

# The effect of nitrogen application time on infection and development of the Wheat Streak Mosaic Virus (WSMV)

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# 1 Introduction

## 2 Statistical Experience and Assistance Needed

Nar B. Ranabhat is a PhD candidate in the Department of Land Resources and Environmental Science. He is currently working on several projects regarding the spread and development of the Wheat Streak Mosaic Virus (WSMV) in Winter Wheat. Nar has taken Statistics 511 and 512 here at Montana State University, and he is currently auditing Statistics 448. Nar has asked for our help in building a model, fitting it in R, and interpreting the results.

## 3 Objectives and Questions

Nar is interested in the effect of variety, nitrogen application time, and inoculation on the probability of virus infection in Winter Wheat. He is primarily interested in the effect of nitrogen application time, and he wants to assess evidence for all possible two and three way interactions. Nar's goal is to select the simplest possible model, and then use this model to describe the effects of the above variables.

We address the following questions in this report:

- Is there evidence that the interaction between nitrogen application and variety differs between inoculated and control plots?
- Is there evidence that the nitrogen application effect varies across varieties? Is there evidence that the nitrogen application effect differs between inoculated and control?
- Is there evidence that the variety effect differs between inoculated and control?
- Conditional on the results to the above questions, how do we appropriately describe the estimated effects for variety, nitrogen application time, and inoculation on the probability of virus infection?

## 4 Study Design/Data collection

Nar conducted this experiment in a MSU field at the base of the Bridger Mountains. He chose one area in the field and divided it into six 31.5 by 27.5 meter blocks. The blocks were then divided into five rows, with 5 meters of space between each row. Each row was randomly assigned to a variety of Winter Wheat with separate randomizations in each block. The rows then were divided into six 1.5 by 5 meter plots. Each plot was randomly assigned to one of six combinations of nitrogen application time and inoculation status, with separate randomizations in each row. The combinations were fall/inoculated, fall/control, early spring/inoculated, early spring/control, late spring/inoculated, and late spring/control. All plots were planted in the **FALL?** and allowed to grow over the winter. The following

**September?**, 30 plants were chosen from each plot to be taken to the lab and tested for presence of the virus. The selection of the plants from each plot was not random nor technically systematic. The **undergraduate?** technicians were instructed to collect a sample of plants spread evenly throughout the field. **different undergraduates doing picking? picking done all at the same time? is there visual evidence of the disease?** A total of 180 plants were collected. The plants were then sent to the lab and screened for the virus via the ELISA procedure. This is a three year study. The data are collected for the first year, and the second year plants are waiting to be analyzed in the lab **describe how they are stored during waiting time**. Planting for the third year will begin this fall **I think?**.

Plots that were assigned to the inoculated treatment had five infected plants transplanted to the middle of the plot. These plants were infected **as adults?** in the greenhouse by clipping an infected leaf to the healthy plant. The Wheat Streak Mosaic Virus is transmitted by the wheat curl mite, tiny organisms that are nearly invisible to the naked eye. They can easily move from leaf to leaf on a plant, and they are transported from plant to plant by the wind (Sloderbeck 2008). It is our understanding that once the infected mites were introduced to the study area, they were considered to be everywhere in the air and that all plants in the field were exposed to the infected mites.

## 5 Recommendations

### 5.1 Binomial Generalized Linear Mixed Model

Because the response variable is a binomial count, and because the design includes random assignment to rows within blocks, a binomial generalized linear mixed model is appropriate. This model will estimate the probability of infection for each **variety:treatment:status** combination while controlling for differences between blocks and between rows in the same block.

