# Using asreml and asremlPlus for the Ladybird example from Welham et al. (2014)

Chris Brien

28 October, 2024

# Introduction

This vignette shows how to use asremlPlus (Brien, 2024a), and dae (Brien, 2024b), for exploring and presenting predictions from a linear mixed model analysis, the predictions having been produced using asreml (Butler et al., 2023). Here, asremlPlus, dae and asreml are packages for the R Statistical Computing environment (R Core Team, 2024).

The context is a three-factor factorial experiment on ladybirds (Welham et al., 2014, Example 8.2) that aims to answer the question "Will ladybirds transfer fungus to aphids on plants?" The experiment consists of 2 runs of 36 containers, each with a plant and aphids. There are three factors that results in 12 treatments: Host plant (beans, trefoil), infected Cadavers (5, 10, 20), Ladybird (-, +). These are randomized to the containers within a run so that each is replicated 3 times within a run. The response to be analysed is the logit of the proportion of live aphids that were infected.

### Initialize

```
library(knitr)
opts_chunk$set("tidy" = FALSE, comment = NA)
suppressMessages(library(asrem1))

## Offline License checked out Mon Oct 28 10:56:56 2024

packageVersion("asrem1")

## [1] '4.2.0.332'
suppressMessages(library(asrem1Plus))
packageVersion("asrem1Plus")

## [1] '4.4.40'
suppressMessages(library("dae"))
packageVersion("dae")

## [1] '3.2.29'
```

```
options(width = 95, show.signif.stars = FALSE)
```

### Get data available in asremlPlus

```
data("Ladybird.dat")
```

# Do an ANOVA of logits

```
Error: Run
            Sum Sq Mean Sq F value Pr(>F)
Residuals 1 0.06766 0.06766
Error: Run:Plant
                     Df Sum Sq Mean Sq F value
                                               Pr(>F)
Host
                      1 13.599 13.599 59.172 1.82e-10
                               8.514 37.044 3.78e-11
Cadavers
                      2 17.027
Ladybird
                      1 11.091 11.091 48.257 3.33e-09
                      2 0.308 0.154 0.670 0.5158
Host:Cadavers
Host:Ladybird
                      1 0.228 0.228 0.992
                                               0.3234
Cadavers:Ladybird
                      2 1.735
                                0.867
                                        3.774
                                               0.0287
Host:Cadavers:Ladybird 2 0.200
                              0.100
                                       0.435
                                               0.6493
Residuals
                     59 13.560
                                0.230
```

The anova table gives the F-tests for the three-factor effects and interactions. Note the Residuals Mean Sq value for Run: Plant of 0.230. Also, it is clear that the Run component is negative, given that the Residuals Mean Sq value for Run is less than that for Run: Plant; it is (0.06766 - 0.230) / 36). From the table it is seen that the only significant interaction is Cadavers: Ladybird and that the Host main effect is significant.

# Use asreml to analyse the logits

# Mixed model analysis of logits

```
ASReml Version 4.2 28/10/2024 10:56:57

LogLik Sigma2 DF wall
1 3.071301 0.2262391 60 10:56:57 ( 1 restrained)
```

```
2
       3.642605
                     0.2269125
                                   60
                                        10:56:57 ( 1 restrained)
                                                      1 restrained)
3
                     0.2271056
       3.712498
                                   60
                                         10:56:57
                                                   (
                                                      1 restrained)
4
       3.717213
                     0.2271206
                                   60
                                         10:56:57
5
                                                      1 restrained)
       3.717512
                     0.2271216
                                   60
                                         10:56:57
```

#### summary(m)\$varcomp

```
component std.error z.ratio bound %ch Run 2.298309e-08 NA NA B NA Run:Plant!R 2.271216e-01 0.04156985 5.463612 P 0
```

As expected the Run component is bound (B) at approximately zero. This results in a change in the estimate of the residual variance to 0.227. To allow for a negative estimate we will unconstrain the Run component. As Littell et al. (2006, p.150) say

if you do not set the negative variance component estimate to zero, but allow it to remain negative, you get better control over Type I error and, for cases of negative wholeplot error variance estimates, greater power. Therefore, this is the recommended procedure.

