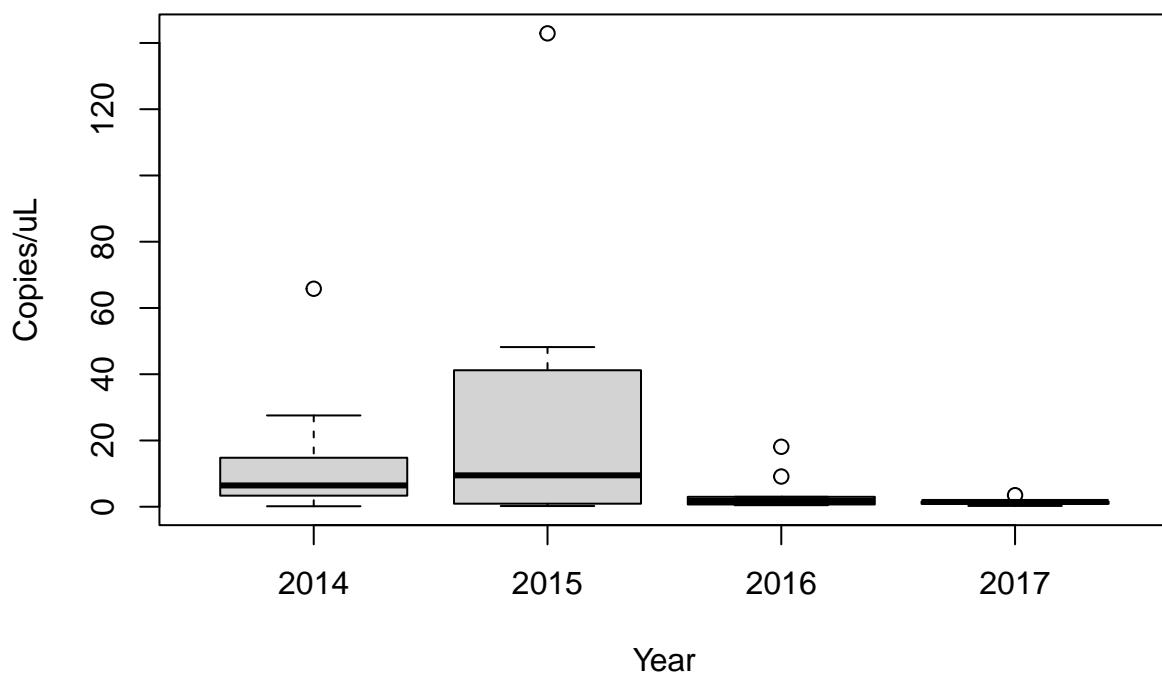
Abundance by Inoculation



```
#Year  
boxplot(Copies_ul ~ Year, data = SUBSET,  
        xlab = "Year", ylab = "Copies/uL", main = "Abundance by Year")  
mtext("(e)", side = 3, line = 1, adj = 0, font = 2)
```

