Testing leaf surface effect on interction with Botrytis

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This notebook aims to compile and annotate the R scripts used for the analysis of the Leaf surface-Botrytis dataset.

This notebook charges R scripts that contain all the code. The main result figures are plotted directly in the notebook.

Experimental design:

Sixteen Eudicot species and 20 A.thaliana genotypes were analyzed. Detached leaves were inoculated with Botrytis in 'experimental trays', that constitutes a micro-environment for a randomized collection of isolates. After 72h, pictures of all trays were taken. Image analysis for calculation of lesion area (and many other parameters) was conducted in R.

For image analysis R codes, see the Image_analysis_pipeline_Final R notebook.

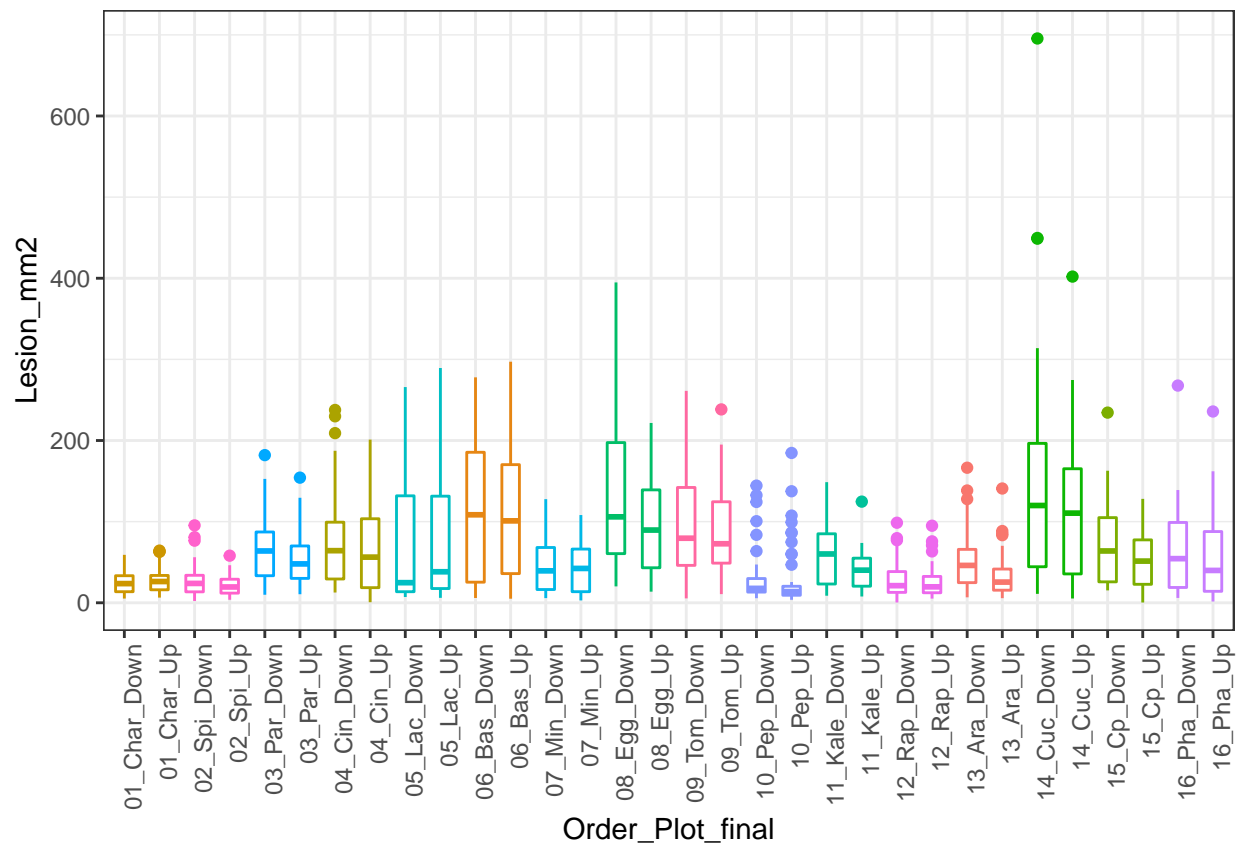## 1. Testing the leaf surface effect across 16 eudicot species

Associated files:

- Eudi16_72h_all1.txt

- Stomata_forPlot.txt

- Eudicot_Stomata_counts_col.txt

R Code:

- Eudi_UD_analysis_final.R

```
## Figure S1A
ggplot(data=Eudi_UD, aes(x=Order_Plot_final, y=Lesion_mm2, color=Species)) +
  geom_boxplot(width=0.5, show.legend = FALSE)+
  theme_bw()+
  theme(axis.text.x = element_text(angle = 90, hjust = 1))
```

```
ggplot(data=Asterids, aes(x=Order_Plot, y=Lesion_mm2, color=Species)) +
  geom_boxplot()+
  theme_bw()+
  theme(axis.text.x = element_text(angle = 90, hjust = 1))+
  ggtitle("Asterids")
```

Asterids
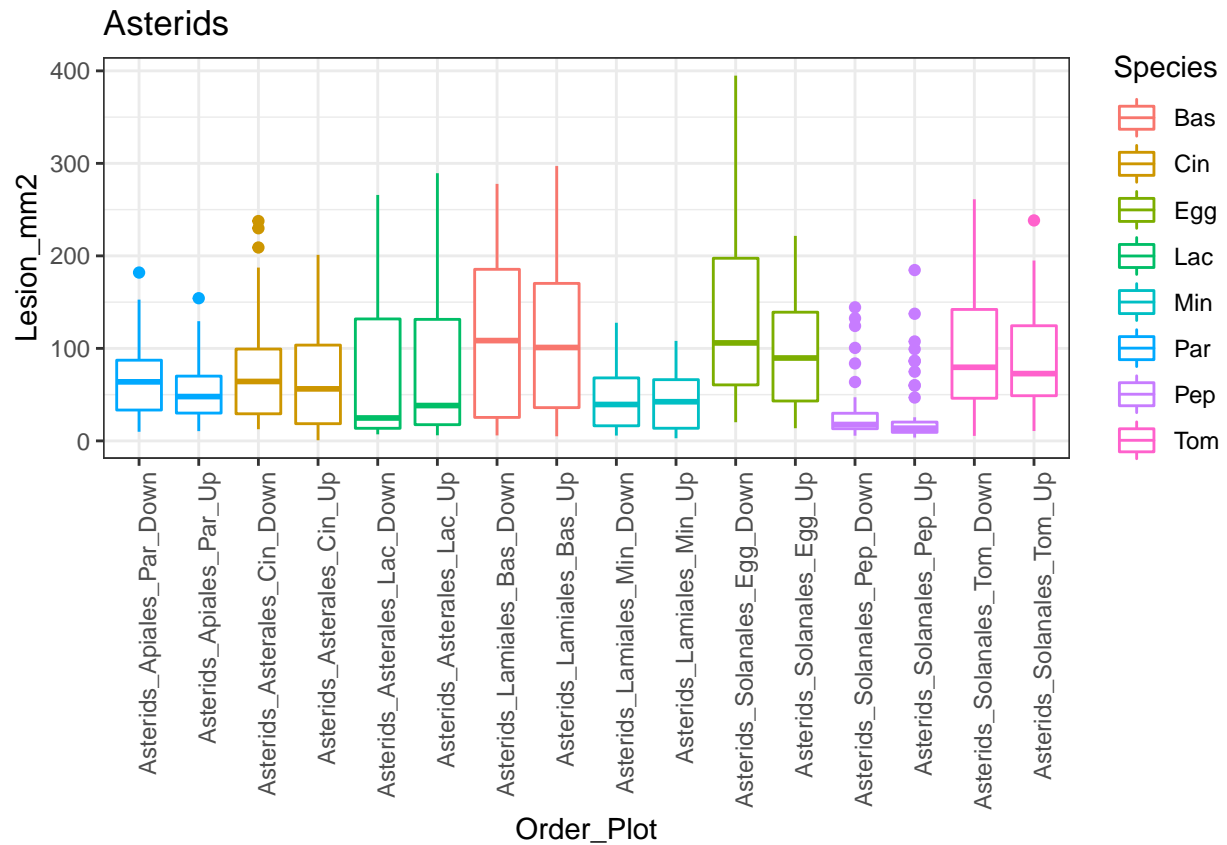

```
ggplot(data=Rosids, aes(x=Order_Plot, y=Lesion_mm2, color=Species)) +
  geom_boxplot()+
  theme_bw()+
  theme(axis.text.x = element_text(angle = 90, hjust = 1))+
  ggtitle("Rosids")
```

Rosids
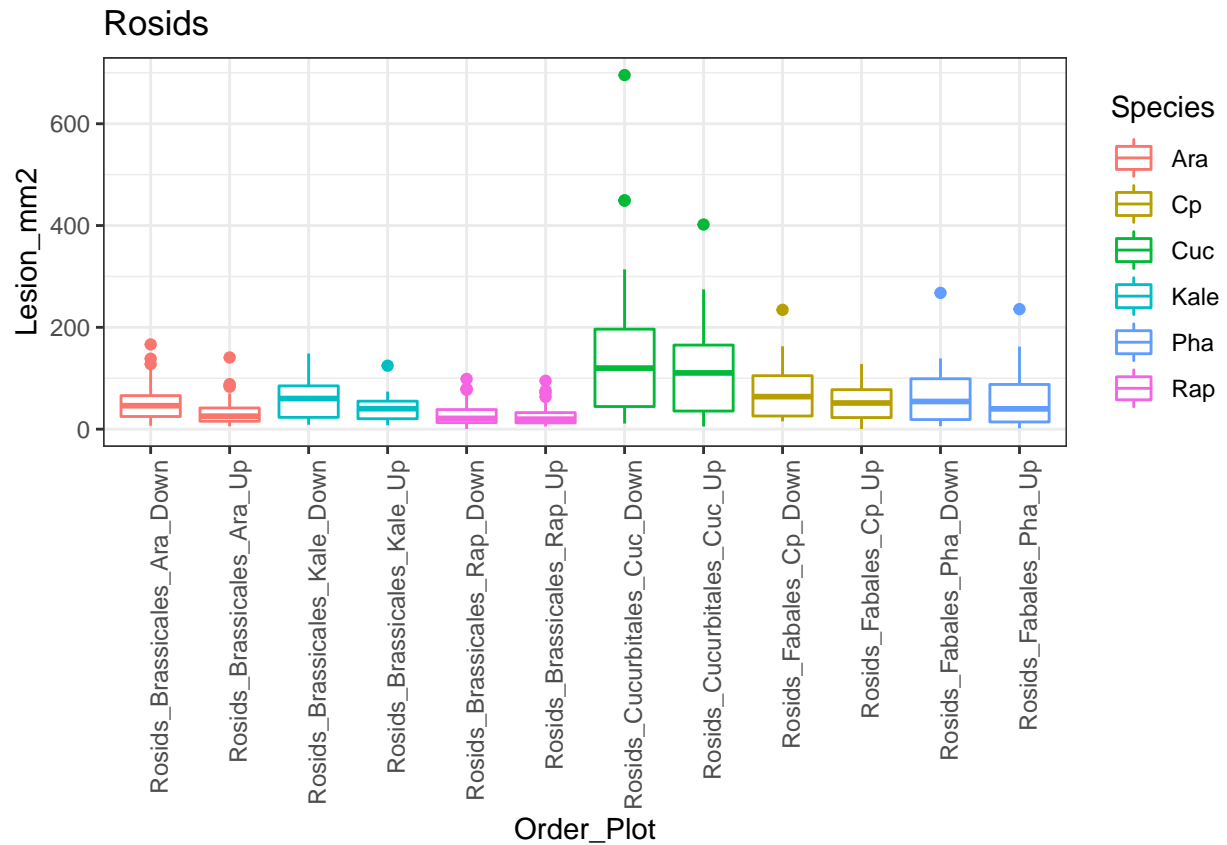

```r
ggplot(data=Core, aes(x=Order_Plot, y=Lesion_mm2, color=Species)) +
  geom_boxplot()+
  theme_bw()+
  theme(axis.text.x = element_text(angle = 90, hjust = 1))+
  ggtitle("Caryophyllales")
```