#### Unconstrain Reps to make the analysis equivalent to ANOVA

```
m <- setvarianceterms(m$call, terms = "Run", bounds = "U")</pre>
```

```
ASReml Version 4.2 28/10/2024 10:56:57
                                    DF
          LogLik
                         Sigma2
                                            wall
1
        3.071301
                      0.2262391
                                    60
                                          10:56:57
                                                      1 restrained)
                                                    (
 2
        3.642605
                      0.2269125
                                    60
                                          10:56:57
                                                    (
                                                       1 restrained)
 3
        3.802834
                      0.2274541
                                    60
                                          10:56:57
                                                    ( 1 restrained)
 4
        3.839487
                      0.2334176
                                    60
                                          10:56:57
 5
        3.955683
                      0.2309710
                                    60
                                         10:56:57
 6
        3.973334
                      0.2300353
                                    60
                                          10:56:57
 7
        3.974051
                      0.2298346
                                    60
                                          10:56:57
```

```
summary(m)$varcomp
```

```
        component
        std.error
        z.ratio
        bound
        %ch

        Run
        -0.004504789
        0.002896281
        -1.555370
        U 0.1

        Run:Plant!R
        0.229834648
        0.042316936
        5.431269
        P 0.0
```

Now the Run component is negative and the Run:Plant variance estimate is now equal to that for the Residuals Mean Sq for Run:Plant from the anova table.

### Set up an asrtests object

```
current.asrt <- as.asrtests(m)
print(current.asrt, which = "pseudoanova")</pre>
```

#### #### Pseudo-anova table for fixed terms

Wald tests for fixed effects.

Response: logitP

	Df	${\tt denDF}$	F.inc	Pr
(Intercept)	1	1	1550.00	0.0162
Host	1	59	59.17	0.0000
Cadavers	2	59	37.04	0.0000
Ladybird	1	59	48.26	0.0000
Host:Cadavers	2	59	0.67	0.5158
Host:Ladybird	1	59	0.99	0.3234
Cadavers:Ladybird	2	59	3.77	0.0287
Host:Cadavers:Ladybird	2	59	0.44	0.6493

The asrtests object contains a wald.tab component which can be printed by specifying that the pseudoanova is printed. The F-values for the fixed terms in this table are the same as those in the anova table.

## Obtain the marginality matrix for the fixed terms

The pstructure function from the dae package (Brien, 2024b) produce the marginality matrix for a formula as a side effect and we take advantage of that to obtain the matrix required here.

	Host	Cadavers	Host:Cadavers	Ladybird	Host:Ladybird	Cadavers:Ladybird
Host	1	0	1	0	1	0
Cadavers	0	1	1	0	0	1
Host:Cadavers	0	0	1	0	0	0
Ladybird	0	0	0	1	1	1
Host:Ladybird	0	0	0	0	1	0
Cadavers:Ladybird	0	0	0	0	0	1
Host:Cadavers:Ladybird	. 0	0	0	0	0	0

	Host:Cadavers:Ladybird
Host	1
Cadavers	1
Host:Cadavers	1
Ladybird	1
Host:Ladybird	1
Cadavers:Ladybird	1
Host:Cadavers:Ladybird	1

This marginality matrix is interpreted by taking a row term and noting that it is marginal to any column term with a one in this row.

#### Choose marginality-compliant model

```
chosen <- chooseModel(current.asrt, terms.marginality = HCL.marg)</pre>
current.asrt <- chosen$asrtests.obj</pre>
print(current.asrt, which = "test", omit.columns = c("AIC", "BIC"))
     Sequence of model investigations
                   terms DF denDF
                                                  action
1 Host:Cadavers:Ladybird 2
                               59 0.6493 Nonsignificant
2
       Cadavers:Ladybird 2
                               59 0.0287
                                             Significant
3
           Host:Ladybird 1
                               59 0.3234 Nonsignificant
           Host:Cadavers 2
4
                               59 0.5158 Nonsignificant
5
                               59 0.0000
                                             Significant
                    Host 1
(chosen$sig.terms)
```

```
[[1]]
[1] "Cadavers:Ladybird"

[[2]]
[1] "Host"
```

The chooseModel function produces a list with components sig.terms, a list with the terms in the marginality-compliant model, and asrtests.obj, the asrtests object resulting from the model selection. In particular, the asrtests object contains a test.summary that details the tests performed in choosing the model; the AIC and BIC columns are omitted from test.summary because their inclusion has not been requested. Note that chooseModel does not test the main effects for Cadavers or Ladybird, because these are marginal to the significant two-factor interaction Cadavers:Ladybird.