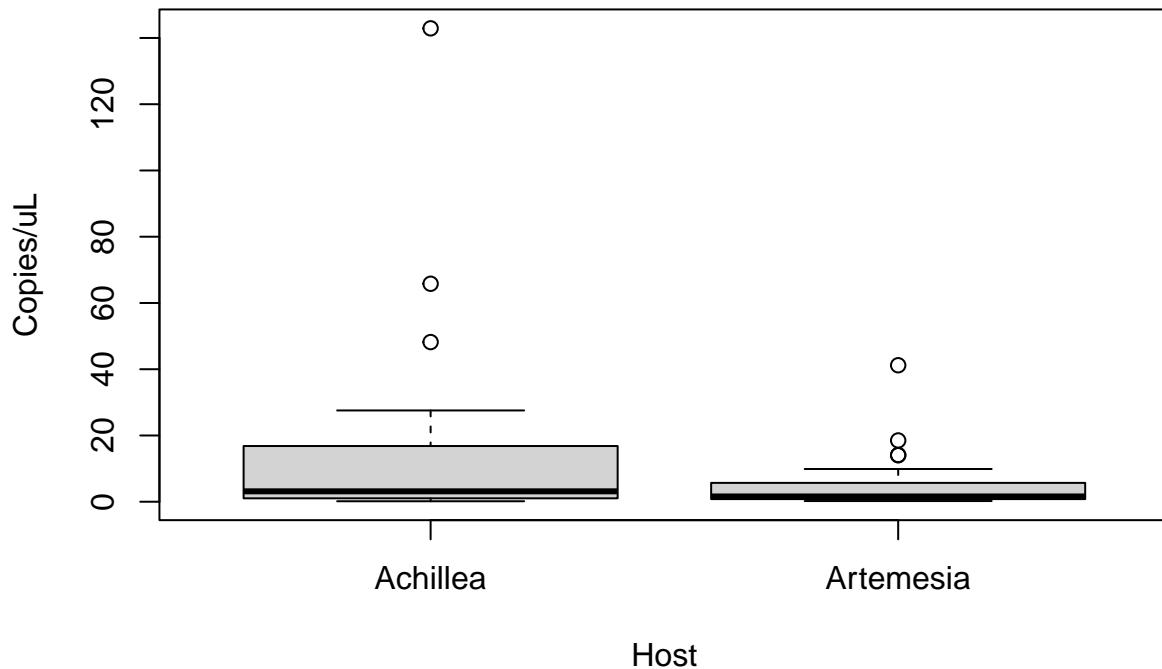
(e)

Abundance by Year



```
#HostID
boxplot(Copies_ul ~ hostID, data = SUBSET,
        xlab = "Host",
        ylab = "Copies/uL",
        main = "Abundance by Host")
mtext("(f)", side = 3, line = 1, adj = 0, font = 2)
```

(f)

Abundance by Host

```

dev.off()

## null device
##           1

#Check Possible Interactions
par(mfrow = c(1,1)) #Revert plot to 1 per row and column

#inoculation x hostID
interaction.plot(data$inoculation, data$hostID, data$Presence,
                 main= "UBCO hostID*inoculation",
                 xlab= "Inoculation",
                 ylab= "Mean presence",
                 legend= TRUE,
                 col= 1:length(unique(data$hostID)),
                 pch= 1:length(unique(data$hostID)),
                 type= "b")
#Likely interaction

#inoculation x Year
interaction.plot(data$Year, data$inoculation, data$Presence,
                 main= "UBCO hostID*inoculation",
                 xlab= "Year",
                 ylab= "Mean presence",
                 legend= TRUE,

```

```

            col= 1:length(unique(data$inoculation)),
            pch= 1:length(unique(data$inoculation)),
            type= "b")
#Possible interaction

#Binomial Model (Presence)
#Note: Plot is often singular or collinear with Site at UBCO; omit.
#Start with the simplest model
#Year as a predictor
binom_FIXED_1 <- glm(Presence ~ Year_centered,
                      family = binomial(link = "logit"),
                      data = data)
summary(binom_FIXED_1)

##
## Call:
## glm(formula = Presence ~ Year_centered, family = binomial(link = "logit"),
##      data = data)
##
## Coefficients:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept)  2.9426    0.9558   3.079  0.00208 **
## Year_centered -0.6595    0.4197  -1.571  0.11608
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
## Null deviance: 39.880 on 47 degrees of freedom
## Residual deviance: 37.047 on 46 degrees of freedom
## AIC: 41.047
##
## Number of Fisher Scoring iterations: 5

#Year+inoculation
binom_FIXED_2 <- glm(Presence ~ Year_centered + inoculation,
                      family = binomial(link = "logit"),
                      data = data)
summary(binom_FIXED_2)

##
## Call:
## glm(formula = Presence ~ Year_centered + inoculation, family = binomial(link = "logit"),
##      data = data)
##
## Coefficients:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept)  3.6554    1.1904   3.071  0.00214 **
## Year_centered -0.6835    0.4290  -1.593  0.11106
## inoculation1 -1.1299    0.9212  -1.226  0.22001
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##

