data_exploration

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Data import

Background information

This is the data of the whitefly development bioassay on grafts of MM and LA1840. The nymphs in all developmental stages (first to fourth instar) were counted every other day. After the fourth instar stage, whiteflies develop into adults, leaving behind the larval skin called exuviae. Nymphs in the last phase of fourth instar stage and exuviae were removed from the leaf after each count to prevent a whitefly outbreak in the greanhouse.

See metadata file for more information.

The raw data

genotype	place	date	day	stage	number	$eggs_start$	hatched
LA1840	1	26/11/2021	2	0_egg	60	60	40
LA1840	1	26/11/2021	2	1_first_instar	0	60	40
LA1840	1	26/11/2021	2	2 _second_instar	0	60	40
LA1840	1	26/11/2021	2	3_third_instar	0	60	40
LA1840	1	26/11/2021	2	4_early_fourth_instar	0	60	40
LA1840	1	26/11/2021	2	5 _late_fourth_instar	0	60	40
LA1840	1	26/11/2021	2	6_exuviea	0	60	40
MM	2	26/11/2021	2	0 _egg	66	66	46
MM	2	26/11/2021	2	1 _first_instar	0	66	46
MM	2	26/11/2021	2	2_second_instar	0	66	46

Preparing the data for analysis

A few improvements to the data should be made before analysis.

First, three plants must be excluded from analysis, based on observations during counting (see metadata file).

Next, I make data wide for access to separate life stages.

Because the fourth instars are the end point and taken of after counting, combine fourth instars and exuviae and make their numbers cumulative over time.

Remove separate and non-cumulative late fourth instar and exuviae columns.

Now the data can be made long again.

Add the relative number of nymphs as percentage from the number of eggs and as percentage from the number of hatched eggs

Add a new dataframe for the total number of nymphs per day without percentage, based on "flies" and make the data wide.

Add the total number of nymphs per sample per day and the relative total as percentage from the number of eggs and as percentage from the number of hatched eggs

Different developmental stages, different effects

In the original dataset of Arjen with ungrafted plants, you can see different effects of LA1840 on the different whitefly lifestages. The number of eggs was higher on LA1840 than on MM, while the number of fourth instar nymphs was drasticly decreased on LA1840. While the number of eggs is probably the result of the effect the plant has on adult flies, the nymph development is more likely to be influenced by the diet of the nymphs themselves. Another effect the plants could have, is affecting the hatching of the eggs. This can however not be analysed from Arjens data.

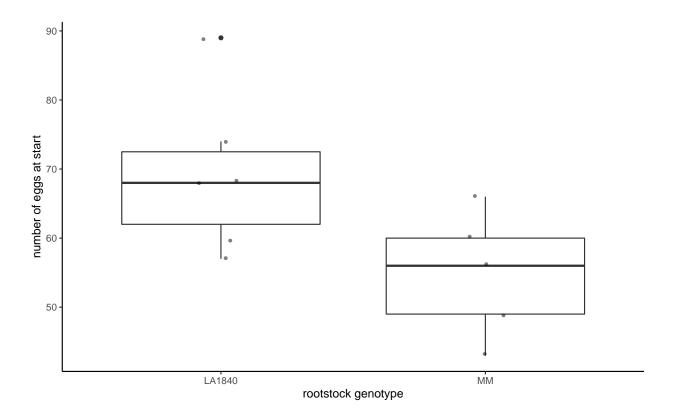
Because I want to have a clear view of those different effects, I will go through each part separately.

Eggs

On normal LA1840 plants, the number of eggs is increased compared to MM plants. Can we see the same effect when an LA1840 rootstock is used with a MM scion?

First a quick overview of the data:

And now visualized in a boxplot:



It seems like the number of eggs might be higher on the grafts with an LA1840 rootstock.

Before testing this, check the assumptions: - normality - homogeneity of variance - no significant outliers

```
##
## Shapiro-Wilk normality test
##
## data: resid(aov(flies_wide$'0_egg' ~ flies_wide$genotype))
## W = 0.95145, p-value = 0.6625
```

The p-value of the Shapiro-Wilk test is >0.05, so the data is normally distributed

```
## Levene's Test for Homogeneity of Variance (center = median)
## Df F value Pr(>F)
## group 1 0.0447 0.8372
## 9
```

The Levene's test also has p>0.05, so the variance is equal between the two groups.