The model is

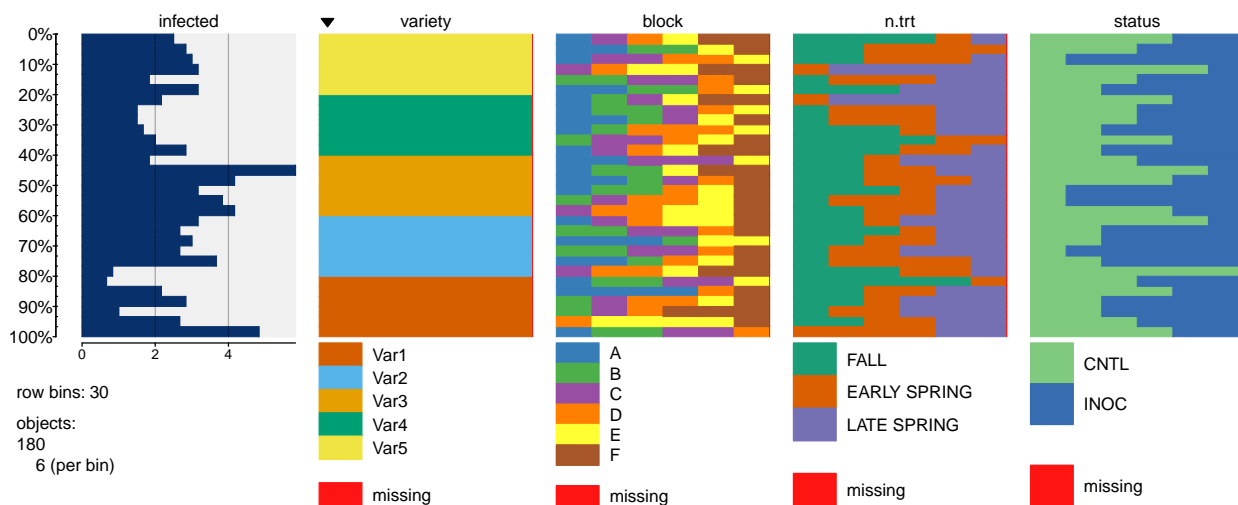
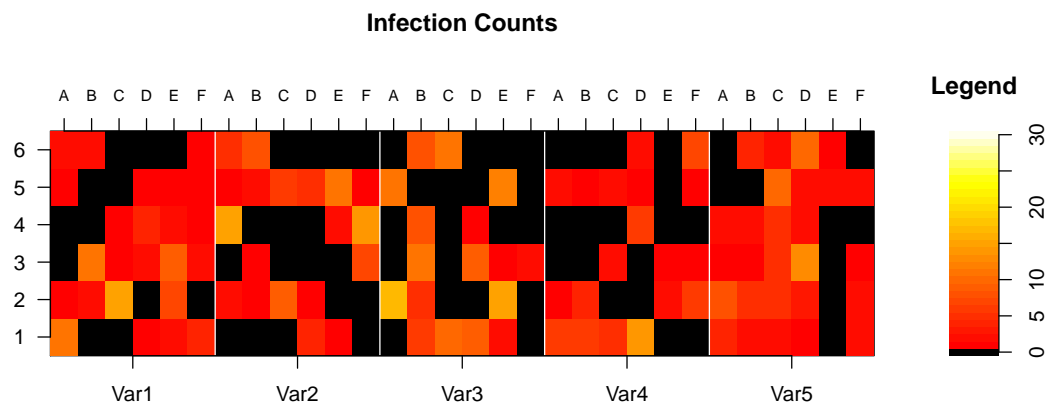
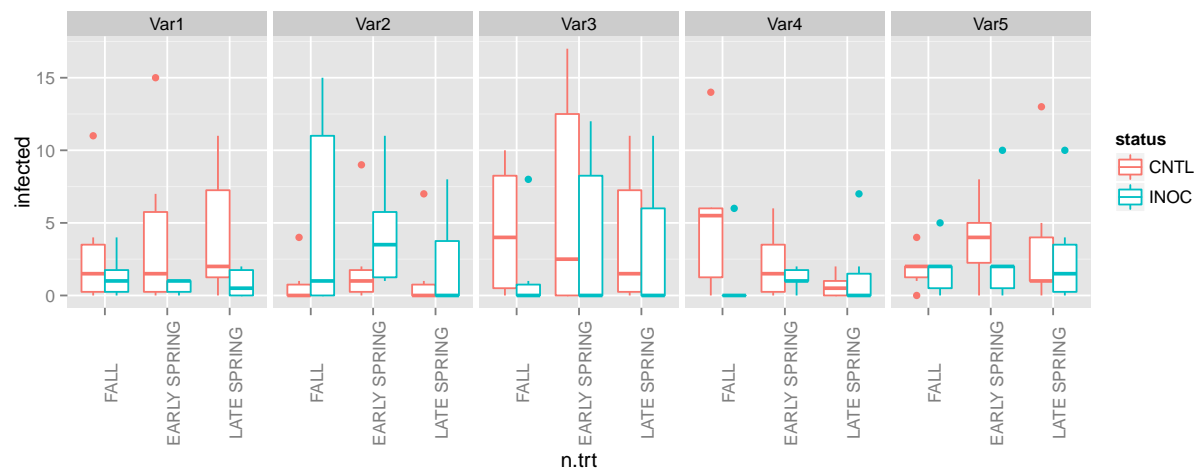
$$y_i \sim \text{Binomial}(30, p_i),$$