```

```

## (Dispersion parameter for binomial family taken to be 1)
##
## Null deviance: 39.880 on 47 degrees of freedom
## Residual deviance: 35.402 on 45 degrees of freedom
## AIC: 41.402
##
## Number of Fisher Scoring iterations: 5

#Year+inoculation+hostID
binom_FIXED_3 <- glm(Presence ~ Year_centered + inoculation + hostID,
                      family = binomial(link = "logit"), data = data)
summary(binom_FIXED_3)

```

```

##
## Call:
## glm(formula = Presence ~ Year_centered + inoculation + hostID,
##      family = binomial(link = "logit"), data = data)
##
## Coefficients:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept) 3.4890    1.2406   2.812  0.00492 **
## Year_centered -0.6867    0.4302  -1.596  0.11043
## inoculation1 -1.1353    0.9237  -1.229  0.21900
## hostIDArtemesia 0.3732    0.8693   0.429  0.66772
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
## Null deviance: 39.880 on 47 degrees of freedom
## Residual deviance: 35.216 on 44 degrees of freedom
## AIC: 43.216
##
## Number of Fisher Scoring iterations: 5

```

```

#Compare cv binary
cv.binary(binom_FIXED_1)

```

```

##
## Fold: 3 10 2 6 5 4 9 8 7 1
## Internal estimate of accuracy = 0.854
## Cross-validation estimate of accuracy = 0.854

```

```

cv.binary(binom_FIXED_2)

```

```

##
## Fold: 4 6 8 7 1 2 5 3 9 10
## Internal estimate of accuracy = 0.854
## Cross-validation estimate of accuracy = 0.854

```

```

cv.binary(binom_FIXED_3)

## 
## Fold: 7 10 6 5 8 4 3 9 2 1
## Internal estimate of accuracy = 0.854
## Cross-validation estimate of accuracy = 0.833

#All comparable, year is likely driving presence

#Test whether adding the interaction improves model fit
binom_FIXED_4 <- glm(Presence ~ Year_centered * inoculation,
                      family = binomial(link = "logit"), data = data)
summary(binom_FIXED_4)

## 
## Call:
## glm(formula = Presence ~ Year_centered * inoculation, family = binomial(link = "logit"),
##      data = data)
##
## Coefficients:
##                               Estimate Std. Error z value Pr(>|z|)
## (Intercept)                 4.6532    2.4673   1.886   0.0593 .
## Year_centered              -1.1171    0.9623  -1.161   0.2457
## inoculation1               -2.4136    2.6830  -0.900   0.3683
## Year_centered:inoculation1  0.5823    1.0798   0.539   0.5897
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
## Null deviance: 39.880  on 47  degrees of freedom
## Residual deviance: 35.075  on 44  degrees of freedom
## AIC: 43.075
##
## Number of Fisher Scoring iterations: 6

#Mixed-Effects Binomial Models
#Add a random effects structure to account for repeated measure across years
binom_RI <- glmer(Presence ~ Year_centered + inoculation + hostID +
                    + (1 | SampleID),
                    family = binomial(link = "logit"),
                    data = data)
summary(binom_RI)

## Generalized linear mixed model fit by maximum likelihood (Laplace
## Approximation) [glmerMod]
## Family: binomial ( logit )
## Formula: Presence ~ Year_centered + inoculation + hostID + (1 | SampleID)
## Data: data
##
##      AIC      BIC  logLik deviance df.resid
##      45.2     54.5    -17.6     35.2       43

```

```

## 
## Scaled residuals:
##    Min     1Q Median     3Q    Max
## -3.2641  0.1538  0.2880  0.4398  0.9481
## 
## Random effects:
##   Groups   Name        Variance Std.Dev.
##   SampleID (Intercept) 0.2727   0.5222
##   Number of obs: 48, groups: SampleID, 12
## 
## Fixed effects:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept)  3.6559    1.5149   2.413   0.0158 *
## Year_centered -0.7118    0.4528  -1.572   0.1159
## inoculation1 -1.1556    0.9948  -1.162   0.2454
## hostIDArtemesia  0.3212    0.9672   0.332   0.7398
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## 
## Correlation of Fixed Effects:
##          (Intr) Yr_cnt inclt1
## Year_centrld -0.721
## inoculatin1 -0.539  0.114
## hostIDArtms -0.382  0.021  0.049