Now the outliers:

```
## # A tibble: 1 x 18
     genotype place date
                                 day eggs_start hatched '0_egg' '1_first_instar'
##
     <chr>>
              <int> <chr>
                                <int>
                                           <int>
                                                   <int>
                                                           <int>
                                                                             <int>
                  7 26/11/2021
## 1 LA1840
                                   2
                                              89
                                                      57
## # ... with 10 more variables: 2_second_instar <int>, 3_third_instar <int>,
       4_early_fourth_instar <int>, 5_completed_lifecycle <int>,
       6_total_fourth_instars <int>, total <dbl>, percentage_from_eggs <dbl>,
       percentage_from_hatched <dbl>, is.outlier <lgl>, is.extreme <lgl>
## #
```

There is one outlier, but it is not extreme.

Because all assumptions for a t-test are met, we can do a Students t-test.

```
## # A tibble: 1 x 9
                                                            p p.signif
           group1 group2
                                   n2 statistic
     .y.
                             n1
## * <chr> <chr>
                                           <dbl> <dbl>
                  <chr>
                          <int>
                                <int>
                                                        <dbl> <chr>
## 1 0_egg LA1840 MM
                             78
                                   65
                                           2.30
                                                     9 0.0468 *
## # A tibble: 1 x 7
           group1 group2 effsize
                                           n2 magnitude
                                     n1
                            <dbl> <int> <int> <ord>
## * <chr> <chr>
                  <chr>>
## 1 0_egg LA1840 MM
                             1.39
                                     78
                                           65 large
```

The difference in number of eggs on MM and LA1840 grafts is significant (p=0.0468) and the effectsize of the rootstock genotype on the number of eggs is large (d=1.39).

The d-value indicates the difference between the two means in number of standard deviations. So the mean of LA1840 grafts is 1.39 standard deviations higher than that of MM grafts.

Effect of rootstock genotype on the number of eggs

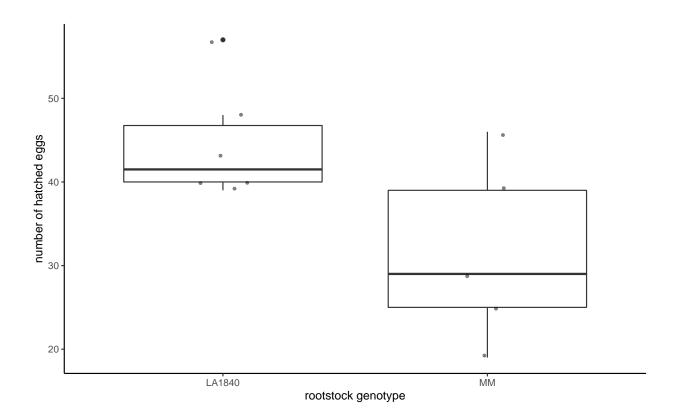
Similar to the previous findings on normal LA1840 and MM plants, the number of eggs are increased on grafts with an LA1840 rootstock compared to grafts with a MM rootstock. This indicates that the signal responsible for the increased oviposition is transported from the LA1840 rootstock to the MM scion.

Hatching

Is there an effect of rootstock genotype on the hatching of the eggs? For this I will use the absolute number of hatched eggs, as well as the percentage.

absolute number of hatched eggs

An overview of the data:



Shapiro-Wilk test:

Levene's test

```
## # A tibble: 1 x 4
## df1 df2 statistic p
## <int> <dbl> <dbl> <dbl> ## 1 1 9 0.858 0.378
```

Identifying extreme outliers:

```
## # A tibble: 1 x 18
##
     genotype place date
                                      eggs_start hatched '0_egg' '1_first_instar'
                               day
                                                                             <int>
     <chr>>
              <int> <chr>
                                <fct>
                                           <int>
                                                   <int>
                                                           <int>
## 1 LA1840
                  7 26/11/2021 2
                                              89
                                                      57
                                                              89
## # ... with 10 more variables: 2_second_instar <int>, 3_third_instar <int>,
       4_early_fourth_instar <int>, 5_completed_lifecycle <int>,
       6_total_fourth_instars <int>, total <dbl>, percentage_from_eggs <dbl>,
       percentage_from_hatched <dbl>, is.outlier <lgl>, is.extreme <lgl>
```

The assumptions for a t-test are met.