Caryophyllales
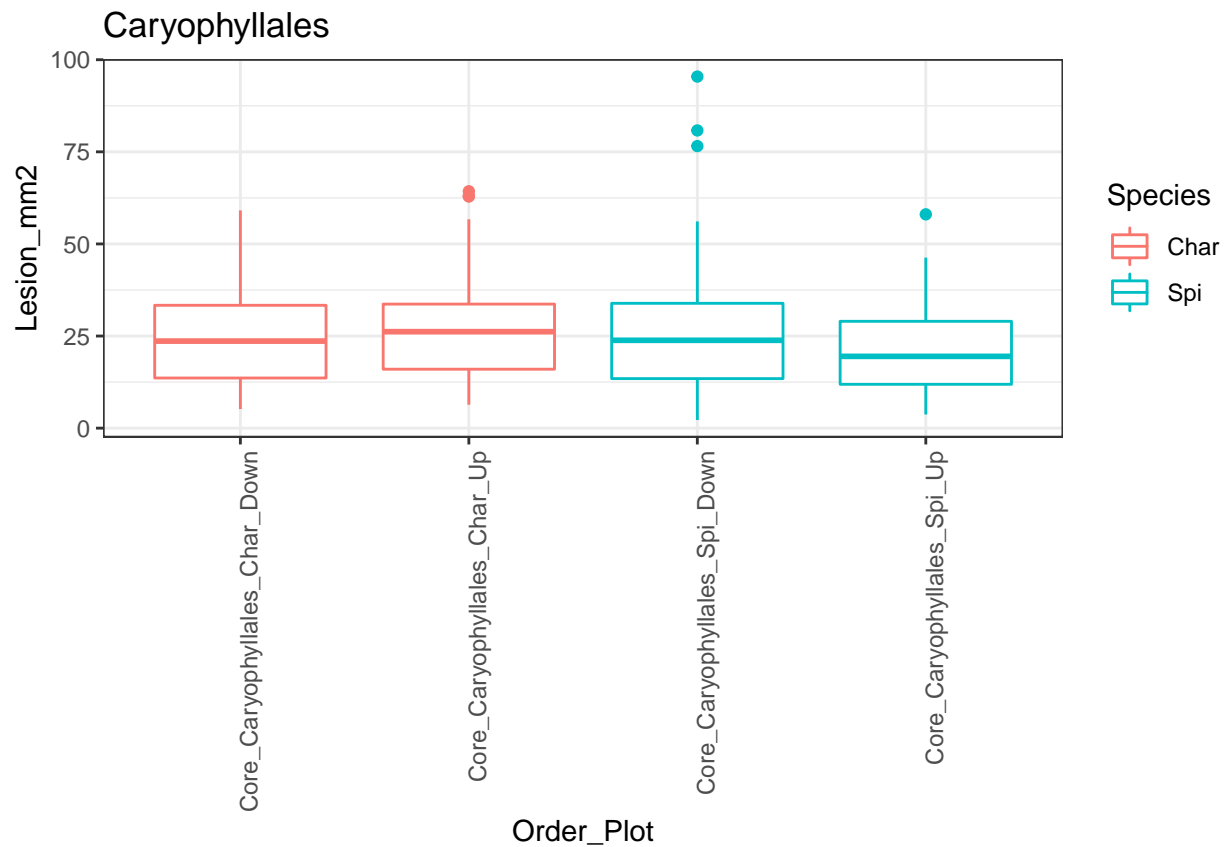

```
## Figure S1B
ggplot(ModelRes, aes(Species, PercVar, fill=Param1)) +
  geom_col()+
  scale_fill_manual(values=c("#f1b6da", "#7b3294", "#92c5de","#bababa","#ffffbf"))+
  geom_text(aes(label=PercVar1), position=position_stack(vjust=0.5), size=3)+
  theme_minimal()+
  theme(axis.text.x = element_text(angle = 90, vjust = 0.5, hjust=1))
```
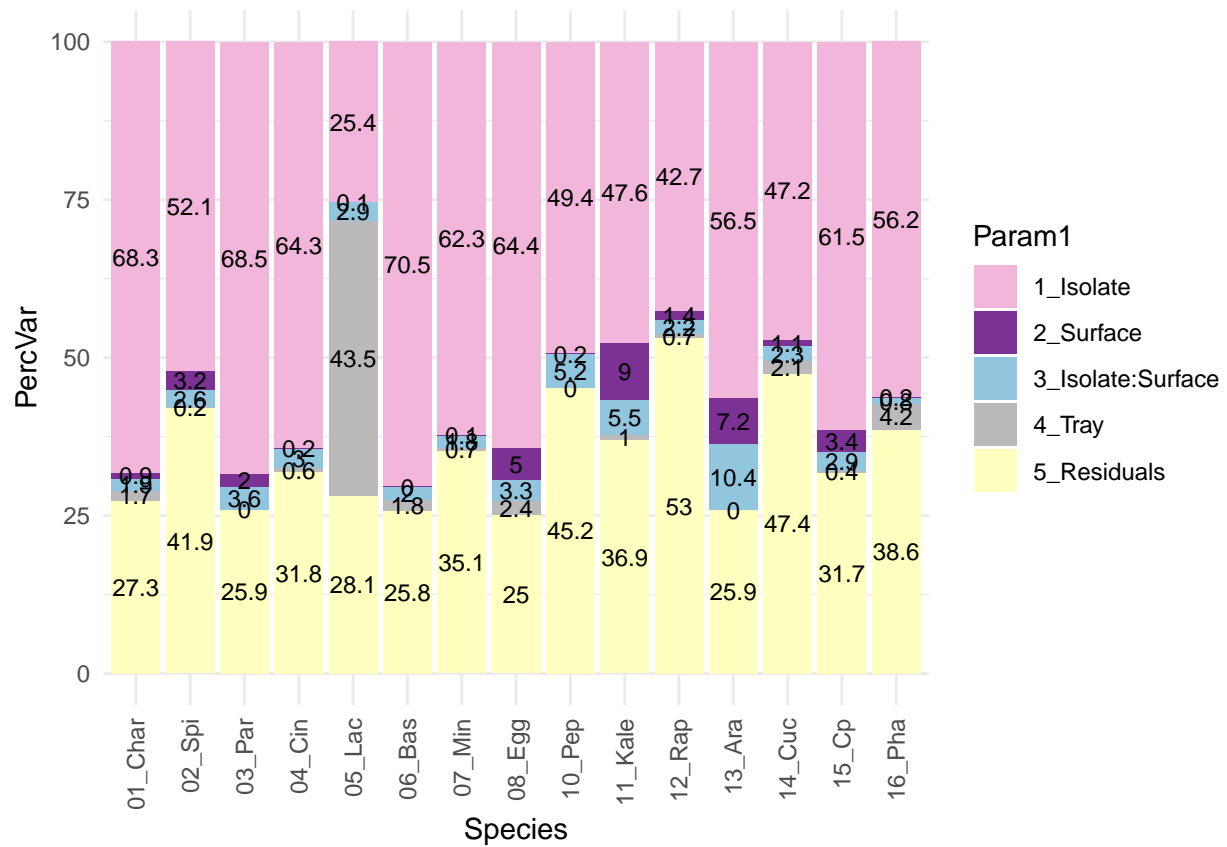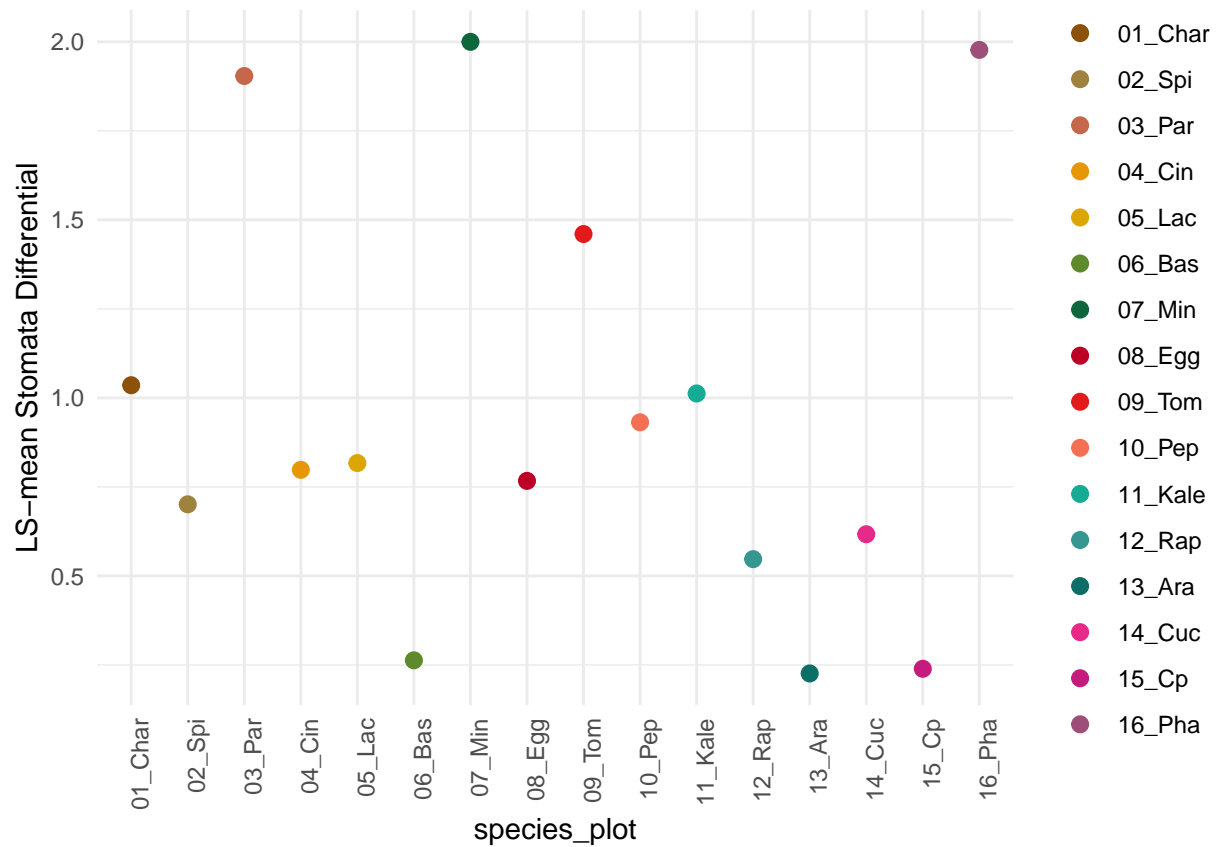
```r
ggplot(Surface_diff_LSM, aes(species_plot, Std_Diff, color=species_plot)) +
  geom_point(size=2.5, show.legend = FALSE)+
  scale_color_manual(values=Eudi16_color)+
  theme_minimal()+
  theme(axis.text.x = element_text(angle = 90, hjust = 1))+
  ylab("LS-mean Surface Differential")
```
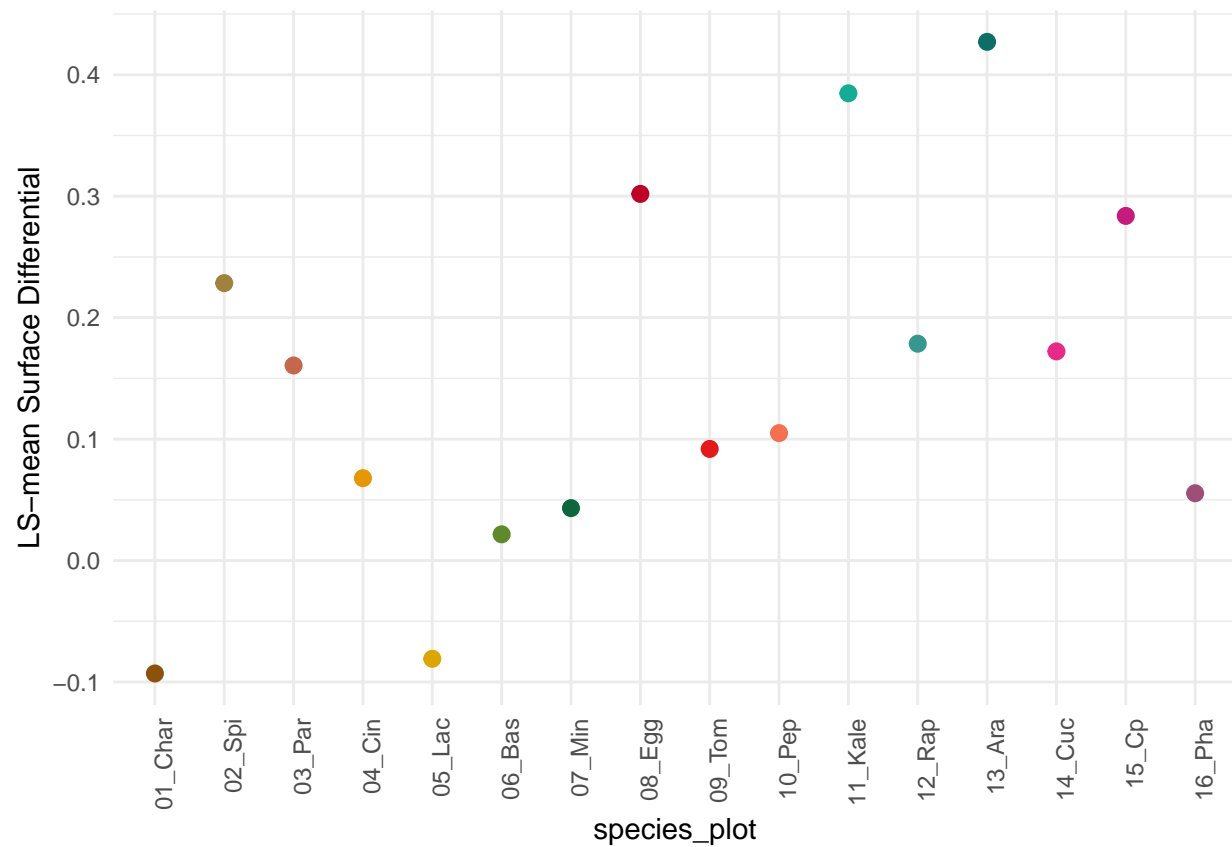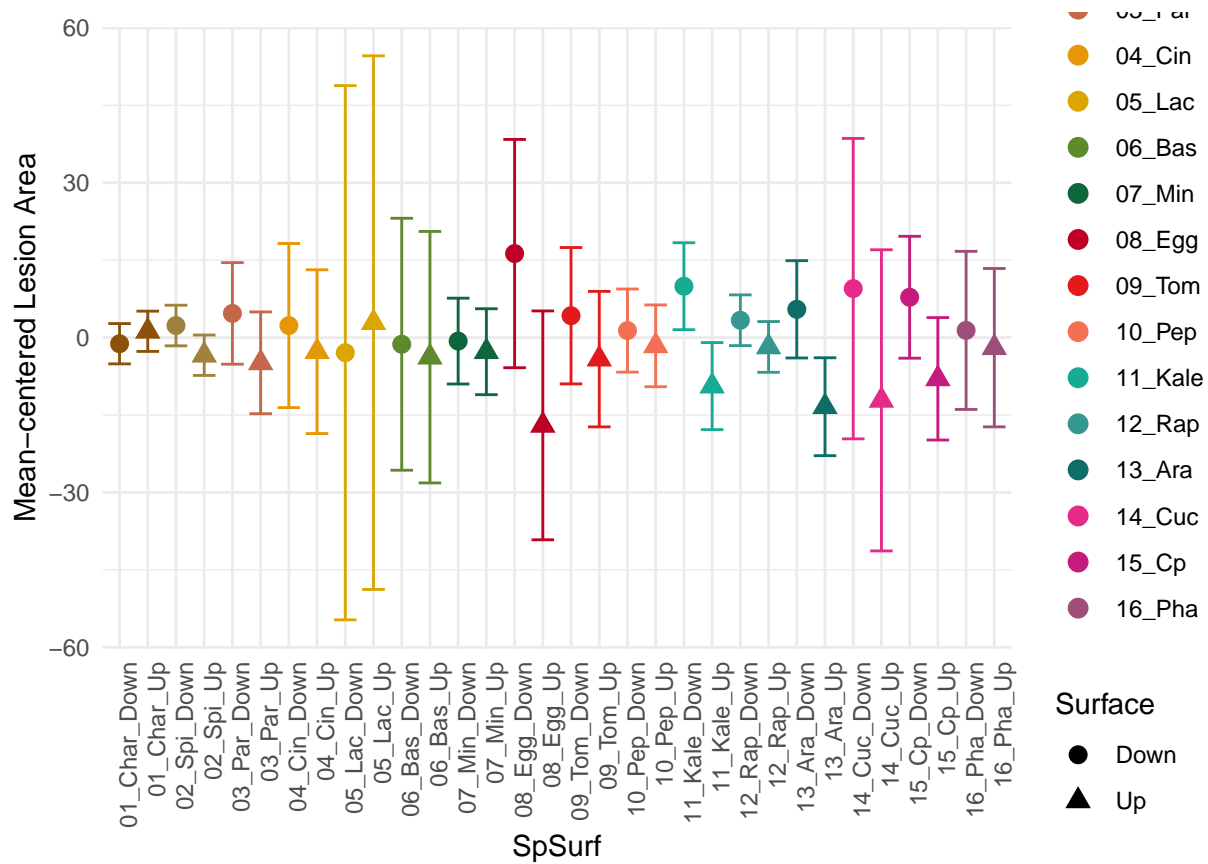
```r
ggplot(Surface_diff_LSM, aes(species_plot, emmean_sp, color=species_plot)) +
    geom_point(size=2.5)+
    scale_color_manual(values=Eudi16_color)+
    theme_minimal()+
    theme(axis.text.x = element_text(angle = 90, hjust = 1))+
    ylab("LS-mean Lesion Area [mm2]")
```