#### Form formula for selected model

~Cadavers:Ladybird + Host

```
chosen.mod <- paste(unlist(chosen$sig.terms), collapse = " + ")
(chosen.mod <- as.formula(paste("~", chosen.mod)))</pre>
```

# Obtain predictions under the chosen model and form an alldiffs object

```
Joining with 'by = join_by(fac.comb)'
Joining with 'by = join_by(Host)'
```

#### Predictions for logitP transform(s) from Host:Ladybird:Cadavers

#### Notes:

The original predictions, obtained as described below, have been linearly transformed to form estimated marginal means.

- The predictions are obtained by averaging across the hypertable calculated from model terms constructed solely from factors in the averaging and classify sets.
- Use 'average' to move ignored factors into the averaging set.
- The ignored set: Run

	Host	Ladybird	Cadavers	<pre>predicted.value</pre>	${\tt standard.error}$	<pre>upper.halfLeastSignificant.limit</pre>
1	bean	-	5	-1.6038338	0.1417454	-1.4080222
2	bean	-	10	-1.1454308	0.1417454	-0.9496192
3	bean	-	20	-0.7448097	0.1417454	-0.5489981
4	bean	+	5	-1.0195475	0.1417454	-0.8237359
5	bean	+	10	-0.5983440	0.1417454	-0.4025323
6	bean	+	20	0.4786704	0.1417454	0.6744820
7	trefoil	-	5	-2.4730339	0.1417454	-2.2772223
8	trefoil	_	10	-2.0146309	0.1417454	-1.8188193
9	trefoil	-	20	-1.6140098	0.1417454	-1.4181982
10	trefoil	+	5	-1.8887476	0.1417454	-1.6929360
11	trefoil	+	10	-1.4675441	0.1417454	-1.2717325
12	trefoil	+	20	-0.3905297	0.1417454	-0.1947181
	lower ha	lfI asetQ.	ianificant	t limit oct ctati	16	

lower.halfLeastSignificant.limit est.status

1	-1.7996454	Estimable
2	-1.3412425	Estimable
3	-0.9406214	Estimable
4	-1.2153592	Estimable
5	-0.7941556	Estimable
6	0.2828588	Estimable
7	-2.6688455	Estimable
8	-2.2104426	Estimable
9	-1.8098215	Estimable
10	-2.0845593	Estimable
11	-1.6633557	Estimable
12	-0.5863414	Estimable

## LSD values

```
minimum LSD = 0.3916233 0.3916233

mean LSD = 0.3916233 0.3916233

maximum LSD = 0.3916233 0.3916233

(sed range / mean sed = 9.92e-16 8.5e-16)
```

Setting the terms argument to Host:Ladybird:Cadavers requests predictions for all combinations of the three factors and the linear.transformation argument is used to obtain estimated marginal means (emm) that conform to the chosen model. The wald.tab is supplied so that it can be used to get the degrees of freedom for the t-value to be used in calculating the LSD; the degrees of freedom to the source for the terms argument will be used. The error.intervals argument has been set to "halfLeast", the LSDtype argument to "factor.combination" and the LSDby argument to "Host" so that the average LSD will be calculated for each Host. This necessary because, under the chosen model, the LSDs differ between Hosts. It results in lower.halfLeastSignificant.limit and upper.halfLeastSignificant.limit being added to the predictions component of the alldiffs object.

Or, caclulate predictions to check first and then transform to conform to chosen model

```
Joining with 'by = join_by(fac.comb)'
Joining with 'by = join_by(Host)'
```

#### Predictions for logitP transform(s) from Host:Ladybird:Cadavers

#### Notes:

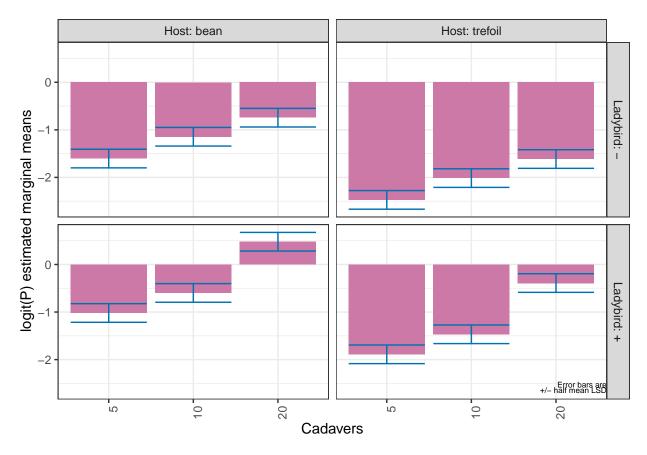
The original predictions, obtained as described below, have been linearly transformed to form estimated marginal means.