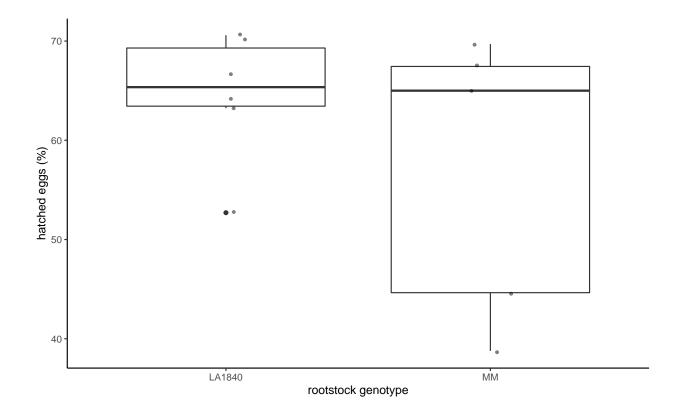
```
## # A tibble: 1 x 9
             group1 group2
     .у.
                                     n2 statistic
                                                      df
                                                              p p.signif
                               n1
                           <int> <int>
                                            <dbl> <dbl>
                                                          <dbl> <chr>
## * <chr>
             <chr> <chr>
## 1 hatched LA1840 MM
                                6
                                      5
                                             2.39
                                                       9 0.0403 *
## # A tibble: 1 x 7
                                             n2 magnitude
##
             group1 group2 effsize
                                       n1
## * <chr>
             <chr> <chr>
                              <dbl> <int> <int> <ord>
## 1 hatched LA1840 MM
                               1.45
                                        6
                                              5 large
```

Again, there is a significant difference between LA1840 and MM grafts (p=0.04) with a large effectsize of the rootstock genotype (d=1.45). This could, however, be caused by the difference in number of eggs. Therefore, I will also check the success of hatching relative to the amount of eggs.

Percentage of hatching

An overview of the data:

```
## # A tibble: 2 x 5
     genotype variable
##
                               n mean
##
     <chr>>
              <chr>>
                            <dbl> <dbl> <dbl>
                                   64.6 6.56
## 1 LA1840
              perc_hatched
                                6
## 2 MM
              perc_hatched
                                5
                                   57.1 14.3
```



Shapiro-Wilk test:

Levene's test

```
## # A tibble: 1 x 4
## df1 df2 statistic p
## <int> <int> <dbl> <dbl> <dbl> ## 1 1 9 1.45 0.260
```

Identifying extreme outliers:

```
## # A tibble: 1 x 19
                                      eggs_start hatched '0_egg' '1_first_instar'
##
     genotype place date
                                day
##
     <chr>>
              <int> <chr>
                                <fct>
                                           <int>
                                                    <int>
                                                            <int>
                                                                             <int>
                  6 26/11/2021 2
                                              74
                                                               74
## 1 LA1840
                                                      39
                                                                                 0
## # ... with 11 more variables: 2_second_instar <int>, 3_third_instar <int>,
       4_early_fourth_instar <int>, 5_completed_lifecycle <int>,
       6_total_fourth_instars <int>, total <dbl>, percentage_from_eggs <dbl>,
       percentage_from_hatched <dbl>, perc_hatched <dbl>, is.outlier <lgl>,
## #
       is.extreme <lgl>
```

The assumptions for a t-test are met.

```
## # A tibble: 1 x 9
    .у.
                  group1 group2
                                   n1
                                          n2 statistic
                                                                 p p.signif
## * <chr>
                  <chr> <chr> <int> <int>
                                                 <dbl> <dbl> <dbl> <chr>
## 1 perc_hatched LA1840 MM
                                     6
                                           5
                                                  1.15
                                                             0.28 ns
## # A tibble: 1 x 7
                  group1 group2 effsize
    .у.
                                            n1
                                                  n2 magnitude
## * <chr>
                  <chr>
                         <chr>
                                   <dbl> <int> <int> <ord>
## 1 perc_hatched LA1840 MM
                                  0.696
                                                   5 moderate
```

There is no difference in the percentage of hatched eggs between the two rootstock genotypes. There is, however, a large variation in hatching between plants, as can be seen in the above boxplot.

```
## ANOVA Table (type III tests)
##
##
             Effect DFn DFd
## 1
           genotype
                       1
                           9
                                6.344 3.30e-02
                                                    * 0.378
## 2
                           9 120.586 1.63e-06
                                                    * 0.650
               stage
## 3 genotype:stage
                       1
                           9
                                0.139 7.17e-01
                                                      0.002
```

The same result is visible in a two-way repeated measures anova. Although the number of eggs and of hatched eggs differs between the rootstock genotypes and the number of hatched eggs is lower than the initial number of eggs, there is no genotype*stage effect. This means that the rootstock genotype does not influence the hatching rate.

Effect of rootstock genotype on hatching

Although the LA1840 grafts have a higher number of hatched eggs, the grafting itself does not seem to be influenced by the rootstock, as the percentage of hatched eggs is equal on the LA1840 and MM grafts.

Nymph development

For the nymph development, I want to focus on the development from first instar to fourth instar. To cancel-out effect on oviposition and variation in hatching, take percentage of later nymphs from first instars.