```

#Sample ID does explain some variance

```

#Remove hostID to decrease model complexity
binom_RI_2 <- glmer(Presence ~ Year_centered + inoculation
+ (1 | SampleID),
family = binomial(link = "logit"),
data = data)
summary(binom_RI_2)

```

```

## Generalized linear mixed model fit by maximum likelihood (Laplace
## Approximation) [glmerMod]
## Family: binomial ( logit )
## Formula: Presence ~ Year_centered + inoculation + (1 | SampleID)
## Data: data
## 
##      AIC      BIC  logLik deviance df.resid
##      43.3     50.8    -17.6     35.3      44
## 
## Scaled residuals:
##    Min     1Q Median     3Q    Max
## -2.9797  0.1634  0.2788  0.3996  0.9336
## 
## Random effects:
##   Groups   Name        Variance Std.Dev.
##   SampleID (Intercept) 0.4132   0.6428
##   Number of obs: 48, groups: SampleID, 12
## 
## Fixed effects:
##             Estimate Std. Error z value Pr(>|z|)
## 
```

```

## (Intercept) 3.8848    1.4325    2.712  0.00669 **
## Year_centered -0.7198   0.4541   -1.585  0.11295
## inoculation1 -1.1856   1.0328   -1.148  0.25098
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr) Yr_cnt
## Year_centrld -0.760
## inoculatin1 -0.585  0.124

#model with and without hostID are near identical, exclude host ID

#Compare AIC of each model
AIC(binom_FIXED_1, binom_FIXED_2, binom_FIXED_3, binom_RI, binom_RI_2)

##           df      AIC
## binom_FIXED_1 2 41.04744
## binom_FIXED_2 3 41.40221
## binom_FIXED_3 4 43.21621
## binom_RI      5 45.16251
## binom_RI_2    4 43.26681

#No statistical difference between binom_RI_2 and all other models (delta AIC<2)
#Continue with the mixed effects model that includes inoculation and year as
#the two fixed effect predictors, as these are the two predictors of interest

#Residual Diagnostics (DHARMA)
#Simluate residuals using DHARMA
binom_RI_res_2 <- simulateResiduals(binom_RI)
#Plot simulated residuals
plot(binom_RI_res_2)#No issues detected
#Test dispersion
print(testDispersion(binom_RI_res_2)) #No significant issues

## 
##  DHARMA nonparametric dispersion test via sd of residuals fitted vs.
##  simulated
##
##  data: simulationOutput
##  dispersion = 1.0674, p-value = 0.84
##  alternative hypothesis: two.sided

#Test for significant outliers
print(testOutliers(binom_RI_res_2))#No significant issues

## 
##  DHARMA bootstrapped outlier test
##
##  data: binom_RI_res_2
##  outliers at both margin(s) = 0, observations = 48, p-value = 1
##  alternative hypothesis: two.sided

```

```

## percent confidence interval:
## 0 0
## sample estimates:
## outlier frequency (expected: 0.000208333333333333 ) 0
## 0

#Test for temporal autocorrelation
testTemporalAutocorrelation(
  DHARMa::recalculateResiduals(binom_RI_res_2, group = data$Year_centered),
  time = sort(unique(data$Year_centered))
)

## Durbin-Watson test
## data: simulationOutput$scaledResiduals ~ 1
## DW = 2.0746, p-value = 0.8989
## alternative hypothesis: true autocorrelation is not 0

#Years are not autocorrelated. Residuals suggest a time pattern not captured
#by the model may be present

#Check residuals for each predictor
plotResiduals(binom_RI_res_2, data$Year_centered)
plotResiduals(binom_RI_res_2, data$inoculation)

#Check for collinearity among predictors
performance::check_collinearity(binom_RI_2) #No collinearity

## # Check for Multicollinearity
##
## Low Correlation
##
##           Term   VIF      VIF 95% CI adj. VIF Tolerance Tolerance 95% CI
## Year_centered 1.02 [1.00, 2.61e+05]     1.01      0.98      [0.00, 1.00]
## inoculation   1.02 [1.00, 2.61e+05]     1.01      0.98      [0.00, 1.00]

#Move forward with the mixed effects model (inoculation + year + (1/SampleID)),
#Inoculation and Year are chosen as the final predictors, as these are the
#variables of interest. HostID adds complexity and does not enhance model fit.

#Gamma Model (Abundance)
#Create a histogram of the non-zero observations
hist(SUBSET$Copies_ul,
  breaks=20,
  main="Copies/pL distribution",
  xlab="Copies/pL")

#Start with the simplest model (Year as singular predictor)
gamma_1<- glm(Copies_ul ~ Year_centered,
               family = Gamma(link = "log"),
               data = SUBSET)
summary(gamma_1)