$$\text{logit}(p_i) = \mathbf{x}_i\beta + b_{0,j[i]} + b_{1,j[i],k[i]}$$

where

- $p_i$  is the probability of infection for the  $i$ th observation,
- $\text{logit}(p_i) = \log\left(\frac{p_i}{1-p_i}\right)$  is the natural logarithm of the odds of infection,
- $\mathbf{x}_i$  is a row vector containing indicator variables for the variety, nitrogen application timing, and inoculation status of observation  $i$ , and
- $b_{0,j} \sim N(0, \sigma_{b_0}^2)$  and  $b_{1,j,k} \sim N(0, \sigma_{b_1}^2)$  are random effects for block and row, respectively.

## 5.2 Graphical Data Exploration



The `itableplot` function in the `tabplot` package is another useful tool for visualizing the data and identifying patterns.

## 5.3 Convergeance Warning Messages

```
glmm3way <- glmer(cbind(infected, total) ~ variety*n.trt*status +
  (1|block/row),
  control = glmerControl(optimizer = 'bobyqa'),
  family = binomial, data = fert.sim1)

## Warning in commonArgs(par, fn, control, environment()): maxfun < 10 * length(par)^2 is
not recommended.

summary(glmm3way)

## Generalized linear mixed model fit by maximum likelihood (Laplace Approximation) ['glmerMod']
## Family: binomial ( logit )
## Formula: cbind(infected, total) ~ variety * n.trt * status + (1 | block/row)
## Data: fert.sim1
## Control: glmerControl(optimizer = "bobyqa")
##
##          AIC      BIC    logLik deviance df.resid
##          988      1090     -462      924      148
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.500 -1.253 -0.723  0.866  4.220
##
## Random effects:
## Groups Name Variance Std.Dev.
## row:block (Intercept) 2.09e-01 4.57e-01
## block (Intercept) 5.81e-17 7.62e-09
## Number of obs: 180, groups: row:block, 30; block, 6
##
## Fixed effects:
##
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept)      -2.3475     0.3104  -7.56  4.0e-14
## varietyVar2       -1.2770     0.5786  -2.21  0.02731
## varietyVar3         0.3391     0.4194   0.81  0.41874
## varietyVar4         0.4860     0.4137   1.17  0.24001
## varietyVar5        -0.5822     0.4797  -1.21  0.22486
## n.trtEARLY SPRING    0.3103     0.3268   0.95  0.34243
## n.trtLATE SPRING     0.3199     0.3267   0.98  0.32750
## statusINOC          -0.8125     0.4361  -1.86  0.06244
## varietyVar2:n.trtEARLY SPRING 0.6436     0.6261   1.03  0.30390
## varietyVar3:n.trtEARLY SPRING -0.0188     0.4286  -0.04  0.96496
## varietyVar4:n.trtEARLY SPRING -1.1626     0.4771  -2.44  0.01483
## varietyVar5:n.trtEARLY SPRING 0.4305     0.5032   0.86  0.39232
## varietyVar2:n.trtLATE SPRING 0.1359     0.6627   0.21  0.83749
## varietyVar3:n.trtLATE SPRING -0.4922     0.4468  -1.10  0.27055
## varietyVar4:n.trtLATE SPRING -2.3563     0.6305  -3.74  0.00019
## varietyVar5:n.trtLATE SPRING 0.2878     0.5074   0.57  0.57058
## varietyVar2:statusINOC 2.6038     0.6568   3.96  7.4e-05
## varietyVar3:statusINOC -0.3158     0.5916  -0.53  0.59351
## [ reached getOption("max.print") -- omitted 12 rows ]
```