First, I'll make a new dataframe for the total number of nymphs that passed a developmental stage for each plant

genotype	place	stage	number
LA1840	1	eggs_start	60
LA1840	1	hatched	40
LA1840	1	$second_instars$	37
LA1840	1	$third_instars$	36
LA1840	1	$fourth_instars$	35
MM	2	eggs_start	66
MM	2	hatched	46
MM	2	$second_instars$	45
MM	2	$third_instars$	41
MM	2	$fourth_instars$	40

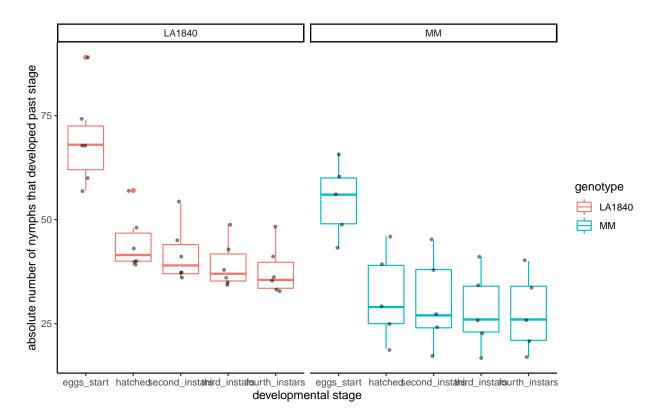
And also for the numbers relative to eggs and hatched eggs

genotype	place	stage	perc_from_eggs
LA1840	1	eggs	100.00000
LA1840	1	hatched_eggs	66.66667
LA1840	1	second	61.66667
LA1840	1	third	60.00000
LA1840	1	fourth	58.33333
MM	2	eggs	100.00000
MM	2	$hatched_eggs$	69.69697
MM	2	second	68.18182
MM	2	third	62.12121
MM	2	fourth	60.60606

genotype	place	stage	perc_from_hatched
LA1840	1	eggs	150.00000
LA1840	1	$hatched_eggs$	100.00000
LA1840	1	second	92.50000
LA1840	1	third	90.00000
LA1840	1	fourth	87.50000
MM	2	eggs	143.47826
MM	2	hatched_eggs	100.00000
MM	2	second	97.82609
MM	2	third	89.13043

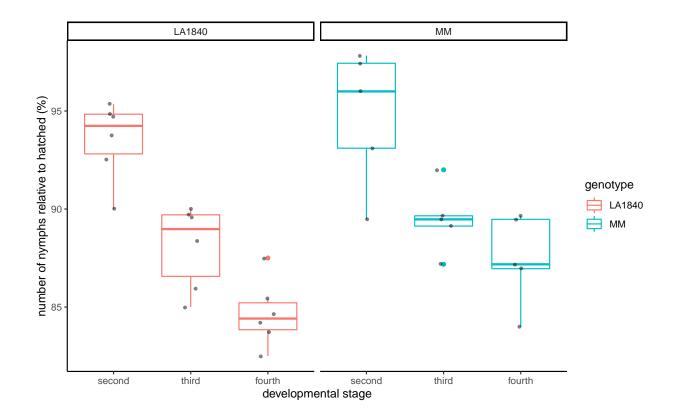
genotype	place	stage	perc_from_hatched
MM	2	fourth	86.95652

What happens in the absolute numbers through the development?



The number of nymphs appears to be higher on LA1840 grafts, but this could be the result of the higher number of eggs, as discussed above.

What happens if we look at the number of nymphs relative to the number of hatched eggs?



Now it seems like a larger percentage of eggs which hatched on MM grafts developed into fourth instar nymph than on LA1840 grafts.

Is this a significant difference?

An overview of the data:

```
## # A tibble: 2 x 5
     genotype variable
##
                                                 sd
                                        mean
     <chr>
              <chr>
                                 <dbl> <dbl> <dbl>
## 1 LA1840
              perc_from_hatched
                                     6
                                        84.7
                                               1.70
## 2 MM
              perc_from_hatched
                                        87.5
                                     5
                                              2.30
```

Shapiro-Wilk test:

Levene's test

```
## # A tibble: 1 x 4
## df1 df2 statistic p
## <int> <int> <dbl> <dbl> <dbl> ## 1 1 9 0.343 0.572
```

Identifying extreme outliers:

The assumptions for a t-test are met.

```
## # A tibble: 1 x 9
##
     .y.
                        group1 group2
                                          n1
                                                n2 statistic
                                                                          p p.signif
                                       <int>
                                                                      <dbl> <chr>
## * <chr>
                        <chr>
                               <chr>
                                             <int>
                                                        <dbl> <dbl>
                                                        -2.32
                                                                  9 0.0455 *
## 1 perc_from_hatched LA1840 MM
                                           6
                                                 5
## # A tibble: 1 x 7
##
                        group1 group2 effsize
                                                         n2 magnitude
     .у.
                                                   n1
## * <chr>
                        <chr>
                               <chr>>
                                         <dbl> <int> <int> <ord>
## 1 perc_from_hatched LA1840 MM
                                         -1.40
                                                          5 large
```

The percentage of hatched eggs that develop into fourth instar nymphs is indeed lower on LA1840 grafts than on MM grafts (p=0.046). The rootstock genotype has a large effect (d=1.40) on the development of nymphs into fourth instars after hatching.