- The predictions are obtained by averaging across the hypertable calculated from model terms constructed solely from factors in the averaging and classify sets.
- Use 'average' to move ignored factors into the averaging set.
- The ignored set: Run

	Host	Ladybird	Cadavers	<pre>predicted.value</pre>	standard.error	upper.halfLeastSignificant.limit
1	bean	-	5	-1.6038338	0.1417454	-1.4080222
2	bean	-	10	-1.1454308	0.1417454	-0.9496192
3	bean	-	20	-0.7448097	0.1417454	-0.5489981
4	bean	+	5	-1.0195475	0.1417454	-0.8237359
5	bean	+	10	-0.5983440	0.1417454	-0.4025323
6	bean	+	20	0.4786704	0.1417454	0.6744820
7	trefoil	-	5	-2.4730339	0.1417454	-2.2772223
8	trefoil	-	10	-2.0146309	0.1417454	-1.8188193
9	trefoil	_	20	-1.6140098	0.1417454	-1.4181982
10	trefoil	+	5	-1.8887476	0.1417454	-1.6929360
11	${\tt trefoil}$	+	10	-1.4675441	0.1417454	-1.2717325

```
12 trefoil
                                 -0.3905297
                                                                                 -0.1947181
                         20
                                                 0.1417454
   lower.halfLeastSignificant.limit est.status
                        -1.7996454 Estimable
1
2
                        -1.3412425 Estimable
3
                        -0.9406214 Estimable
4
                        -1.2153592 Estimable
5
                        -0.7941556 Estimable
6
                         0.2828588 Estimable
7
                        -2.6688455 Estimable
8
                        -2.2104426 Estimable
9
                        -1.8098215 Estimable
10
                        -2.0845593 Estimable
                        -1.6633557 Estimable
11
12
                        -0.5863414 Estimable
LSD values
minimum LSD = 0.3916233 0.3916233
mean LSD = 0.3916233 0.3916233
maximum LSD = 0.3916233 0.3916233
(sed range / mean sed = 9.92e-16 8.5e-16)
```

# Plot the predictions

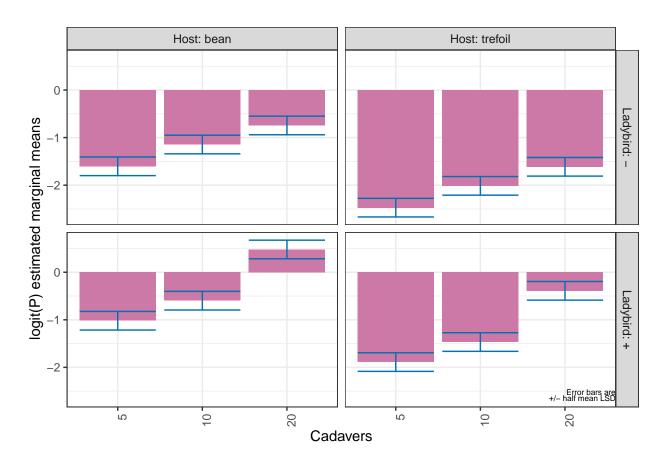


The function plotPredictions uses ggplot to produce the plot and the ggplotFuncs argument allows the addition of ggplot functions to modify the plot. In this case, the facet.grid function is respecified to include prepender functions that modify the labels of the facets to include the factor names. Note the the error bars in the plots are of  $\pm 0.5 LSD$  so that pairs of prediction with nonoverlapping bars are significantly different (Snee, 1981).

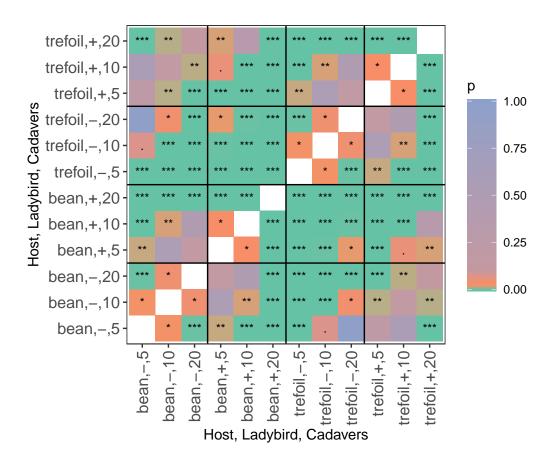
### Get and plot the predictions with a single function call

The predictPresent function combines the functionality of predictPlus and plotPredictions, as demonstrated now. Also, the use of plotPvalues to plot the pairwise p-values is displayed. The predictPresent function has the capability of producing alldiffs objects for multiple terms and these are stored in a list each of which is named for the term whose alldiffs object it stores. Thus, the term has to be specified in referencing components of diffs.