```
##
## Correlation matrix not shown by default, as p = 30 > 20.
## Use print(x, correlation=TRUE) or
## vcov(x) if you need it

## convergence code: 0
## maxfun < 10 * length(par)^2 is not recommended.

glmm2way <- glmer(cbind(infected, total) ~ variety*n.trt + variety*status +
                  n.trt*status + (1|block/row),
                  control = glmerControl(optimizer = 'bobyqa'),
                  family = binomial, data = fert.sim1)
summary(glmm2way)

## Generalized linear mixed model fit by maximum likelihood (Laplace Approximation) ['glmerMod']
## Family: binomial ( logit )
## Formula: cbind(infected, total) ~ variety * n.trt + variety * status +      n.trt * status + (1 | bl
## Data: fert.sim1
## Control: glmerControl(optimizer = "bobyqa")
##
##          AIC          BIC    logLik deviance df.resid
##          993          1070     -473     945      156
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.462 -1.334 -0.744  0.782  5.563
##
## Random effects:
## Groups Name Variance Std.Dev.
## row:block (Intercept) 0.211 0.459
## block (Intercept) 0.000 0.000
## Number of obs: 180, groups: row:block, 30; block, 6
##
## Fixed effects:
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept) -2.18009 0.28694 -7.60 3.0e-14
## varietyVar2 -0.78980 0.42736 -1.85 0.06459
## varietyVar3 0.00305 0.39295 0.01 0.99380
## varietyVar4 0.10857 0.39426 0.28 0.78303
## varietyVar5 -0.62022 0.42153 -1.47 0.14119
## n.trtEARLY SPRING 0.08607 0.28844 0.30 0.76540
## n.trtLATE SPRING 0.07060 0.28750 0.25 0.80601
## statusINOC -1.44780 0.31854 -4.55 5.5e-06
## varietyVar2:n.trtEARLY SPRING 0.08115 0.39552 0.21 0.83744
## varietyVar3:n.trtEARLY SPRING 0.44266 0.36554 1.21 0.22591
## varietyVar4:n.trtEARLY SPRING -0.65341 0.40593 -1.61 0.10747
## varietyVar5:n.trtEARLY SPRING 0.50767 0.40144 1.26 0.20601
## varietyVar2:n.trtLATE SPRING -0.73786 0.42333 -1.74 0.08134
## varietyVar3:n.trtLATE SPRING 0.00219 0.37297 0.01 0.99532
## varietyVar4:n.trtLATE SPRING -1.16310 0.43613 -2.67 0.00766
## varietyVar5:n.trtLATE SPRING 0.34709 0.40106 0.87 0.38681
## varietyVar2:statusINOC 2.40291 0.36694 6.55 5.8e-11
## varietyVar3:statusINOC 0.85304 0.33545 2.54 0.01099
## [ reached getOption("max.print") -- omitted 4 rows ]
```

```
##
## Correlation matrix not shown by default, as p = 22 > 20.
## Use print(x, correlation=TRUE) or
## vcov(x) if you need it

Anova(glm3way)

## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: cbind(Infected, Total)
##
```