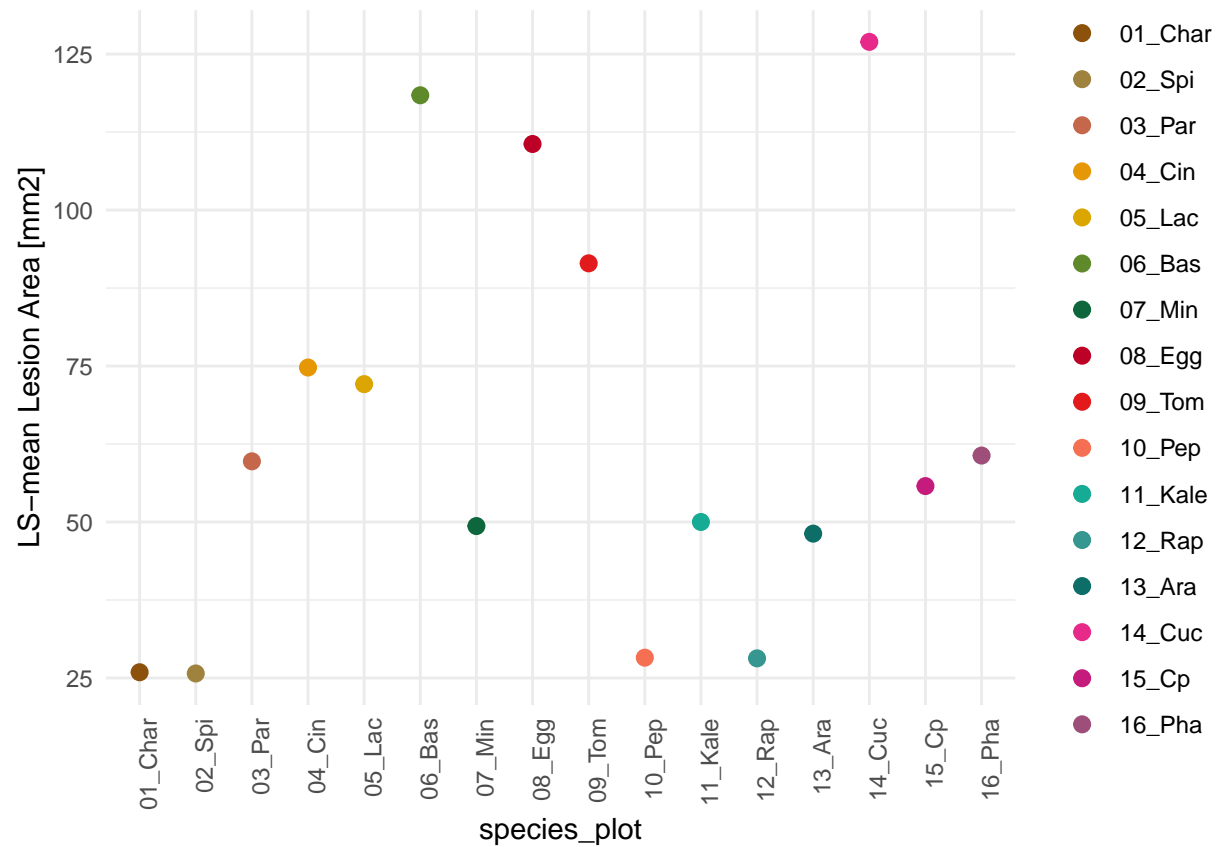
```
ggplot(Surface_diff_LSM, aes(species_plot, Stom_Diff, color=species_plot)) +
    geom_point(size=2.5)+
    scale_color_manual(values=Eudi16_color)+
    theme_minimal()+
    theme(axis.text.x = element_text(angle = 90, hjust = 1))+
    ylab("LS-mean Stomata Differential")
```

```
ggplot(Lesion_LS_centered, aes(SpSurf, emmean, color=species_plot, shape=Surface)) +
  geom_point(size=3)+
  geom_errorbar(aes(ymin=emmean-SE, ymax=emmean+SE), width=.8, position = position_dodge(0.1), show.leg
  scale_color_manual(values=Eudi16_color)+
  theme_minimal()+
  theme(axis.text.x = element_text(angle = 90, hjust = 1))+
  ylab("Mean-centered Lesion Area")
```

```
ggplot(Summary_Stom_col, aes(SpSurf, mean.stom, color=species_plot, shape=Surface)) +
 geom_point(size=3)+
 geom_errorbar(aes(ymin=mean.stom-se.stom, ymax=mean.stom+se.stom), width=.8, position = position_dodge
 scale_color_manual(values=Eudi16_color)+
 theme_minimal()+
 theme(axis.text.x = element_text(angle = 90, hjust = 1))+
 ylab("Mean-centered Stomata Density")
```
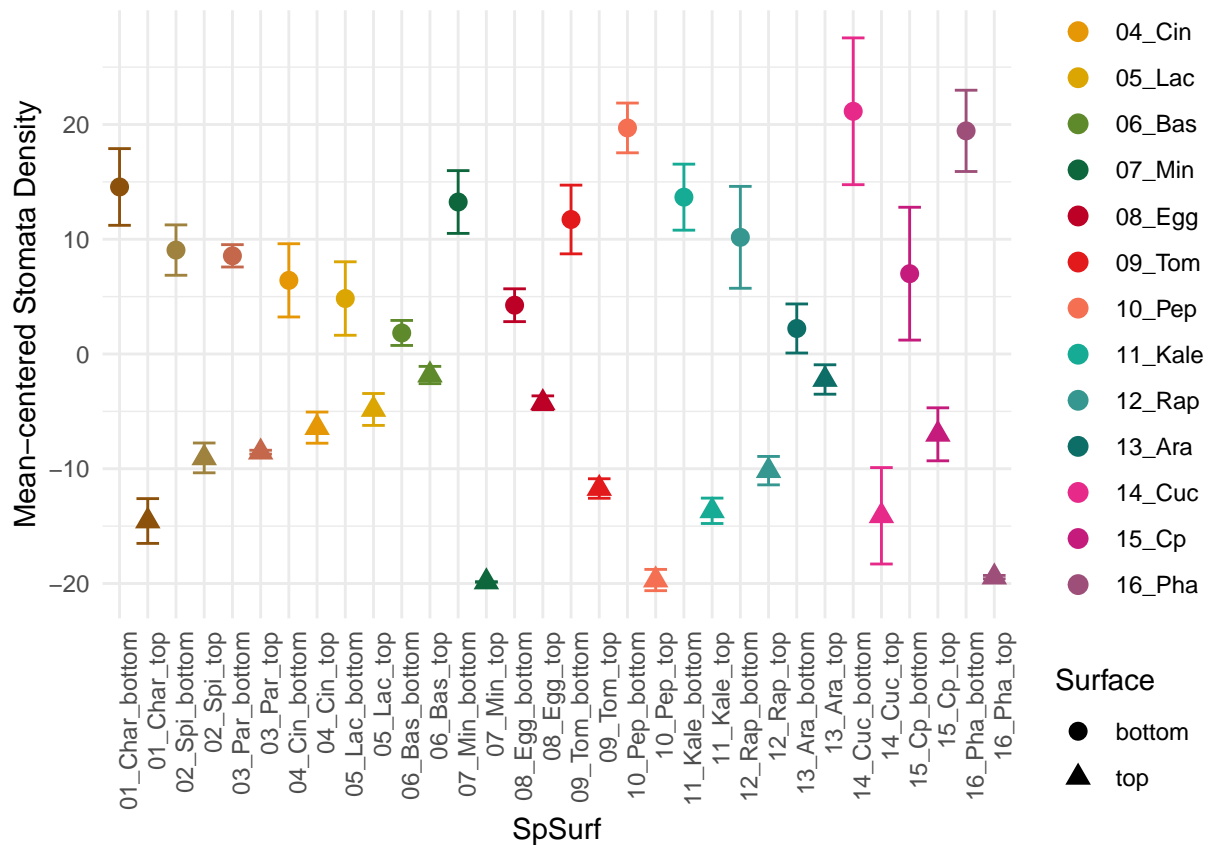
```
rm(list = ls())
```