Effect of rootstock genotype on the nymph development

In line with the previous results on normal LA1840 and MM plants, the nymph development is decreased on the LA1840 grafts compared to the MM grafts. Contrary to the previous findings, however, this is only visible in the development relative to the number of hatched eggs.

First conclusions

overall similar to results of Arjen, but diluted. Two contradicting effects of LA1840 rootstock on whiteflies: - increased oviposition - hampered nymph development

Effects found on MM scion suggests transportable nature of responsible mechanism(s).

Phenotype in detail

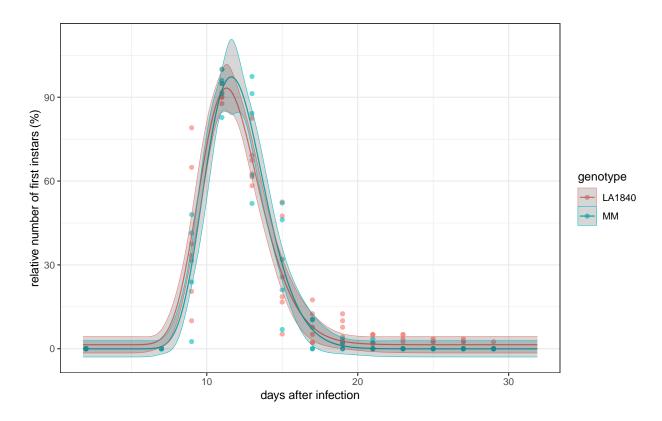
Now, let's have a more detailed look at the development. Because I am specifically interested in the effect of the rootstock genotype on the development of the nymphs and we just saw a variation in hatching and an effect of the rootstock genotype on the number of eggs, I will use nymph numbers relative to the number of hatched eggs.

Hatching (first instars)

I want to use the right model, so that it the first step. The drc package has a function for selection the best fitting model, but needs an initial model to start with.

```
## lgaussian -511.8928 1045.786 0.9997925 80.96022
## lgaussian -511.8928 1045.786 0.9997925 80.96022
## gaussian -519.4618 1060.924 0.4390741 90.00075
```

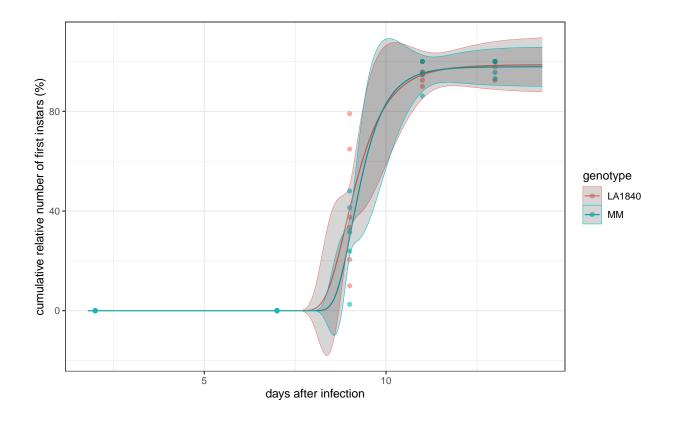
The data follows a skewed bell-shaped pattern, so the best fitting model is lgaussian.



and cumulative:

##		logLik	IC	Lack of fit	Res var
##	W1.3	-201.9921	417.9843	1.0000000	101.7791
##	LL.3	-202.0131	418.0262	0.9998484	101.8566
##	W2.3	-202.3056	418.6113	0.9710982	102.9460
##	W2.3	-202.3056	418.6113	0.9710982	102.9460
##	W1.4	NA	NA	NA	NA
##	W2.4	NA	NA	NA	NA

W1.3 has the best fit.



The two curves are mostly overlapping. Are there any differences?

```
##
## 1st model
            W1.3(names = c("Slope", "Upper Limit", "ED50"))
   pmodels: 1 (for all parameters)
## 2nd model
  fct:
            W1.3(names = c("Slope", "Upper Limit", "ED50"))
   pmodels: ~genotype, ~genotype, ~genotype
## ANOVA table
##
            ModelDf
                        RSS Df F value p value
##
## 2nd model
                 52 5344.1
                 49 4987.2 3 1.1690 0.3311
## 1st model
##
## Model fitted: Weibull (type 1) with lower limit at 0 (3 parms)
##
## Parameter estimates:
##
                             Estimate Std. Error t-value p-value
## Slope:(Intercept)
                           -15.148474
                                       8.136629 -1.8618 0.06864
## Slope:genotypeMM
                            -3.946561 15.812214 -0.2496 0.80395
## Upper Limit:(Intercept)
                           98.733997
                                        4.875073 20.2528 < 2e-16 ***
## Upper Limit:genotypeMM
                           -0.877253
                                        6.908700 -0.1270 0.89948
## ED50:(Intercept)
                             8.925431
                                        0.074395 119.9729 < 2e-16 ***
```