Joining with 'by = join\_by(fac.comb)'
Joining with 'by = join\_by(Host)'



plotPvalues(diffs\$Host.Ladybird.Cadavers, factors.per.grid = 1, show.sig = TRUE)



# options(width = 90) diffs\$Host.Ladybird.Cadavers\$differences

```
bean,-,5 bean,-,10 bean,-,20
                                                bean,+,5 bean,+,10 bean,+,20
bean, -, 5
              0.0000000 - 0.4006211 - 0.1258833 - 0.5470869 - 1.6241012
bean, -, 10
              0.45840297
bean, -, 20
              0.85902408
                         0.4006211 0.0000000
                                              0.2747378 -0.1464657 -1.2234801
bean,+,5
              0.58428627
                         0.1258833 -0.2747378
                                               0.0000000 -0.4212036 -1.4982179
                                                          0.0000000 -1.0770144
bean, +, 10
              1.00548982
                         0.5470869
                                    0.1464657
                                               0.4212036
bean,+,20
              2.08250420
                         1.6241012
                                    1.2234801
                                               1.4982179
                                                          1.0770144 0.0000000
            -0.86920012 -1.3276031 -1.7282242 -1.4534864 -1.8746899 -2.9517043
trefoil,-,5
trefoil,-,10 -0.41079715 -0.8692001 -1.2698212 -0.9950834 -1.4162870 -2.4933014
trefoil,-,20 -0.01017604 -0.4685790 -0.8692001 -0.5944623 -1.0156659 -2.0926802
trefoil,+,5
            -0.28491385 -0.7433168 -1.1439379 -0.8692001 -1.2904037 -2.3674180
             0.13628970 \ -0.3221133 \ -0.7227344 \ -0.4479966 \ -0.8692001 \ -1.9462145
trefoil,+,10
trefoil,+,20
             1.21330408 0.7549011 0.3542800 0.6290178 0.2078143 -0.8692001
             trefoil,-,5 trefoil,-,10 trefoil,-,20 trefoil,+,5 trefoil,+,10 trefoil,+,20
bean, -, 5
               0.8692001
                            0.4107972
                                       0.01017604
                                                    0.2849139
                                                                -0.1362897
                                                                             -1.2133041
bean, -, 10
               1.3276031
                            0.8692001
                                        0.46857901
                                                    0.7433168
                                                                 0.3221133
                                                                             -0.7549011
bean,-,20
               1.7282242
                            1.2698212
                                       0.86920012
                                                    1.1439379
                                                                 0.7227344
                                                                             -0.3542800
bean,+,5
               1.4534864
                            0.9950834
                                       0.59446231
                                                    0.8692001
                                                                 0.4479966
                                                                             -0.6290178
bean,+,10
                                                    1.2904037
                                                                 0.8692001
                                                                             -0.2078143
               1.8746899
                           1.4162870
                                        1.01566586
bean, +, 20
               2.9517043
                            2.4933014
                                        2.09268024
                                                    2.3674180
                                                                 1.9462145
                                                                              0.8692001
trefoil,-,5
               0.0000000
                          -0.4584030
                                      -0.85902408
                                                   -0.5842863
                                                                -1.0054898
                                                                             -2.0825042
trefoil,-,10
               0.4584030
                            0.0000000
                                      -0.40062111
                                                   -0.1258833
                                                                -0.5470869
                                                                             -1.6241012
```

```
trefoil,-,20
               0.8590241
                            0.4006211
                                        0.00000000
                                                     0.2747378
                                                                  -0.1464657
                                                                               -1.2234801
trefoil,+,5
               0.5842863
                            0.1258833
                                       -0.27473781
                                                     0.0000000
                                                                  -0.4212036
                                                                               -1.4982179
               1.0054898
trefoil,+,10
                            0.5470869
                                        0.14646574
                                                     0.4212036
                                                                   0.0000000
                                                                               -1.0770144
trefoil,+,20
                                                                                0.0000000
               2.0825042
                                        1.22348012
                                                                   1.0770144