```

```

##  

## Call:  

## glm(formula = Copies_ul ~ Year_centered, family = Gamma(link = "log"),  

##      data = SUBSET)  

##  

## Coefficients:  

##              Estimate Std. Error t value Pr(>|t|)  

## (Intercept)    3.4827    0.4808   7.243 9.96e-09 ***  

## Year_centered -0.9464    0.2690  -3.518  0.00112 **  

## ---  

## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1  

##  

## (Dispersion parameter for Gamma family taken to be 3.745109)  

##  

## Null deviance: 110.463  on 40  degrees of freedom  

## Residual deviance: 85.714  on 39  degrees of freedom  

## AIC: 257.65  

##  

## Number of Fisher Scoring iterations: 12

```

```

#Year+inoculation
gamma_2<- glm(Copies_ul ~ Year_centered + inoculation,
               family = Gamma(link = "log"),
               data = SUBSET)
summary(gamma_2)

```

```

##  

## Call:  

## glm(formula = Copies_ul ~ Year_centered + inoculation, family = Gamma(link = "log"),  

##      data = SUBSET)  

##  

## Coefficients:  

##              Estimate Std. Error t value Pr(>|t|)  

## (Intercept)    3.6879    0.5161   7.146 1.56e-08 ***  

## Year_centered -0.9384    0.2478  -3.787 0.000529 ***  

## inoculation1  -0.5440    0.5583  -0.974 0.336090  

## ---  

## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1  

##  

## (Dispersion parameter for Gamma family taken to be 3.177182)  

##  

## Null deviance: 110.463  on 40  degrees of freedom  

## Residual deviance: 82.774  on 38  degrees of freedom  

## AIC: 257.83  

##  

## Number of Fisher Scoring iterations: 12

```

```

#Year+inoculation+hostID
gamma_3<- glm(Copies_ul ~ Year_centered + inoculation + hostID,
               family = Gamma(link = "log"),
               data = SUBSET)
summary(gamma_3)

```

```

##
```

```

## Call:
## glm(formula = Copies_ul ~ Year_centered + inoculation + hostID,
##      family = Gamma(link = "log"), data = SUBSET)
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)   3.8874    0.5312   7.319 1.07e-08 ***
## Year_centered -0.9111    0.2257  -4.036 0.000262 ***
## inoculation1 -0.2202    0.5119  -0.430 0.669541
## hostIDArtemesia -0.9400    0.5113  -1.838 0.074037 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for Gamma family taken to be 2.62999)
##
## Null deviance: 110.463  on 40  degrees of freedom
## Residual deviance: 75.062  on 37  degrees of freedom
## AIC: 254.77
##
## Number of Fisher Scoring iterations: 11

#Add sample ID as a random effects structure to account for repeated measures
#over years
gamma_4<-glmer(Copies_ul ~ Year_centered + inoculation + hostID
                  + (1|SampleID),
                  family = Gamma(link = "log"),
                  data = SUBSET)
summary(gamma_4)