	Chisq	Df	Pr(>Chisq)
variety	3.72	4	0.445
n.trt	3.41	2	0.182
status	9.52	1	0.002
variety:n.trt	19.90	8	0.011
variety:status	41.86	4	1.8e-08
n.trt:status	1.01	2	0.602
variety:n.trt:status	20.07	8	0.010

```
##
Anova(glm2way)

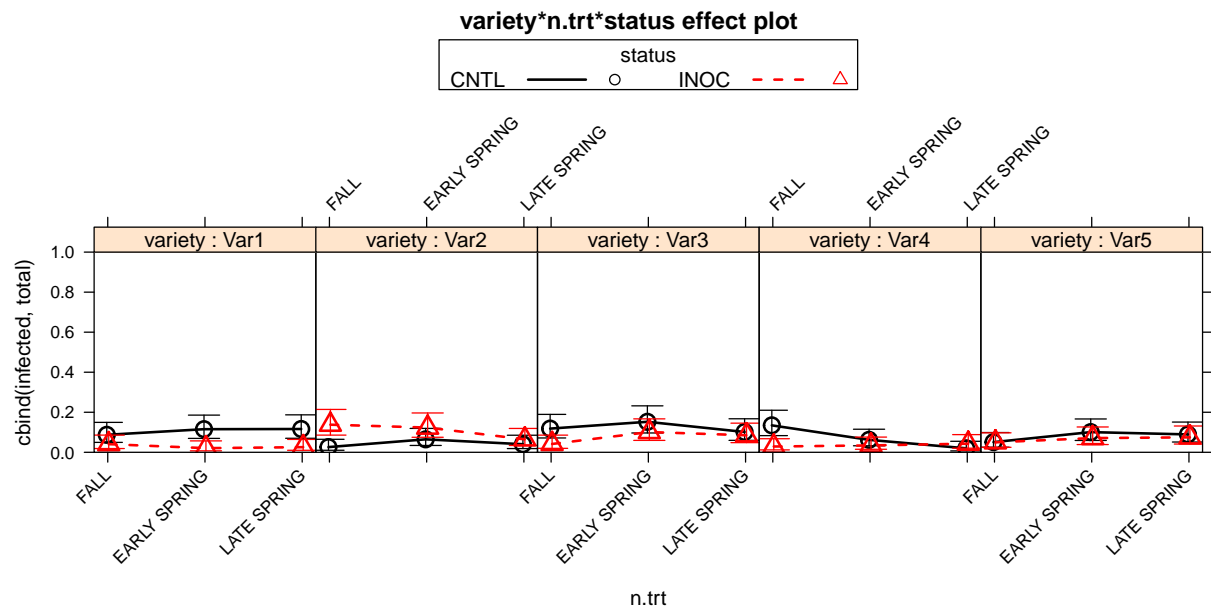
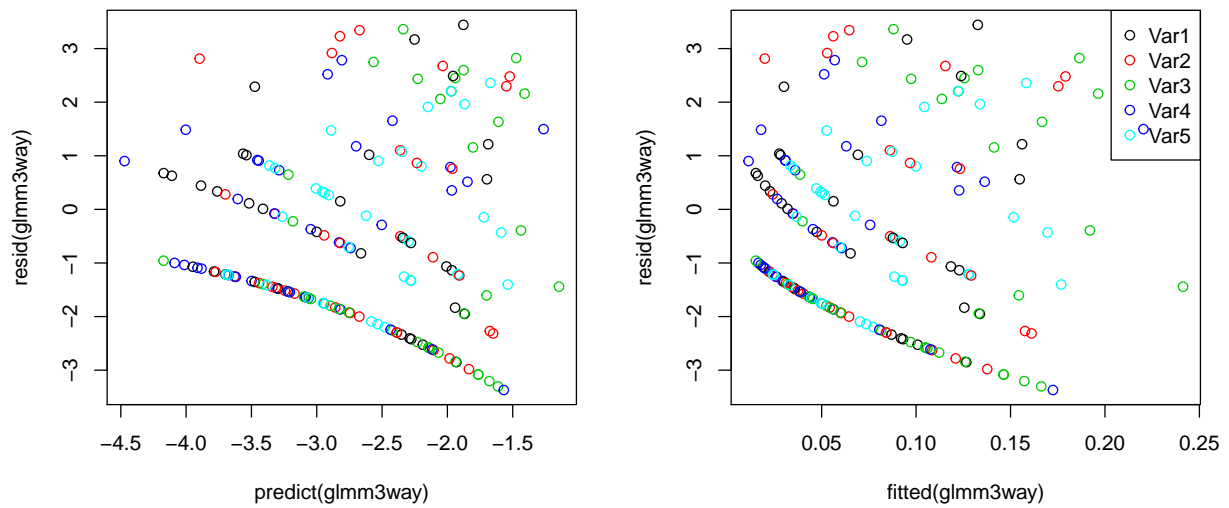
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: cbind(Infected, Total)
##
```