                            1.6241012
                                                     1.4982179
options(width = 90)
print(diffs$Host.Ladybird.Cadavers$sed)
              bean,-,5 bean,-,10 bean,-,20 bean,+,5 bean,+,10 bean,+,20 trefoil,-,5
                    NA 0.1957142 0.1957142 0.1957142 0.1957142 0.1957142
                                                                            0.1129957
bean, -, 5
bean,-,10
             0.1957142
                              NA 0.1957142 0.1957142 0.1957142 0.1957142
                                                                            0.2259913
bean, -, 20
             0.1957142 0.1957142
                                        NA 0.1957142 0.1957142 0.1957142
                                                                            0.2259913
             0.1957142 0.1957142 0.1957142
                                                  NA 0.1957142 0.1957142
                                                                            0.2259913
bean,+,5
bean,+,10
             0.1957142 0.1957142 0.1957142 0.1957142
                                                            NA 0.1957142
                                                                            0.2259913
bean,+,20
             0.1957142 \ 0.1957142 \ 0.1957142 \ 0.1957142 \ 0.1957142
                                                                            0.2259913
trefoil,-,5 0.1129957 0.2259913 0.2259913 0.2259913 0.2259913 0.2259913
                                                                                   NA
trefoil,-,10 0.2259913 0.1129957 0.2259913 0.2259913 0.2259913 0.2259913
                                                                            0.1957142
trefoil,-,20 0.2259913 0.2259913 0.1129957 0.2259913 0.2259913 0.2259913
                                                                            0.1957142
trefoil,+,5 0.2259913 0.2259913 0.1129957 0.2259913 0.2259913
                                                                            0.1957142
trefoil,+,10 0.2259913 0.2259913 0.2259913 0.1129957 0.2259913
                                                                            0.1957142
trefoil,+,20 0.2259913 0.2259913 0.2259913 0.2259913 0.2259913 0.1129957
                                                                            0.1957142
             trefoil,-,10 trefoil,-,20 trefoil,+,5 trefoil,+,10 trefoil,+,20
                             0.2259913
                                         0.2259913
                                                      0.2259913
                                                                    0.2259913
bean, -, 5
                0.2259913
                             0.2259913
                                         0.2259913
                                                      0.2259913
                                                                    0.2259913
bean,-,10
                0.1129957
bean,-,20
                0.2259913
                             0.1129957
                                         0.2259913
                                                      0.2259913
                                                                    0.2259913
bean,+,5
                0.2259913
                             0.2259913
                                         0.1129957
                                                      0.2259913
                                                                    0.2259913
bean,+,10
                0.2259913
                             0.2259913
                                         0.2259913
                                                      0.1129957
                                                                    0.2259913
bean,+,20
                0.2259913
                             0.2259913
                                         0.2259913
                                                      0.2259913
                                                                    0.1129957
trefoil,-,5
                0.1957142
                             0.1957142
                                         0.1957142
                                                      0.1957142
                                                                    0.1957142
trefoil,-,10
                             0.1957142
                                         0.1957142
                                                      0.1957142
                                                                    0.1957142
                       NA
                                         0.1957142
trefoil,-,20
                0.1957142
                                    NA
                                                      0.1957142
                                                                    0.1957142
trefoil,+,5
                0.1957142
                             0.1957142
                                                NA
                                                      0.1957142
                                                                    0.1957142
trefoil,+,10
                0.1957142
                             0.1957142
                                         0.1957142
                                                              NA
                                                                    0.1957142
trefoil,+,20
                0.1957142
                             0.1957142
                                         0.1957142
                                                      0.1957142
                                                                           NA
```

### Perform the analysis with just selected model fitted

The model with nonsignificant fixed terms dropped is obtained in order to compare it with the fit when they are retained and the estimated marginal means for the chosen model are obtained.

#### #### Pseudo-anova table for fixed terms

Wald tests for fixed effects.