# 2. Testing the leaf surface effect within Arabidopsis thaliana

Associated files:

- G24I10_72H.txt
- G24I10_96h.txt
- G24I10_UD_48h.txt
- Genotype_Emmeans_Cat
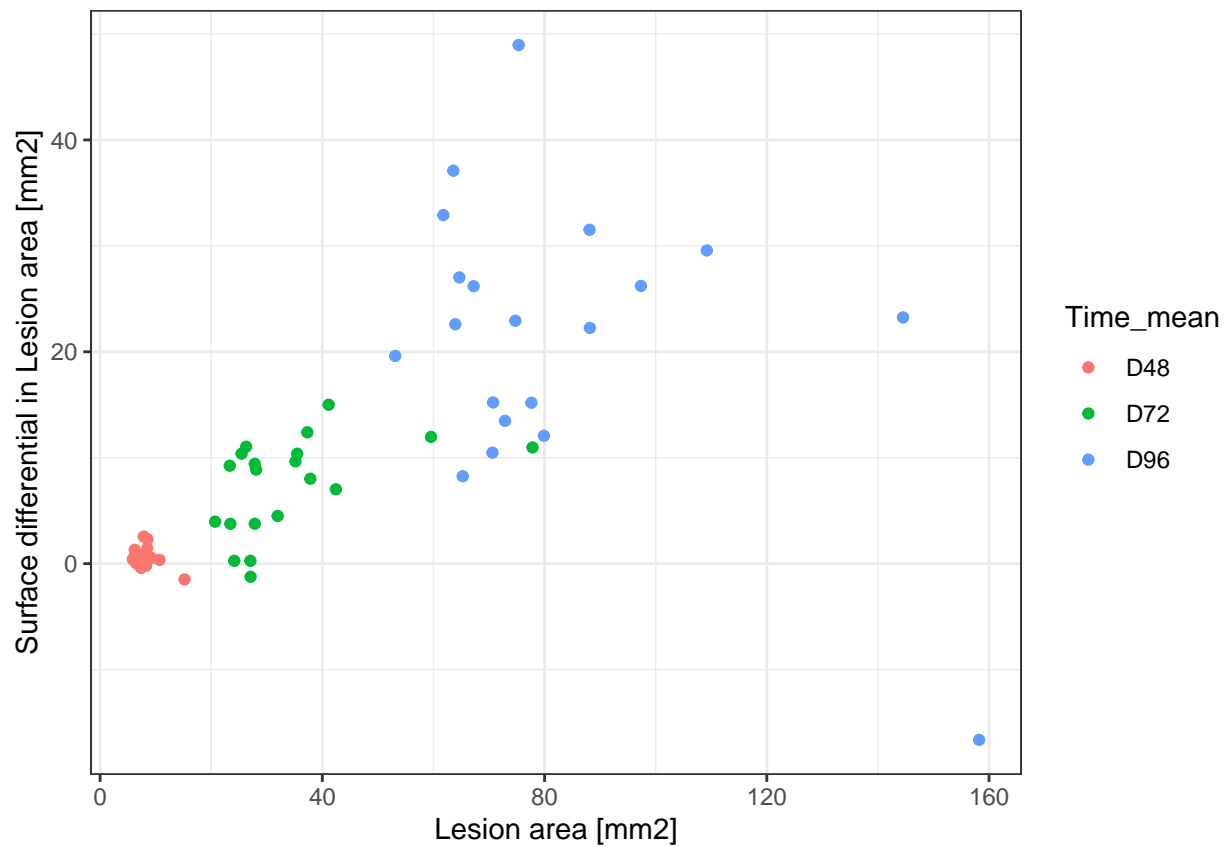- Data_Plot_Lesion_GT.txt

R Code:

- Ara20_72h_all.R

```
ggplot(Lesion_LS_centered, aes(plot_name, emmean, shape=Surface, color=Plot_Cat)) +
  geom_point(size=3)+
  geom_errorbar(aes(ymin=emmean-SE, ymax=emmean+SE), width=.8, position = position_dodge(0.1), show.leg
  theme_minimal()+
  theme(axis.text.x = element_text(angle = 90, hjust = 1))+
  ylab("Mean-centered Lesion Area")
```
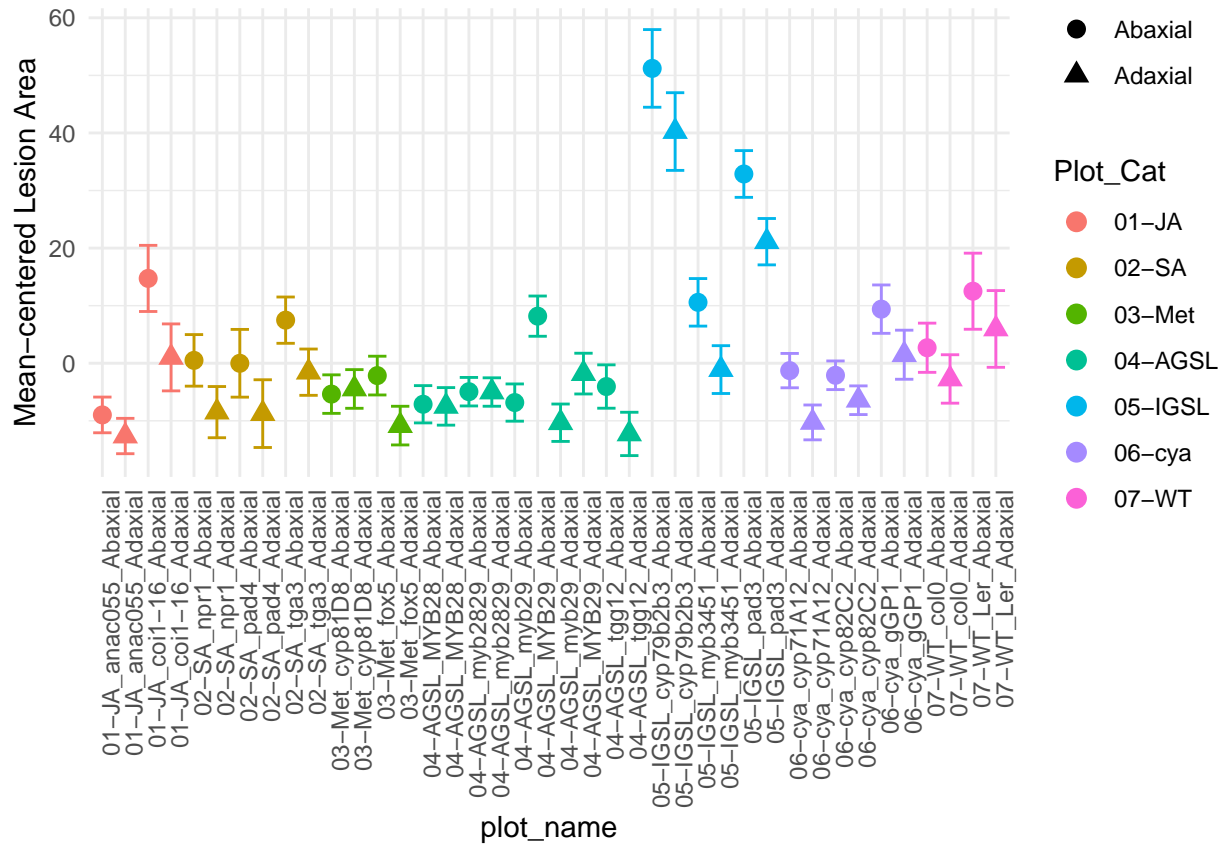
```
ggplot(data=Data_Plot_Lesion_GTL, aes(Mean_lesion, Surface_Diff)) +
  geom_point(aes(color=Time_mean)) +
  scale_fill_manual(values=PaletteP)+
  ylab("Surface differential in Lesion area [mm2]")+
  xlab("Lesion area [mm2]")+
  theme_bw()
```

```
q<- ggplot(data=Data_Plot_Lesion_GTL, aes(Mean_lesion, Surface_Diff)) +
  geom_line(aes( group= Genotype.x, color=Genotype.x), linetype=2)+
  scale_color_manual(values=PaletteN)+
  theme_bw()+
  ylab("Surface differential in Lesion area [mm2]")+
  xlab("Lesion area [mm2]")

direct.label(q,"last.points")
```
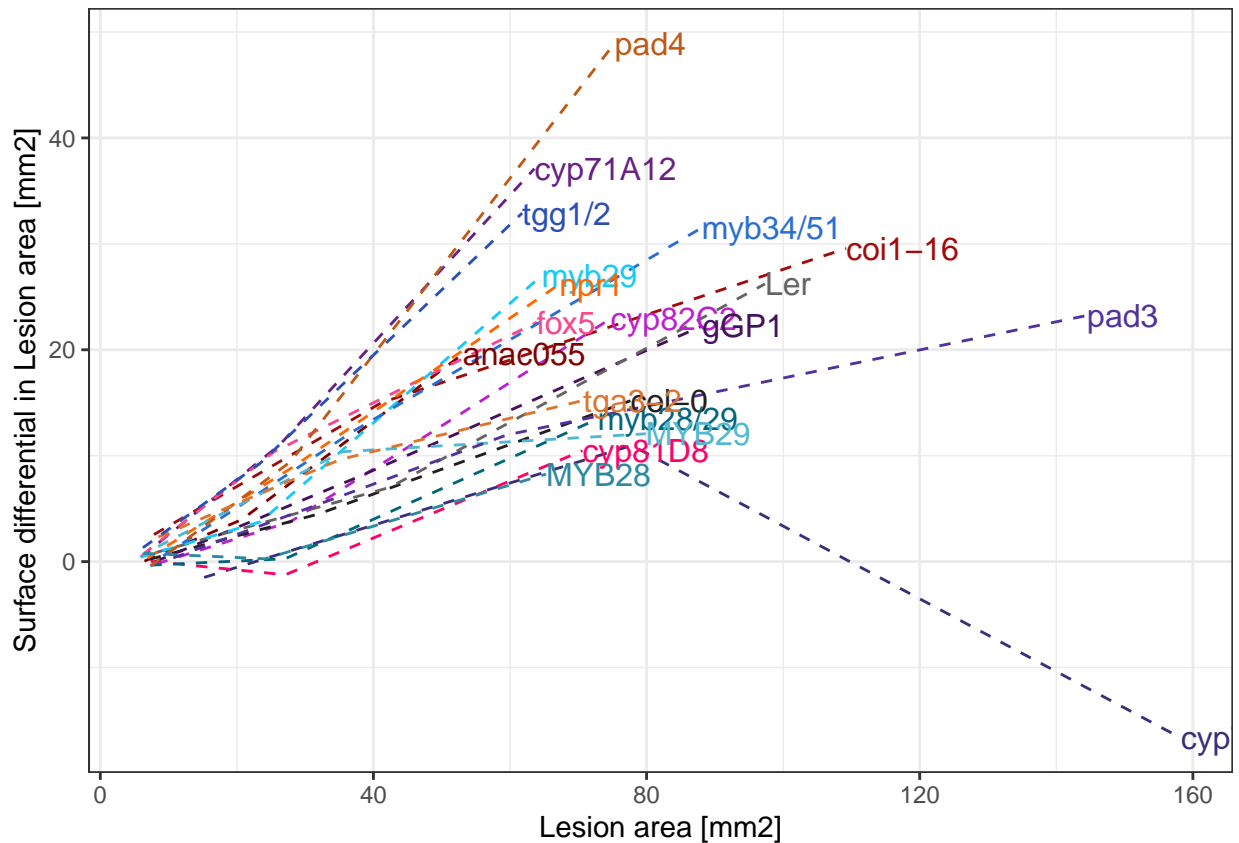
```
rm(list = ls())
```