```

```

## Generalized linear mixed model fit by maximum likelihood (Laplace
## Approximation) [glmerMod]
## Family: Gamma ( log )
## Formula: Copies_ul ~ Year_centered + inoculation + hostID + (1 | SampleID)
## Data: SUBSET
##
##      AIC      BIC  logLik deviance df.resid
## 249.6    259.9   -118.8    237.6      35
##
## Scaled residuals:
##      Min      1Q  Median      3Q     Max
## -0.9565 -0.5855 -0.2762  0.3239  2.5643
##
## Random effects:
## Groups   Name        Variance Std.Dev.
## SampleID (Intercept) 1.125    1.061
## Residual           1.016    1.008
## Number of obs: 41, groups: SampleID, 12
##
## Fixed effects:
##              Estimate Std. Error t value Pr(>|z|)
## (Intercept)   3.1176    0.6146   5.073 3.92e-07 ***
## Year_centered -0.8452    0.1441  -5.866 4.46e-09 ***
## inoculation1 -0.6641    0.6776  -0.980   0.327
## hostIDArtemesia -0.4460    0.6771  -0.659   0.510

```

```

## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr) Yr_cnt inclt1
## Year_centerd -0.359
## inoculatin1 -0.497  0.018
## hostIDArtms -0.556  0.047 -0.058

```

#Compare the mixed effects model with and without hostID

```

gamma_5<-glmer(Copies_ul ~ Year_centered + inoculation
+ (1|SampleID),
family = Gamma(link = "log"),
data = SUBSET)
summary(gamma_5)

```

```

## Generalized linear mixed model fit by maximum likelihood (Laplace
## Approximation) [glmerMod]
## Family: Gamma  ( log )
## Formula: Copies_ul ~ Year_centered + inoculation + (1 | SampleID)
## Data: SUBSET
##
##      AIC      BIC  logLik deviance df.resid
## 248.0   256.6  -119.0    238.0      36
##
## Scaled residuals:
##    Min     1Q Median     3Q    Max
## -0.9590 -0.5693 -0.2837  0.2808  2.4364
##
## Random effects:
## Groups   Name        Variance Std.Dev.
## SampleID (Intercept) 1.205    1.098
## Residual            1.015    1.007
## Number of obs: 41, groups: SampleID, 12
##
## Fixed effects:
##             Estimate Std. Error t value Pr(>|z|)
## (Intercept)  2.8917    0.5237  5.522 3.35e-08 ***
## Year_centered -0.8409    0.1435 -5.858 4.69e-09 ***
## inoculation1 -0.6905    0.6925 -0.997  0.319
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr) Yr_cnt
## Year_centerd -0.389
## inoculatin1 -0.646  0.021

```

#Compare AIC values

```
AIC(gamma_1, gamma_2, gamma_3, gamma_4, gamma_5)
```

```

##      df      AIC
## gamma_1 3 257.6541

```

```

## gamma_2 4 257.8287
## gamma_3 5 254.7656
## gamma_4 6 249.5772
## gamma_5 5 247.9954

#Gamma 4 and gamma 5 are the best AIC fit, exclude host ID to decrease model complexity. This does not affect conclusions and retains the two predictors of highest interest.

#DHARMa Diagnostics for the selected Gamma Model
#Simulate residuals
res_gamma_5 <- simulateResiduals(gamma_5)
#Plot residuals
plot(res_gamma_5)
#Test dispersion
print(testDispersion(res_gamma_5))

##
## DHARMa nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.5957, p-value = 0.6
## alternative hypothesis: two.sided

#Test for significant outliers
print(testOutliers(res_gamma_5))

##
## DHARMa outlier test based on exact binomial test with approximate
## expectations
##
## data: res_gamma_5
## outliers at both margin(s) = 0, observations = 41, p-value = 1
## alternative hypothesis: true probability of success is not equal to 0.007968127
## 95 percent confidence interval:
## 0.00000000 0.08604384
## sample estimates:
## frequency of outliers (expected: 0.00796812749003984 )
## 0

#Check for collinearity among predictors
performance::check_collinearity(gamma_5)

## # Check for Multicollinearity
##
## Low Correlation
##
##
## Term VIF VIF 95% CI adj. VIF Tolerance Tolerance 95% CI
## Year_centered 1.00 [1.00, Inf] 1.00 1.00 [0.00, 1.00]
## inoculation 1.00 [1.00, Inf] 1.00 1.00 [0.00, 1.00]