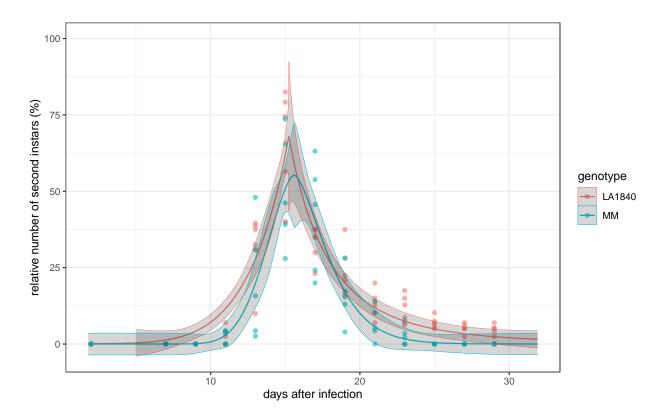
No, the hatching of first instars from the eggs is simmilar for the two rootstock genotypes.

Second instars

I want to use the right model, so that it the first step. The drc package has a function for selection the best fitting model, but needs an initial model to start with.

```
## lgaussian -508.3260 1028.652 0.467282660 74.22996
## lgaussian -508.3260 1028.652 0.467282660 74.22996
## gaussian -515.0255 1042.051 0.009905193 81.52155
```

The data follows a skewed bell-shaped pattern, so the best fitting model is lgaussian.

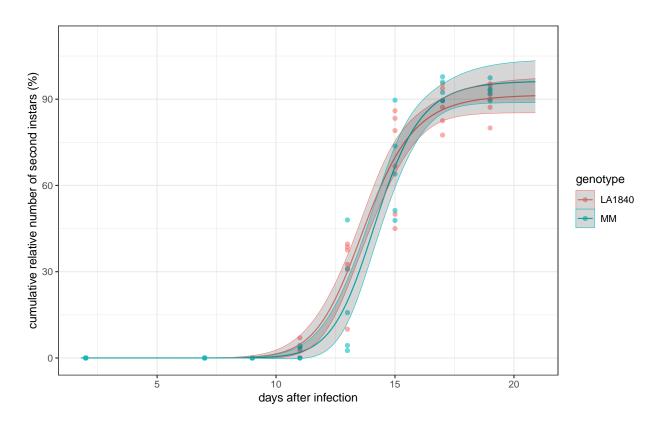


and cumulative:

```
## logLik IC Lack of fit Res var
## LL.3 -306.6068 627.2137 0.9961782 66.75824
```

```
## W2.3 -307.0493 628.0986 0.9860545 67.43292
## W2.3 -307.0493 628.0986 0.9860545 67.43292
## W1.3 -307.3248 628.6497 0.9746333 67.85656
## W1.4 NA NA NA NA NA
```

LL.3 has the best fit.



The two curves are mostly, but not completely overlapping. Are there any differences?

```
##
## 1st model
            LL.3(names = c("Slope", "Upper Limit", "ED50"))
  fct:
   pmodels: 1 (for all parameters)
## 2nd model
            LL.3(names = c("Slope", "Upper Limit", "ED50"))
## fct:
  pmodels: ~genotype, ~genotype, ~genotype
## ANOVA table
##
##
             ModelDf
                       RSS Df F value p value
## 2nd model
                 85 5912.1
## 1st model
                 82 5474.2 3 2.1864 0.0958
##
## Model fitted: Log-logistic (ED50 as parameter) with lower limit at 0 (3 parms)
##
```

```
## Parameter estimates:
##
##
                            Estimate Std. Error t-value
                                                          p-value
## Slope:(Intercept)
                           -13.12247
                                        1.72915 -7.5890 4.528e-11 ***
## Slope:genotypeMM
                            -1.91164
                                        2.66491 -0.7173
                                                          0.47520
## Upper Limit:(Intercept)
                                        3.35378 27.3040 < 2.2e-16 ***
                            91.57163
## Upper Limit:genotypeMM
                                        4.87093 0.9845
                                                          0.32778
                             4.79526
## ED50:(Intercept)
                            13.72869
                                        0.17729 77.4366 < 2.2e-16 ***
                                        0.24289 2.0428
## ED50:genotypeMM
                             0.49619
                                                           0.04428 *
##
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' ' 1
##
## Residual standard error:
##
   8.170572 (82 degrees of freedom)
```