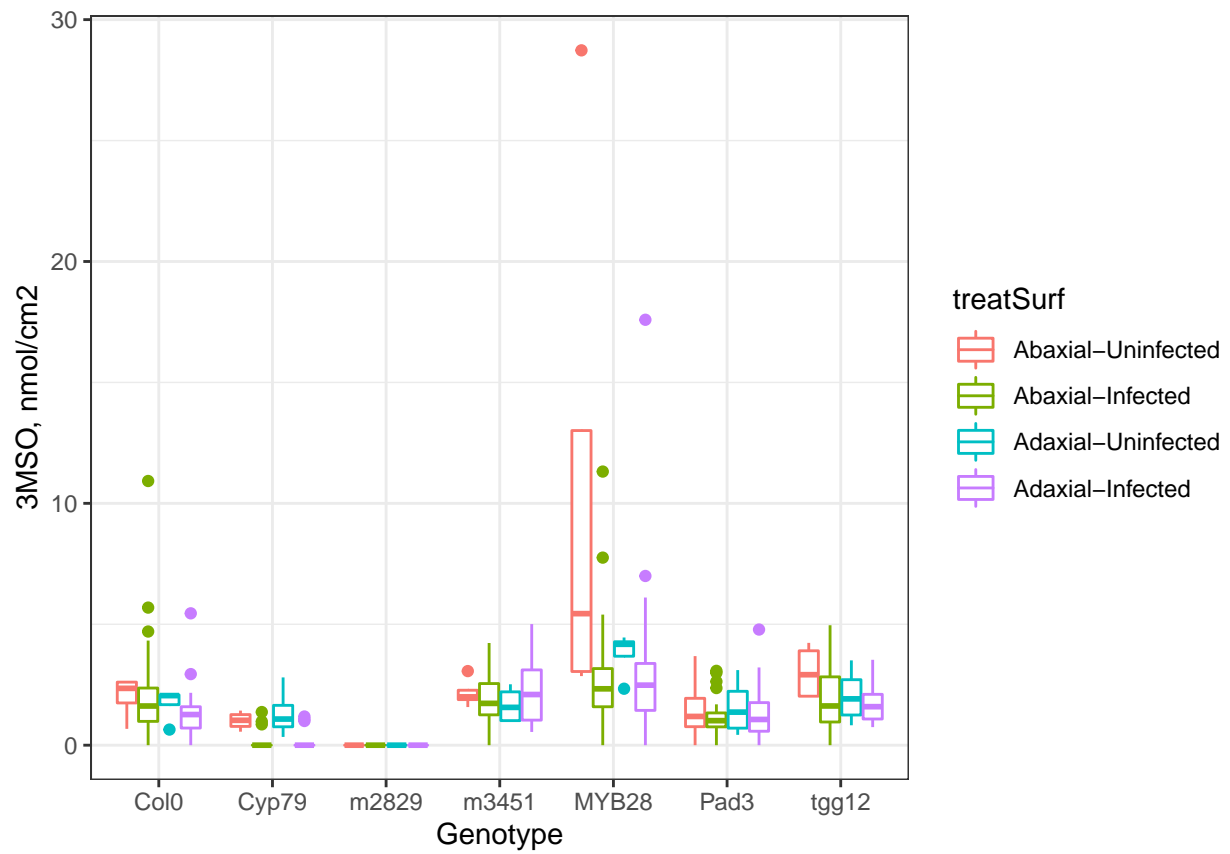## 3. Testing the effect of leaf surface on glucosinolate and camalexin content

Associated files:

- GSL_Leaf_Data1.txt
- Cam_Leaf_Data1.txt
- Chemotype_LM_model_pie.txt
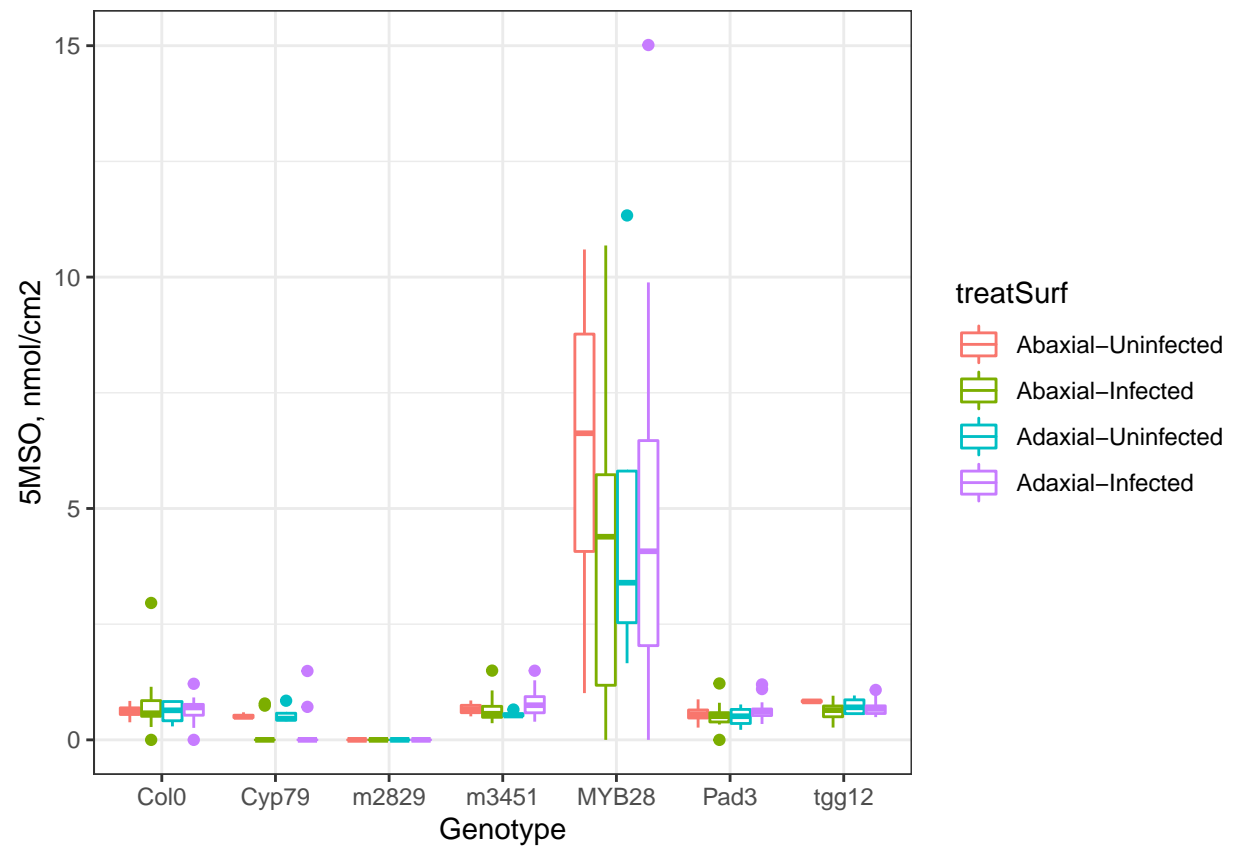
R Code:

- Glucosinolates_Camalexin.R

```
ggplot(GSL_Data, aes(Genotype, QLLf_3MSO*1000, color=treatSurf))+
  geom_boxplot()+
  ylab("3MSO, nmol/cm2")+
  theme_bw()
```
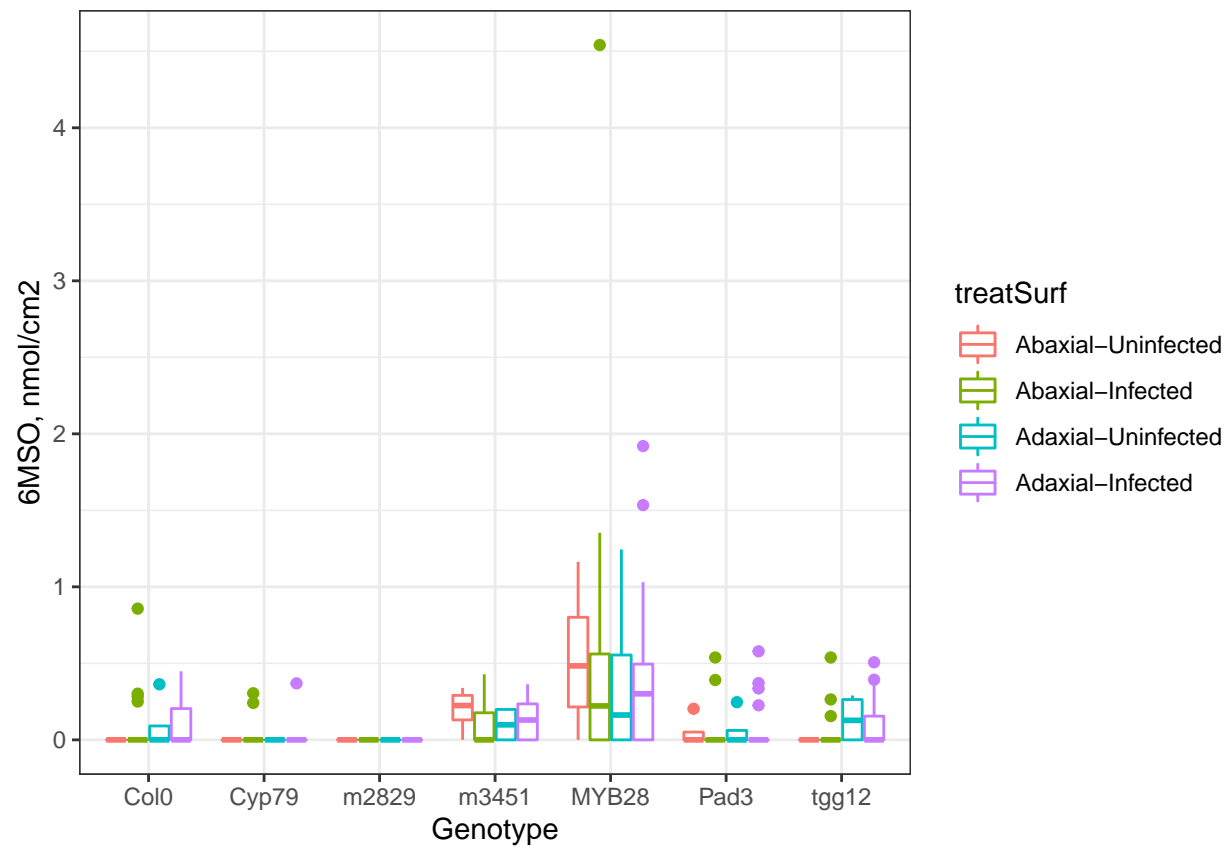
```
ggplot(GSL_Data, aes(Genotype, QLLf_4MSO*1000, color=treatSurf))+
  geom_boxplot()+
  ylab("4MSO, nmol/cm2")+
  theme_bw()
```
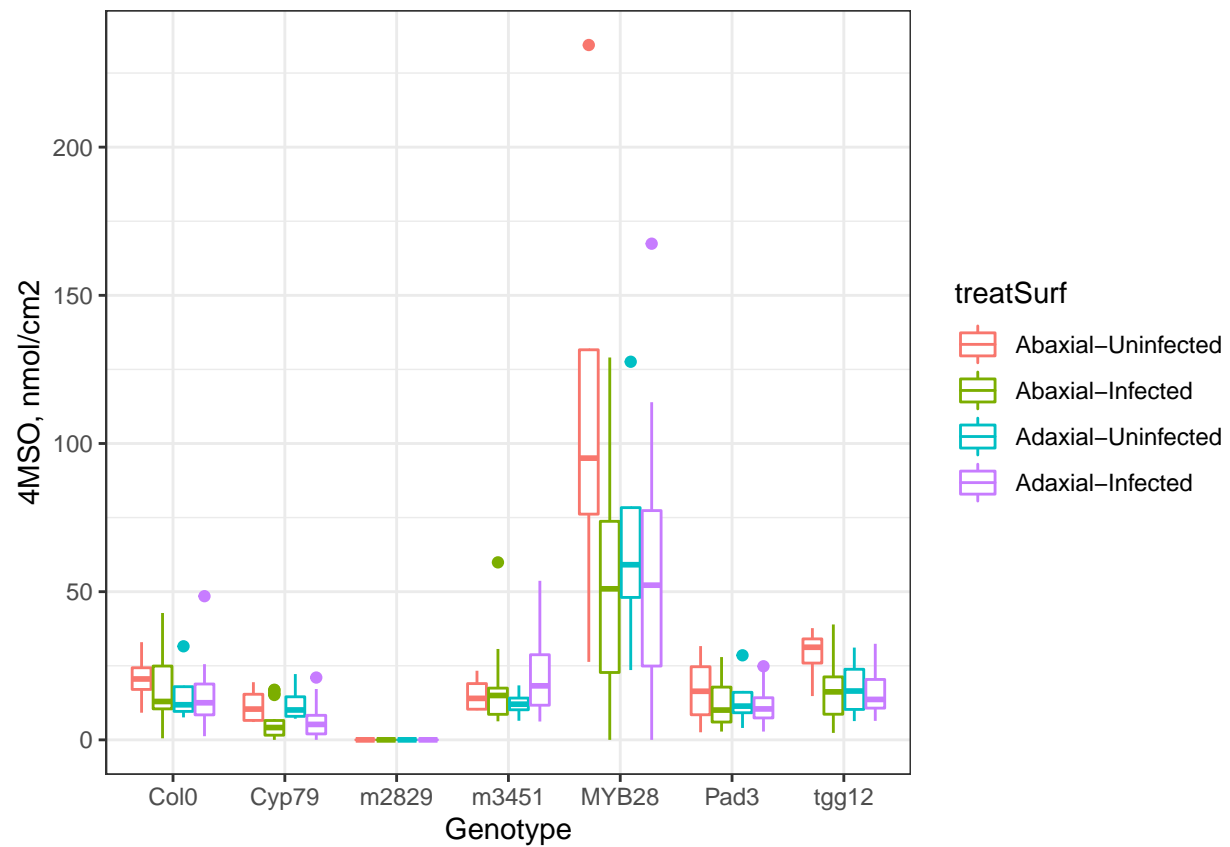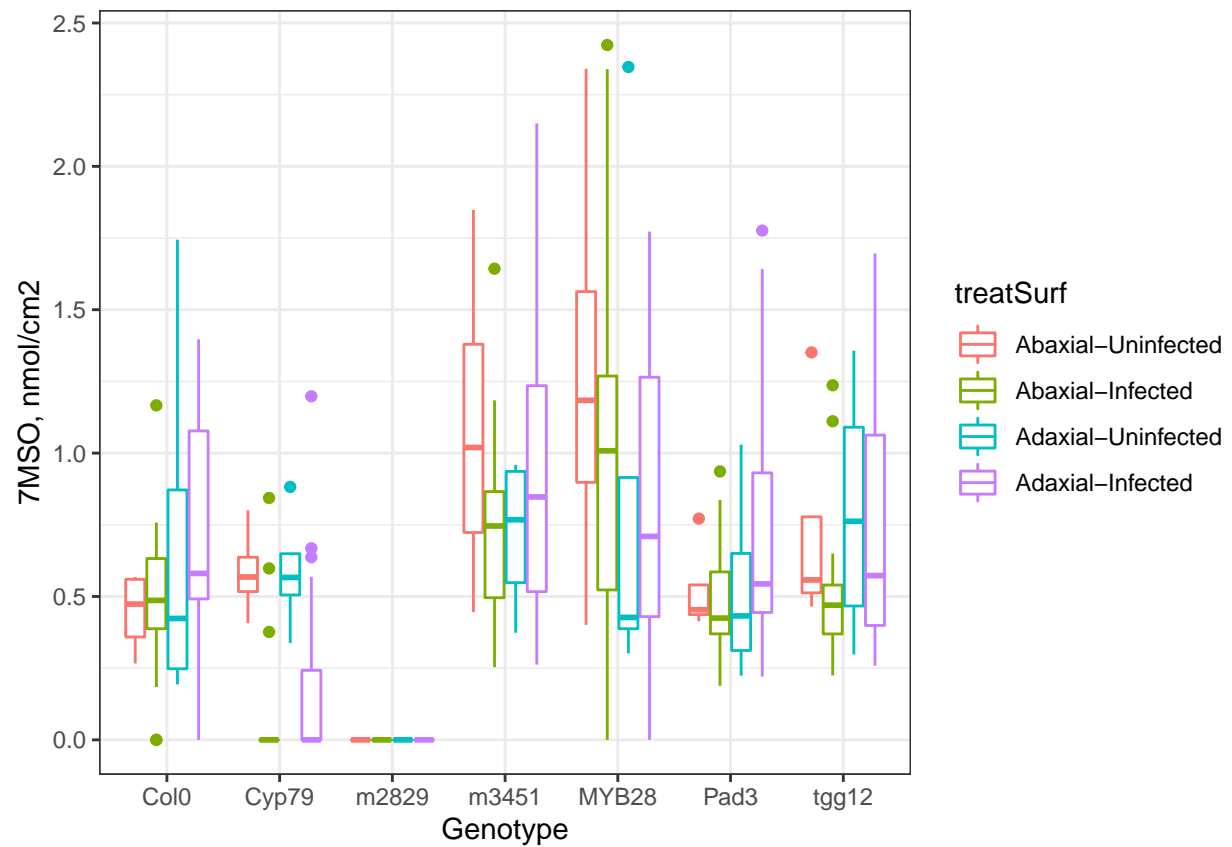
```
ggplot(GSL_Data, aes(Genotype, QLLf_5MSO*1000, color=treatSurf))+
  geom_boxplot()+
  ylab("5MSO, nmol/cm2")+
  theme_bw()
```
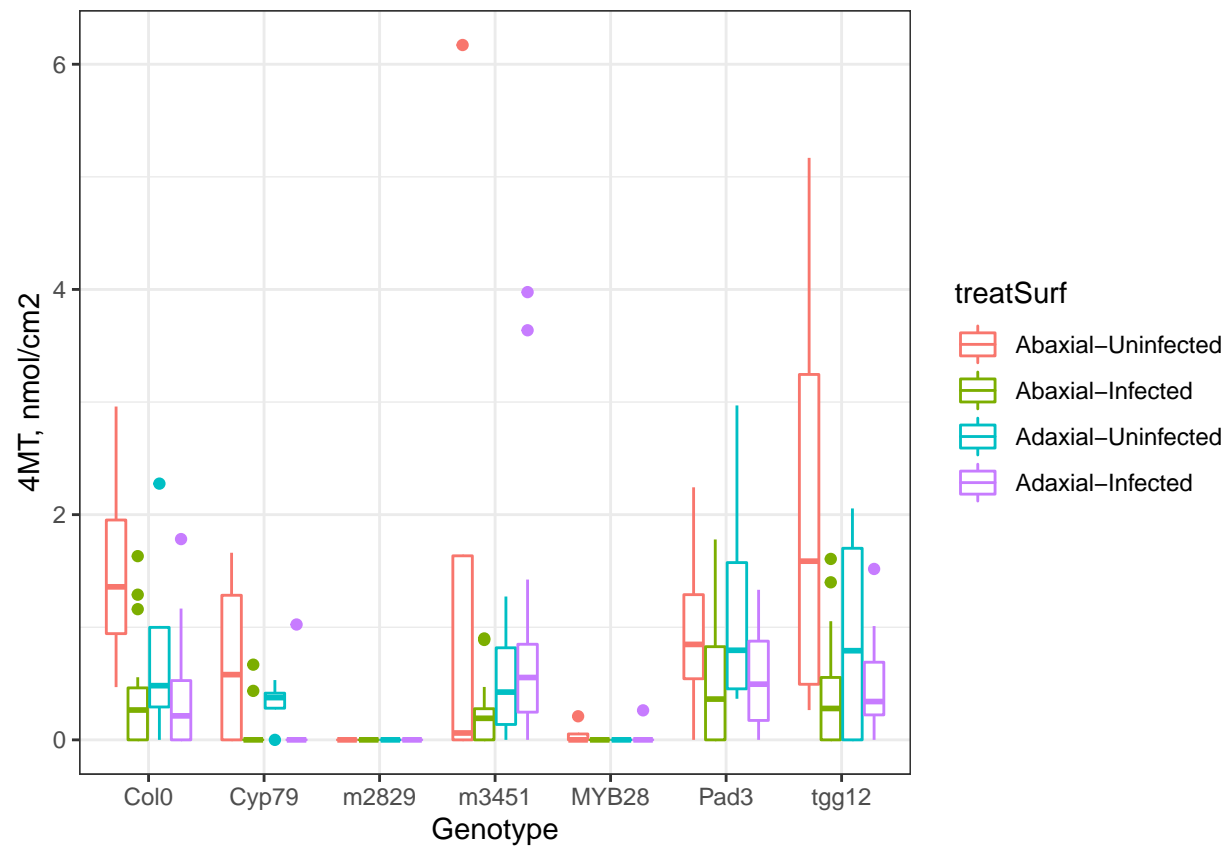
```