```

```

#Check residuals for each predictor
plotResiduals(res_gamma_5,SUBSET$Year_centered)
plotResiduals(res_gamma_5, SUBSET$inoculation)

#Outlier Sensitivity Test
#Filter out the outlier
filtered <- SUBSET %>% filter(Copies_ul <= 100)

#Create the model with the filtered dataset
if (nrow(filtered) >= 10) {
  filtered$Year_centered <- filtered$Year - 2014
  gamma_FILTERED <- glmer(Copies_ul ~ Year_centered + inoculation
    + (1|SampleID),
    family = Gamma(link = "log"),
    data = filtered)
  summary(gamma_FILTERED)
}

## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##   Family: Gamma  ( log )
## Formula: Copies_ul ~ Year_centered + inoculation + (1 | SampleID)
##   Data: filtered
##
##       AIC     BIC   logLik deviance df.resid
##   230.7   239.2   -110.4    220.7      35
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -0.9904 -0.5804 -0.2884  0.4266  2.6755
##
## Random effects:
##   Groups   Name        Variance Std.Dev.
##   SampleID (Intercept) 0.8969   0.9470
##   Residual             0.9456   0.9724
## Number of obs: 40, groups: SampleID, 12
##
## Fixed effects:
##           Estimate Std. Error t value Pr(>|z|)
## (Intercept)  2.7208    0.4813   5.653 1.58e-08 ***
## Year_centered -0.8140    0.1374  -5.924 3.14e-09 ***
## inoculation1 -0.5340    0.6349  -0.841     0.4
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr) Yr_cnt
## Year_centerd -0.411
## inoculatin1 -0.640  0.038

#Removal of the possible outlier does not affect conclusions, model is robust to
#outlier inclusion

```

```

#Summarize final selected models
summary(binom_RI_2)

## Generalized linear mixed model fit by maximum likelihood (Laplace
## Approximation) [glmerMod]
## Family: binomial ( logit )
## Formula: Presence ~ Year_centered + inoculation + (1 | SampleID)
## Data: data
##
##      AIC      BIC  logLik deviance df.resid
##      43.3    50.8   -17.6     35.3      44
##
## Scaled residuals:
##      Min      1Q  Median      3Q      Max
## -2.9797  0.1634  0.2788  0.3996  0.9336
##
## Random effects:
## Groups   Name        Variance Std.Dev.
## SampleID (Intercept) 0.4132   0.6428
## Number of obs: 48, groups: SampleID, 12
##
## Fixed effects:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept)  3.8848    1.4325   2.712  0.00669 **
## Year_centered -0.7198    0.4541  -1.585  0.11295
## inoculation1 -1.1856    1.0328  -1.148  0.25098
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr) Yr_cnt
## Year_centr -0.760
## inoculatin -0.585  0.124

```

```
summary(gamma_5)
```

```

## Generalized linear mixed model fit by maximum likelihood (Laplace
## Approximation) [glmerMod]
## Family: Gamma ( log )
## Formula: Copies_ul ~ Year_centered + inoculation + (1 | SampleID)
## Data: SUBSET
##
##      AIC      BIC  logLik deviance df.resid
##      248.0    256.6   -119.0    238.0      36
##
## Scaled residuals:
##      Min      1Q  Median      3Q      Max
## -0.9590 -0.5693 -0.2837  0.2808  2.4364
##
## Random effects:
## Groups   Name        Variance Std.Dev.
## SampleID (Intercept) 1.205    1.098

```

```
## Residual           1.015   1.007
## Number of obs: 41, groups: SampleID, 12
##
## Fixed effects:
##             Estimate Std. Error t value Pr(>|z|)
## (Intercept)  2.8917    0.5237  5.522 3.35e-08 ***
## Year_centered -0.8409    0.1435 -5.858 4.69e-09 ***
## inoculation1 -0.6905    0.6925 -0.997    0.319
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr) Yr_cnt
## Year_centerd -0.389
## inoculatin1 -0.646  0.021
```