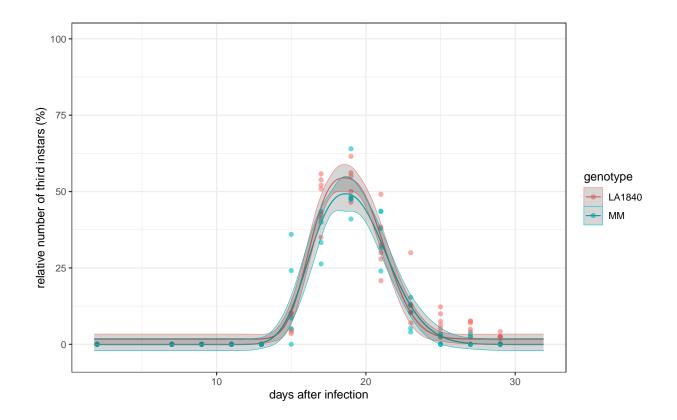
Globally, the curves of the two genotypes are similar, but they do have a different ED50 (p=0.044). The development of 50% of the nymphs to second instar stage is 0.5 (SE=0.2) day faster on LA1840 grafts than on MM grafts.

Third instars

I want to use the right model, so that it the first step. The drc package has a function for selection the best fitting model, but needs an initial model to start with.

```
## lgaussian -444.6945 901.3889 0.66827302 30.48445
## lgaussian -444.6945 901.3889 0.66827302 30.48445
## gaussian -449.8571 911.7142 0.05162815 32.76699
```

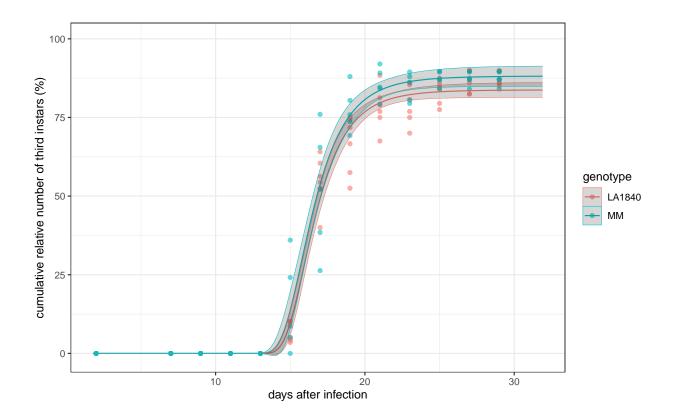
The data follows a skewed bell-shaped pattern, so the best fitting model is lgaussian.



and cumulative:

```
## logLik IC Lack of fit Res var
## W1.3 -458.1157 930.2314 0.5007835830 37.04744
## LL.3 -464.9816 943.9632 0.0503331703 40.78139
## W2.3 -474.2707 962.5415 0.0004724878 46.43920
## W2.3 -474.2707 962.5415 0.0004724878 46.43920
## W1.4 NA NA NA NA NA
## W2.4 NA NA NA NA
```

W1.3 has the best fit.



Although there is stil overlap between the curves, it appears like a difference is starting to form. Are there any significant differences?

```
##
## 1st model
             W1.3(names = c("Slope", "Upper Limit", "ED50"))
  fct:
   pmodels: 1 (for all parameters)
## 2nd model
             W1.3(names = c("Slope", "Upper Limit", "ED50"))
  fct:
   pmodels: ~genotype, ~genotype, ~genotype
## ANOVA table
##
##
             ModelDf
                        RSS Df F value p value
## 2nd model
                 140 5462.4
## 1st model
                 137 5075.5 3 3.4812 0.0177
##
## Model fitted: Weibull (type 1) with lower limit at 0 (3 parms)
##
## Parameter estimates:
##
##
                            Estimate Std. Error
                                                 t-value p-value
## Slope:(Intercept)
                           -11.16269
                                        1.16399
                                                 -9.5900 < 2e-16 ***
## Slope:genotypeMM
                             0.59127
                                        1.51099
                                                  0.3913 0.69617
## Upper Limit:(Intercept)
                            83.75903
                                        1.39649
                                                 59.9784 < 2e-16 ***
## Upper Limit:genotypeMM
                             4.40788
                                         1.99597
                                                  2.2084 0.02888 *
```

```
## ED50:(Intercept) 16.06799 0.10491 153.1538 < 2e-16 ***
## ED50:genotypeMM -0.16151 0.15668 -1.0308 0.30444
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error:
##
## 6.086661 (137 degrees of freedom)</pre>
```