ggplot(GSL_Data, aes(Genotype, QLLf_6MSO*1000, color=treatSurf))+
  geom_boxplot()+
  ylab("6MSO, nmol/cm2")+
  theme_bw()
```
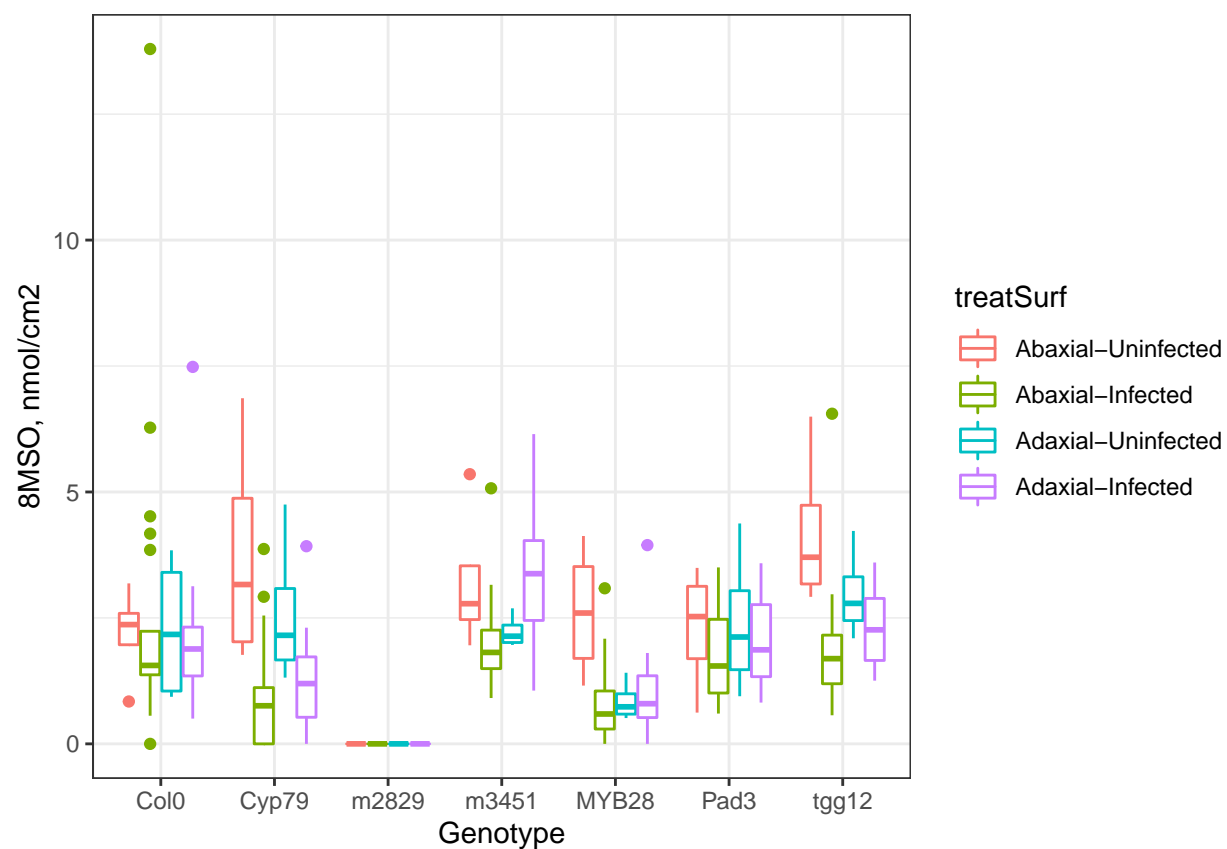
```
ggplot(GSL_Data, aes(Genotype, QLLf_7MSO*1000, color=treatSurf))+
  geom_boxplot()+
  ylab("7MSO, nmol/cm2")+
  theme_bw()
```
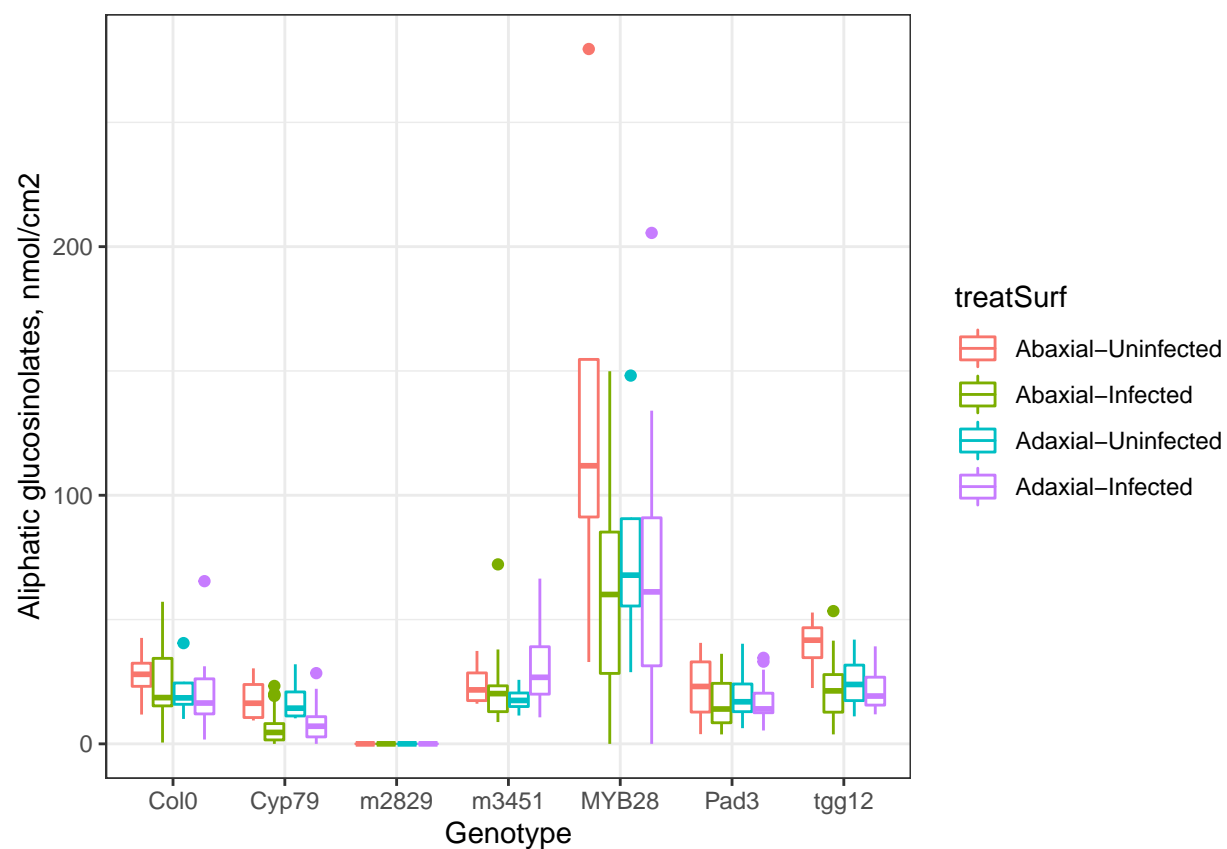
```
ggplot(GSL_Data, aes(Genotype, QLLf_4MT*1000, color=treatSurf))+
  geom_boxplot()+
ylab("4MT, nmol/cm2")+
  theme_bw()
```
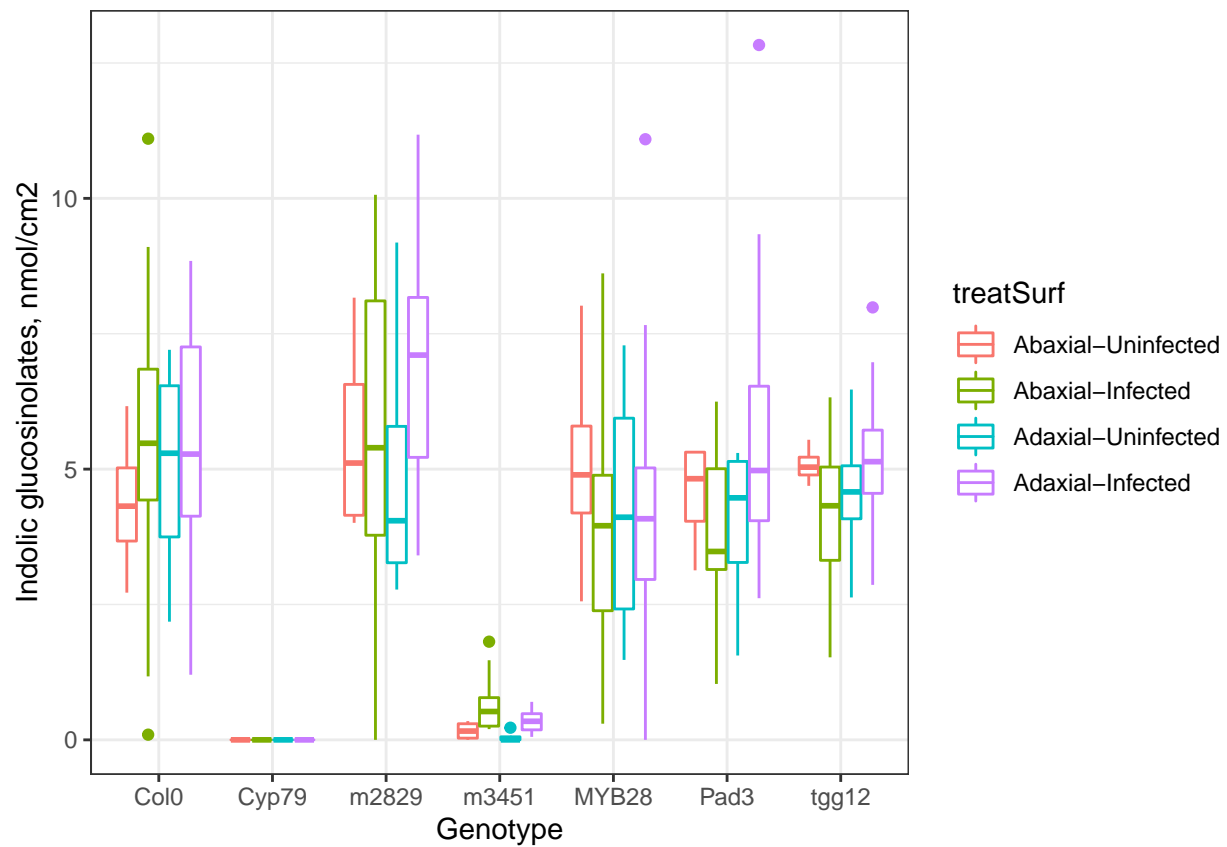
```
ggplot(GSL_Data, aes(Genotype, QLLf_8MSO*1000, color=treatSurf))+
  geom_boxplot()+
  ylab("8MSO, nmol/cm2")+