Response: logitP

	Df	${\tt denDF}$	F.inc	Pr
(Intercept)	1	1	1550.00	0.0162
Host	1	64	60.88	0.0000
Cadavers	2	64	38.12	0.0000
Ladybird	1	64	49.65	0.0000
Cadavers:Ladybird	2	64	3.88	0.0256

```
Joining with 'by = join_by(fac.comb)'
Joining with 'by = join_by(Host)'
```

#### Predictions for logitP from Host:Ladybird:Cadavers

#### Notes:

- The predictions are obtained by averaging across the hypertable calculated from model terms constructed solely from factors in the averaging and classify sets.
- Use 'average' to move ignored factors into the averaging set.
- The ignored set: Run

Host	Ladybird	${\tt Cadavers}$	<pre>predicted.value</pre>	standard.error		
bean	_	5	-1.6038338	0.1398332		
bean	_	10	-1.1454308	0.1398332		
bean	_	20	-0.7448097	0.1398332		
bean	+	5	-1.0195475	0.1398332		
bean	+	10	-0.5983440	0.1398332		
bean	+	20	0.4786704	0.1398332		
trefoil	-	5	-2.4730339	0.1398332		
trefoil	-	10	-2.0146309	0.1398332		
trefoil	-	20	-1.6140098	0.1398332		
trefoil	+	5	-1.8887476	0.1398332		
trefoil	+	10	-1.4675441	0.1398332		
trefoil	+	20	-0.3905297	0.1398332		
upper.halfLeastSignificant.limit lower.halfLeastSignificant						
-1.4107942 -						
	bean bean bean bean bean trefoil trefoil trefoil trefoil trefoil	bean       -         bean       -         bean       +         bean       +         bean       +         trefoil       -         trefoil       -         trefoil       +         trefoil       +         trefoil       +         trefoil       +	bean       -       5         bean       -       20         bean       +       5         bean       +       10         bean       +       20         trefoil       -       5         trefoil       -       20         trefoil       +       5         trefoil       +       5         trefoil       +       10         trefoil       +       20         upper.halfLeastSignificant	bean       -       5       -1.6038338         bean       -       10       -1.1454308         bean       -       20       -0.7448097         bean       +       5       -1.0195475         bean       +       10       -0.5983440         bean       +       20       0.4786704         trefoil       -       5       -2.4730339         trefoil       -       10       -2.0146309         trefoil       -       20       -1.6140098         trefoil       +       5       -1.8887476         trefoil       +       10       -1.4675441         trefoil       +       20       -0.3905297         upper.halfLeastSignificant.limit lower.half	bean       -       10       -1.1454308       0.1398332         bean       -       20       -0.7448097       0.1398332         bean       +       5       -1.0195475       0.1398332         bean       +       10       -0.5983440       0.1398332         bean       +       20       0.4786704       0.1398332         trefoil       -       5       -2.4730339       0.1398332         trefoil       -       10       -2.0146309       0.1398332         trefoil       -       20       -1.6140098       0.1398332         trefoil       +       5       -1.8887476       0.1398332         trefoil       +       10       -1.4675441       0.1398332         trefoil       +       20       -0.3905297       0.1398332         upper.halfLeastSignificant.limit lower.halfLeastSignificant	

```
      upper.halfLeastSignificant.limit
      lower.halfLeastSignificant.limit
      est.status

      1
      -1.4107942
      -1.7968734
      Estimable

      2
      -0.9523913
      -1.3384704
      Estimable

      3
      -0.5517702
      -0.9378493
      Estimable
```

```
4
                         -0.8265080
                                                          -1.2125871 Estimable
                                                          -0.7913835 Estimable
5
                         -0.4053044
6
                          0.6717100
                                                           0.2856308 Estimable
7
                         -2.2799944
                                                          -2.6660735 Estimable
8
                         -1.8215914
                                                          -2.2076705 Estimable
9
                                                          -1.8070494 Estimable
                         -1.4209703
                                                          -2.0817872 Estimable
10
                         -1.6957081
                                                          -1.6605837 Estimable
11
                         -1.2745045
12
                         -0.1974902
                                                          -0.5835693 Estimable
```

#### LSD values

minimum LSD = 0.3860791 0.3860791

mean LSD = 0.3860791 0.3860791

maximum LSD = 0.3860791 0.3860791

(sed range / mean sed = 1.44e-16 1.44e-16)