	Chisq	Df	Pr(>Chisq)
variety	4.09	4	0.3945
n.trt	4.52	2	0.1044
status	10.59	1	0.0011
variety:n.trt	22.70	8	0.0038
variety:status	49.99	4	3.6e-10
n.trt:status	1.31	2	0.5188

```
##
anova(glm2way, glm3way)

## Data: fert.sim1
## Models:
## glm2way: cbind(Infected, Total) ~ variety * n.trt + variety * status +
## glm2way:      n.trt * status + (1 | block/row)
## glm3way: cbind(Infected, Total) ~ variety * n.trt * status + (1 | block/row)
##
```

	Df	AIC	BIC	logLik	deviance	Chisq	Chi	Df	Pr(>Chisq)
glm2way	24	993	1070	-473	945				
glm3way	32	988	1090	-462	924	21.5		8	0.0059



```
table(fert2$infected==0,fert2$variety)
```

```
##
##      MACE PRHN SNMS TAM YSTN
## FALSE  22  31   9  24  27
## TRUE   14   5  27  12   9
```

```
noSNMS3way <- glmer(cbind(infected, total) ~ variety*n.trt*status +
                    (1|block/row), subset = variety != 'SNMS',
                    control = glmerControl(optimizer = 'bobyqa'),
                    family = binomial, data = fert2)
summary(noSNMS3way)
```



```
## Generalized linear mixed model fit by maximum likelihood (Laplace Approximation) ['glmerMod']
## Family: binomial ( logit )
## Formula: cbind(infected, total) ~ variety * n.trt * status + (1 | block/row)
## Data: fert2
## Control: glmerControl(optimizer = "bobyqa")
## Subset: variety != "SNMS"
##
##      AIC      BIC    logLik deviance df.resid
##      854      931     -401     802     118
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.434 -1.330 -0.678  0.986  6.740
##
## Random effects:
## Groups      Name      Variance Std.Dev.
## row:block (Intercept) 0.3141  0.56
## block      (Intercept) 0.0623  0.25
## Number of obs: 144, groups: row:block, 24; block, 6
##
## Fixed effects:
##                                     Estimate Std. Error z value Pr(>|z|)
## (Intercept)                       -2.247     0.337   -6.66  2.7e-11
## varietyPRHN                        0.793     0.429    1.85  0.06441
## varietyTAM                       -0.639     0.497   -1.29  0.19836
## varietyYSTN                      -0.691     0.504   -1.37  0.17002
## n.trtEARLY SPRING                 -0.591     0.361   -1.64  0.10199
## n.trtLATE SPRING                  -0.543     0.347   -1.56  0.11781
## statusINOC                       -0.794     0.380   -2.09  0.03667
## varietyPRHN:n.trtEARLY SPRING      0.441     0.436    1.01  0.31098
## varietyTAM:n.trtEARLY SPRING       0.747     0.545    1.37  0.17041
## varietyYSTN:n.trtEARLY SPRING      1.580     0.517    3.06  0.00224
## varietyPRHN:n.trtLATE SPRING       0.881     0.412    2.14  0.03257
## varietyTAM:n.trtLATE SPRING        1.026     0.518    1.98  0.04746
## varietyYSTN:n.trtLATE SPRING       0.217     0.589    0.37  0.71233
## varietyPRHN:statusINOC             0.106     0.471    0.23  0.82159
## varietyTAM:statusINOC              0.999     0.553    1.81  0.07096
## varietyYSTN:statusINOC             1.801     0.529    3.41  0.00066
## n.trtEARLY SPRING:statusINOC        1.503     0.521    2.89  0.00391
## n.trtLATE SPRING:statusINOC         1.354     0.514    2.63  0.00850
## [ reached getOption("max.print") -- omitted 6 rows ]
##
## Correlation matrix not shown by default, as p = 24 > 20.
## Use print(x, correlation=TRUE) or
## vcov(x) if you need it
Anova(noSNMS3way)