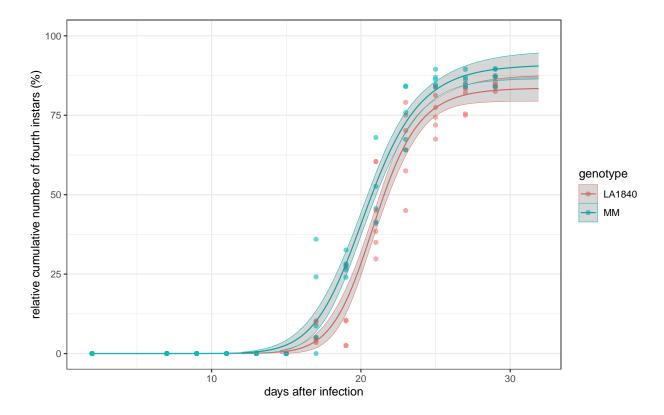
There is a global difference between the curves of the two rootstock genotypes (p=0.018). For the individual parameters, this difference can be found in the upper limit (p=0.029), which is 4.4 (SE=2.0) higher for MM grafts than for LA1840 grafts

Fourth instars

I want to use the right model, so that it the first step. The drc package has a function for selection the best fitting model, but needs an initial model to start with.

```
##
           logLik
                        IC Lack of fit Res var
## LL.3 -456.1016 926.2031
                              0.8835416 36.01838
## W2.3 -457.7544 929.5089
                              0.7313832 36.86073
## W2.3 -457.7544 929.5089
                             0.7313832 36.86073
## W1.3 -461.2309 936.4619
                              0.3476416 38.69727
## W1.4
               NA
                        NA
                                     NA
                                              NA
## W2.4
               NA
                                     NA
                                              NA
                        NA
```

The best fitting model is LL.3.



The curves of the two treatments do not overlap, so let's see if they are indeed different and which specific parameters are influenced strongest.

```
##
## 1st model
##
   fct:
             LL.3(names = c("Slope", "Upper Limit", "ED50"))
   pmodels: 1 (for all parameters)
## 2nd model
             LL.3(names = c("Slope", "Upper Limit", "ED50"))
##
   fct:
   pmodels: ~genotype, ~genotype, ~genotype
## ANOVA table
##
##
             ModelDf
                        RSS Df F value p value
## 2nd model
                 140 6161.7
                 137 4934.5
## 1st model
                             3
                               11.357
                                          0.000
##
## Model fitted: Log-logistic (ED50 as parameter) with lower limit at 0 (3 parms)
##
## Parameter estimates:
##
##
                            Estimate Std. Error
                                                  t-value p-value
## Slope: (Intercept)
                           -14.27595
                                         1.42934
                                                  -9.9878 < 2e-16 ***
## Slope:genotypeMM
                             2.87806
                                         1.74096
                                                   1.6531 0.10059
## Upper Limit:(Intercept)
                            83.62243
                                         2.06794
                                                  40.4375 < 2e-16 ***
## Upper Limit:genotypeMM
                             7.43105
                                         3.08023
                                                   2.4125 0.01717 *
## ED50:(Intercept)
                            20.96204
                                         0.16480 127.1957 < 2e-16 ***
## ED50:genotypeMM
                            -0.58033
                                        0.24862
                                                 -2.3342 0.02104 *
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error:
##
##
   6.001532 (137 degrees of freedom)
```

The ANOVA compares a model based on the pooled data of the two genotypes to the model used to compare the genotypes. This global test shows that the rootstock genotypes indeed have different curves (p<0.0001): The rootstock genotype has an effect on the development to fourth instar nymphs.

The parameters with significant differences are the upper limit (p=0.017) and ED50 (p=0.021). The upper limit of MM is 7.4 (SE=3.1) higher than that of LA1840. The ED50 indicates the day at which 50% of nymphs have reached fourth instar stage and is 0.6 (SE=0.2) day earlier for MM than for LA1840. In other words, nymphs on grafts with a MM rootstock develop faster and more into fourth instar nymphs, than those on grafts with an LA1840 rootstock.

Conclusion on details

Overall, we can see a difference in development between MM and LA1840 grafts that is increasing over time. The hatching happens simultaneously and in the same relative amounts on MM and LA1840 grafts, but the developmental speed and relative amount of nymphs developing to the next stage gradually decrease on LA1840 in the later stages. This leads to the conclusion that an LA1840 has a negative effect on the development of whitefly nymphs on the MM scion.

Final conclusion

On normal LA1840 plants, whitefly adults have an increased oviposition, while the nymphs hatched from those eggs have a strongly hampered development. Here, we used grafts of MM scions on MM and LA1840 rootstocks, to test if the observed phenotype is phloem-mobile. On the LA1840 grafts, we again saw an increased number of eggs and a decreased relative number of fourth instars.