# options(width = 90) print(diffs.red\$sed)

```
bean,-,5 bean,-,10 bean,-,20 bean,+,5 bean,+,10 bean,+,20 trefoil,-,5
bean, -, 5
                   NA 0.1929435 0.1929435 0.1929435 0.1929435 0.1929435
                                                                          0.1113960
bean,-,10
            0.1929435
                             NA 0.1929435 0.1929435 0.1929435 0.1929435
                                                                          0.2227920
            0.1929435 0.1929435
                                       NA 0.1929435 0.1929435 0.1929435
bean,-,20
                                                                          0.2227920
            0.1929435 0.1929435 0.1929435
bean,+,5
                                                 NA 0.1929435 0.1929435
                                                                          0.2227920
bean,+,10
            0.1929435 0.1929435 0.1929435 0.1929435
                                                           NA 0.1929435
                                                                          0.2227920
            0.1929435 0.1929435 0.1929435 0.1929435 0.1929435
bean,+,20
                                                                     NA
                                                                          0.2227920
trefoil,-,5 0.1113960 0.2227920 0.2227920 0.2227920 0.2227920 0.2227920
                                                                                 NA
trefoil,-,10 0.2227920 0.1113960 0.2227920 0.2227920 0.2227920 0.2227920
                                                                          0.1929435
trefoil,-,20 0.2227920 0.2227920 0.1113960 0.2227920 0.2227920 0.2227920
                                                                          0.1929435
trefoil,+,5 0.2227920 0.2227920 0.2227920 0.1113960 0.2227920 0.2227920
                                                                          0.1929435
trefoil,+,10 0.2227920 0.2227920 0.2227920 0.2227920 0.1113960 0.2227920
                                                                          0.1929435
trefoil,+,20 0.2227920 0.2227920 0.2227920 0.2227920 0.2227920 0.1113960
                                                                          0.1929435
            trefoil,-,10 trefoil,-,20 trefoil,+,5 trefoil,+,10 trefoil,+,20
bean, -,5
                0.2227920
                            0.2227920
                                       0.2227920
                                                     0.2227920
                                                                  0.2227920
bean,-,10
                0.1113960
                            0.2227920
                                        0.2227920
                                                     0.2227920
                                                                  0.2227920
bean,-,20
               0.2227920
                            0.1113960
                                       0.2227920
                                                     0.2227920
                                                                  0.2227920
bean,+,5
               0.2227920
                            0.2227920
                                       0.1113960
                                                     0.2227920
                                                                  0.2227920
bean,+,10
               0.2227920
                            0.2227920
                                       0.2227920
                                                     0.1113960
                                                                  0.2227920
bean,+,20
               0.2227920
                            0.2227920
                                       0.2227920
                                                     0.2227920
                                                                  0.1113960
               0.1929435
                            0.1929435 0.1929435
                                                     0.1929435
trefoil,-,5
                                                                  0.1929435
trefoil,-,10
                            0.1929435
                                       0.1929435
                                                     0.1929435
                                                                  0.1929435
                      NA
                                       0.1929435
trefoil,-,20
               0.1929435
                                   NA
                                                     0.1929435
                                                                  0.1929435
trefoil,+,5
               0.1929435
                            0.1929435
                                                     0.1929435
                                                                  0.1929435
                            0.1929435 0.1929435
                                                                  0.1929435
trefoil,+,10
               0.1929435
                                                            NA
trefoil,+,20
               0.1929435
                            0.1929435
                                        0.1929435
                                                     0.1929435
                                                                         NA
```

# References

Brien, C. J. (2024a) asremlPlus: Augments ASReml-R in fitting mixed models and packages generally in exploring prediction differences. Version 4.4.40. https://cran.r-project.org/package=asremlPlus/ or http://chris.brien.name/rpackages/.

Brien, C. J. (2024b) dae: Functions useful in the design and ANOVA of experiments. Version 3.2.29 . https://cran.r-project.org/package=dae/ or http://chris.brien.name/rpackages/.

Butler, D. G., Cullis, B. R., Gilmour, A. R., Gogel, B. J. and Thompson, R. (2023). ASReml-R Reference Manual Version 4.2. VSN International Ltd, https://asreml.kb.vsni.co.uk/.

Littell, R. C., Milliken, G. A., Stroup, W. W., Wolfinger, R. D., & Schabenberger, O. (2006). SAS for Mixed Model. (2nd ed.). Cary, N.C.: SAS Press.

R Core Team (2024) R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. https://www.r-project.org/.

Snee, R. D. (1981). Graphical Display and Assessment of Means. Biometrics, 37, 835–836.

Welham, S. J., Gezan, S. A., Clark, S. J., & Mead, A. (2014). Statistical Methods in Biology: Design and Analysis of Experiments and Regression. Boca Raton: Chapman and Hall/CRC.