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: cbind(infected, total)
##               Chisq Df Pr(>Chisq)
## variety         8.52  3    0.0364
```

```

## n.trt          5.91  2    0.0520
## status         0.03  1    0.8634
## variety:n.trt  22.34  6    0.0011
## variety:status  36.15  3    7e-08
## n.trt:status    1.50  2    0.4724
## variety:n.trt:status 11.99  6    0.0622

noSNMS2way <- glmer(cbind(infected, total) ~ variety*n.trt + variety*status +
  n.trt*status + (1|block/row), subset = variety != 'SNMS',
  control = glmerControl(optimizer = 'bobyqa'),
  family = binomial, data = fert2)

summary(noSNMS2way)

## Generalized linear mixed model fit by maximum likelihood (Laplace Approximation) ['glmerMod']
## Family: binomial ( logit )
## Formula: cbind(infected, total) ~ variety * n.trt + variety * status +      n.trt * status + (1 | bl
## Data: fert2
## Control: glmerControl(optimizer = "bobyqa")
## Subset: variety != "SNMS"
##
##      AIC      BIC    logLik deviance df.resid
##      854      914     -407      814      124
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.668 -1.379 -0.485  0.943  5.816
##
## Random effects:
## Groups      Name                Variance Std.Dev.
## row:block (Intercept) 0.3192     0.565
## block      (Intercept) 0.0621     0.249
## Number of obs: 144, groups: row:block, 24; block, 6
##
## Fixed effects:
##
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept)    -2.5823    0.3346  -7.72  1.2e-14
## varietyPRHN      1.1906    0.4147   2.87  0.00409
## varietyTAM      -0.2378    0.4525  -0.53  0.59923
## varietyYSTN     -0.0727    0.4415  -0.16  0.86918
## n.trtEARLY SPRING -0.0267    0.2728  -0.10  0.92195
## n.trtLATE SPRING  0.0135    0.2710   0.05  0.96018
## statusINOC       0.0441    0.2416   0.18  0.85511
## varietyPRHN:n.trtEARLY SPRING -0.1768    0.3142  -0.56  0.57362
## varietyTAM:n.trtEARLY SPRING  0.2188    0.3586   0.61  0.54171
## varietyYSTN:n.trtEARLY SPRING  0.5321    0.3218   1.65  0.09825
## varietyPRHN:n.trtLATE SPRING  0.2083    0.3053   0.68  0.49508
## varietyTAM:n.trtLATE SPRING  0.2698    0.3590   0.75  0.45237
## varietyYSTN:n.trtLATE SPRING -0.4646    0.3563  -1.30  0.19226
## varietyPRHN:statusINOC -0.9102    0.2536  -3.59  0.00033
## varietyTAM:statusINOC  0.0366    0.2881   0.13  0.89899
## varietyYSTN:statusINOC  0.5427    0.2752   1.97  0.04861
## n.trtEARLY SPRING:statusINOC  0.2957    0.2285   1.29  0.19560
## n.trtLATE SPRING:statusINOC  0.1261    0.2340   0.54  0.58994
##

```

```
## Correlation of Fixed Effects:
##      (Intr) vrPRHN vrtTAM vrYSTN n.tEARLYSPRING n.tLATESPRING stINOC vPRHN:.ES vTAM:.ES vY
## varietyPRHN      -0.709
## varietyTAM        -0.632  0.513
## varietyYSTN        -0.645  0.526  0.485
## n.tEARLYSPRING    -0.386  0.272  0.222  0.205
##      vPRHN:.LS vTAM:.LS vYSTN:.LS vPRHN: vTAM:I vYSTN: n.EARLYSPRING:
## varietyPRHN
## varietyTAM
## varietyYSTN
## n.tEARLYSPRING
## [ reached getOption("max.print") -- omitted 13 rows ]
```

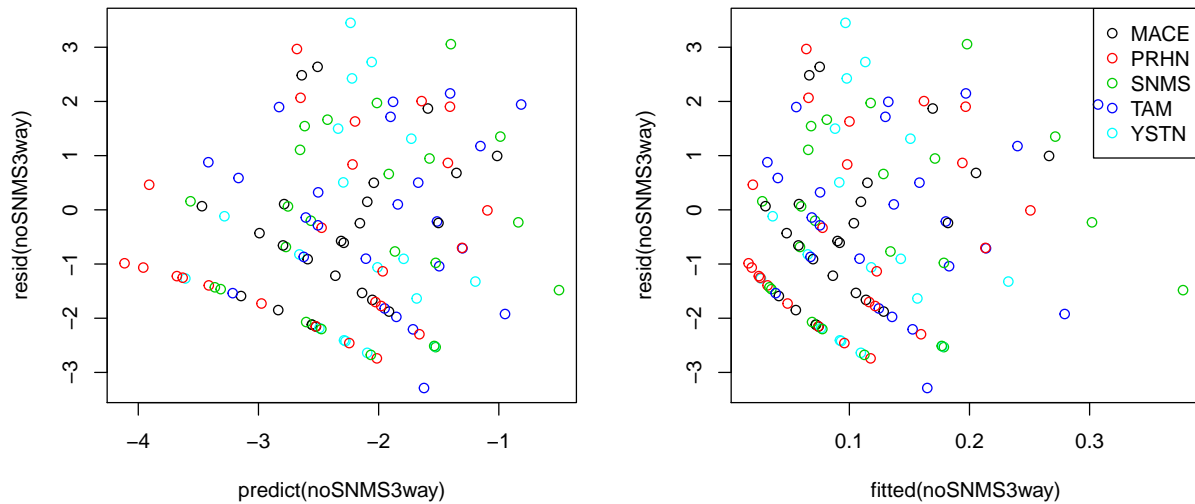
```
Anova(noSNMS2way)
```

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
```

```
##
## Response: cbind(Infected, total)
##      Chisq Df Pr(>Chisq)
## variety      8.69  3    0.0336
## n.trt        6.47  2    0.0395
## status       0.05  1    0.8212
## variety:n.trt 22.23  6    0.0011
## variety:status 37.22  3    4.1e-08
## n.trt:status   1.70  2    0.4268
```

```
anova(noSNMS2way, noSNMS3way)
```

```
## Data: fert2
## Subset: variety != "SNMS"
## Models:
## noSNMS2way: cbind(Infected, total) ~ variety * n.trt + variety * status +
## noSNMS2way:      n.trt * status + (1 | block/row)
## noSNMS3way: cbind(Infected, total) ~ variety * n.trt * status + (1 | block/row)
##      Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
## noSNMS2way 20 854 914   -407      814
## noSNMS3way 26 854 931   -401      802 12.3      6    0.055
```



## 5.4 Model Refinement

## 5.5 Interpretation

# 6 Scope of inference

# 7 Additional Comments

While very low infection rates are, in practice, a desirable result, they present technical and computational problems for analysis. In similar future studies, random sampling should be used within each plot and the sample size should be large enough that the researchers expect at least one infected leaf to be found in each plot.

# 8 References

Gelman, A. and Hill, J. (2007). *Data Analysis Using Regression and Multilevel/Hierarchical Models*. New York, NY: Cambridge University Press.

Sloderbeck, J., Michaud, P., Whitworth, Robert. "Wheat Pests." CurlMite. Kansas State University, 1 May 2008. Web. 18 Sept. 2015. <http://entomology.k-state.edu/extension/insect-info>