  theme_bw()
```
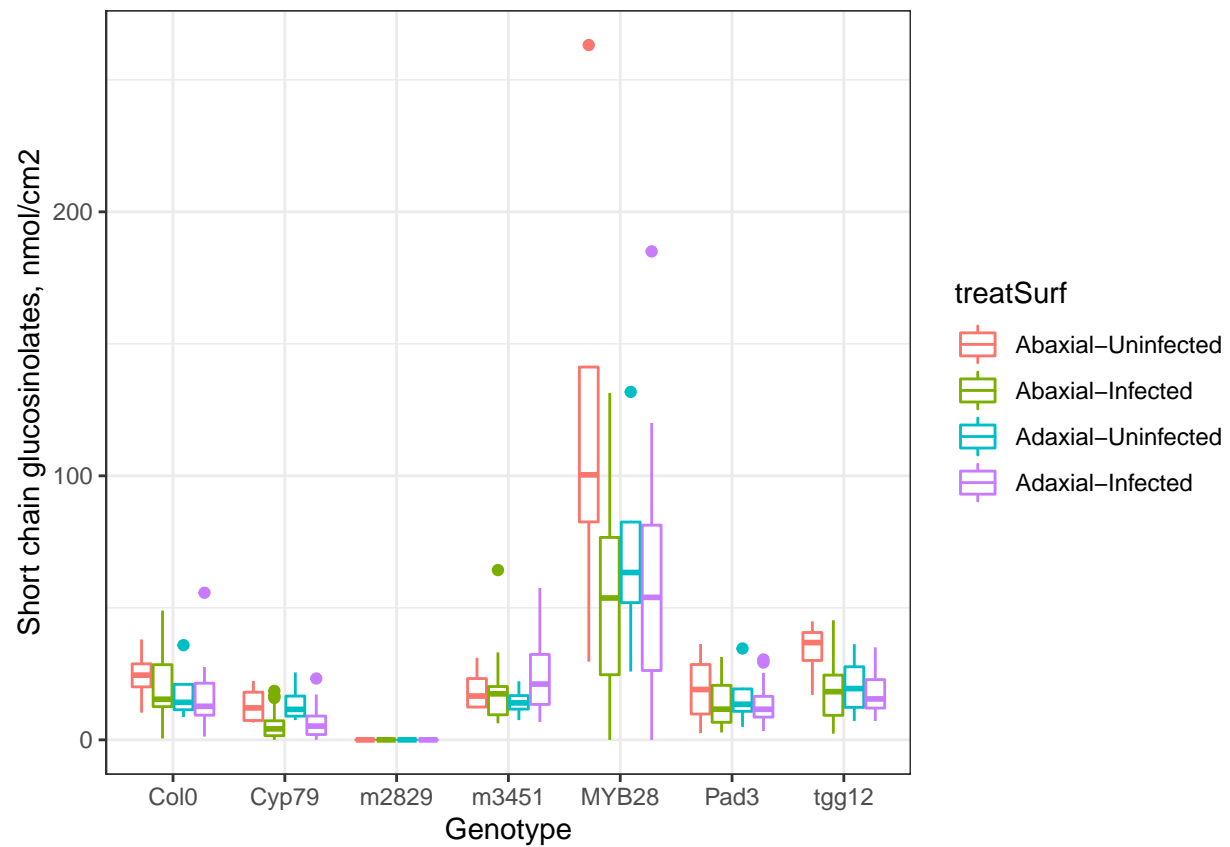
```
ggplot(GSL_Data, aes(Genotype, aliphatic*1000, color=treatSurf))+
  geom_boxplot()+
  ylab("Aliphatic glucosinolates, nmol/cm2")+
  theme_bw()
```
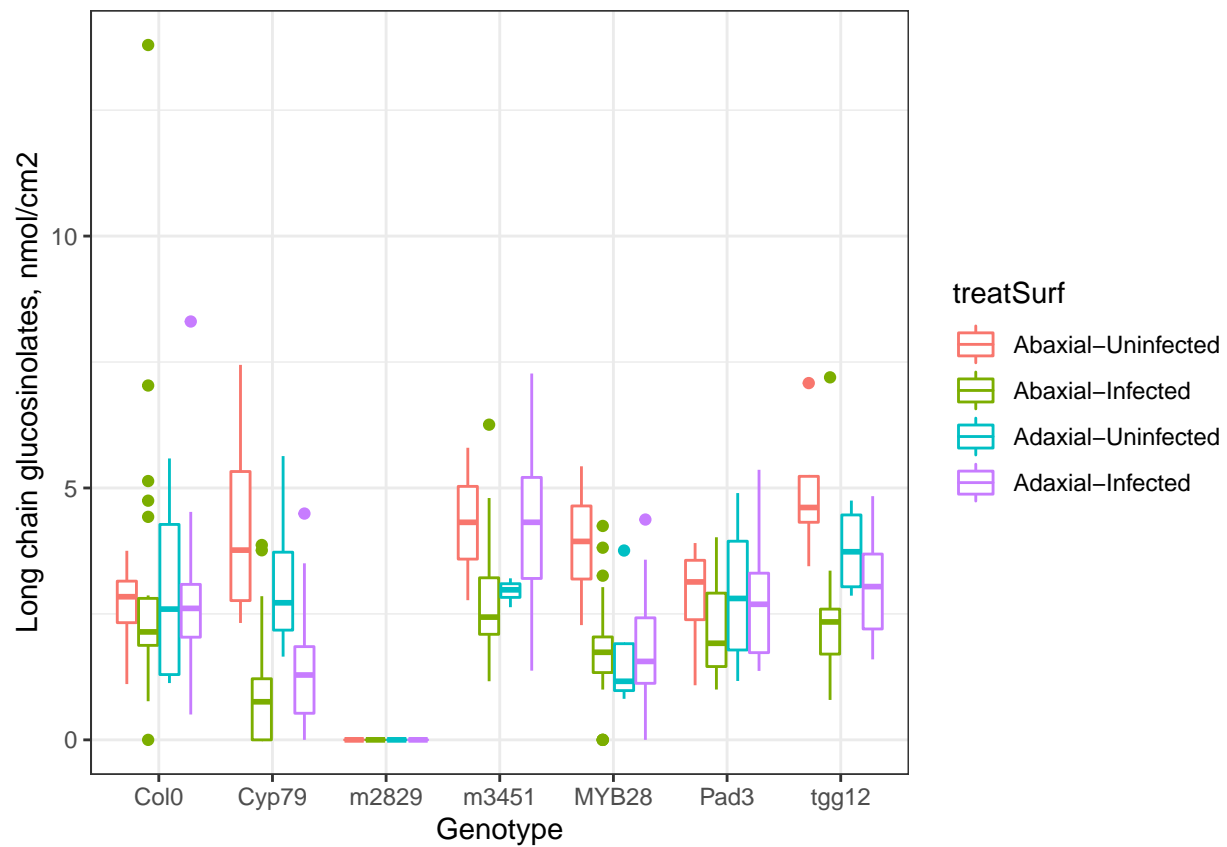
```r
ggplot(GSL_Data, aes(Genotype, indolic*1000, color=treatSurf))+
  geom_boxplot()+
  ylab("Indolic glucosinolates, nmol/cm2")+
  theme_bw()
```
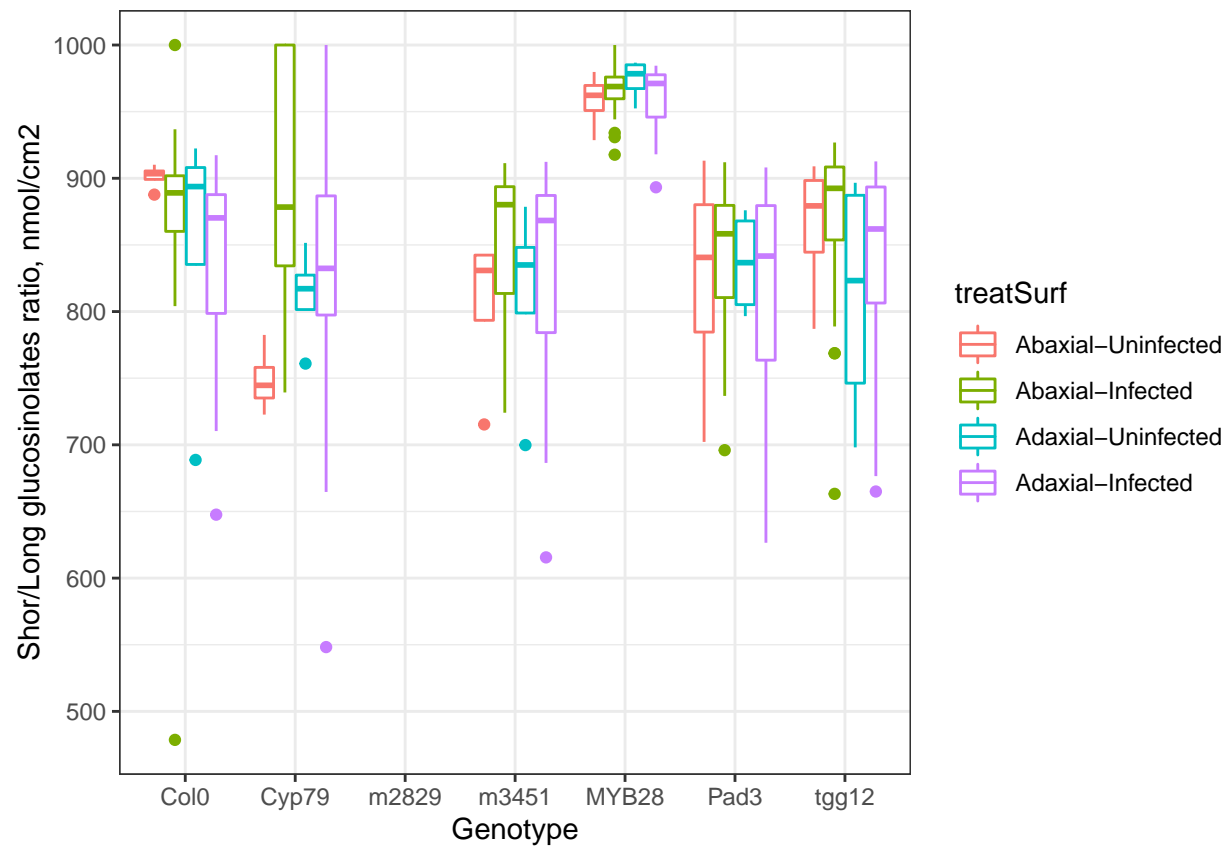
```
ggplot(GSL_Data, aes(Genotype, SC_GSL*1000, color=treatSurf))+
  geom_boxplot()+
  ylab("Short chain glucosinolates, nmol/cm2")+
  theme_bw()
```
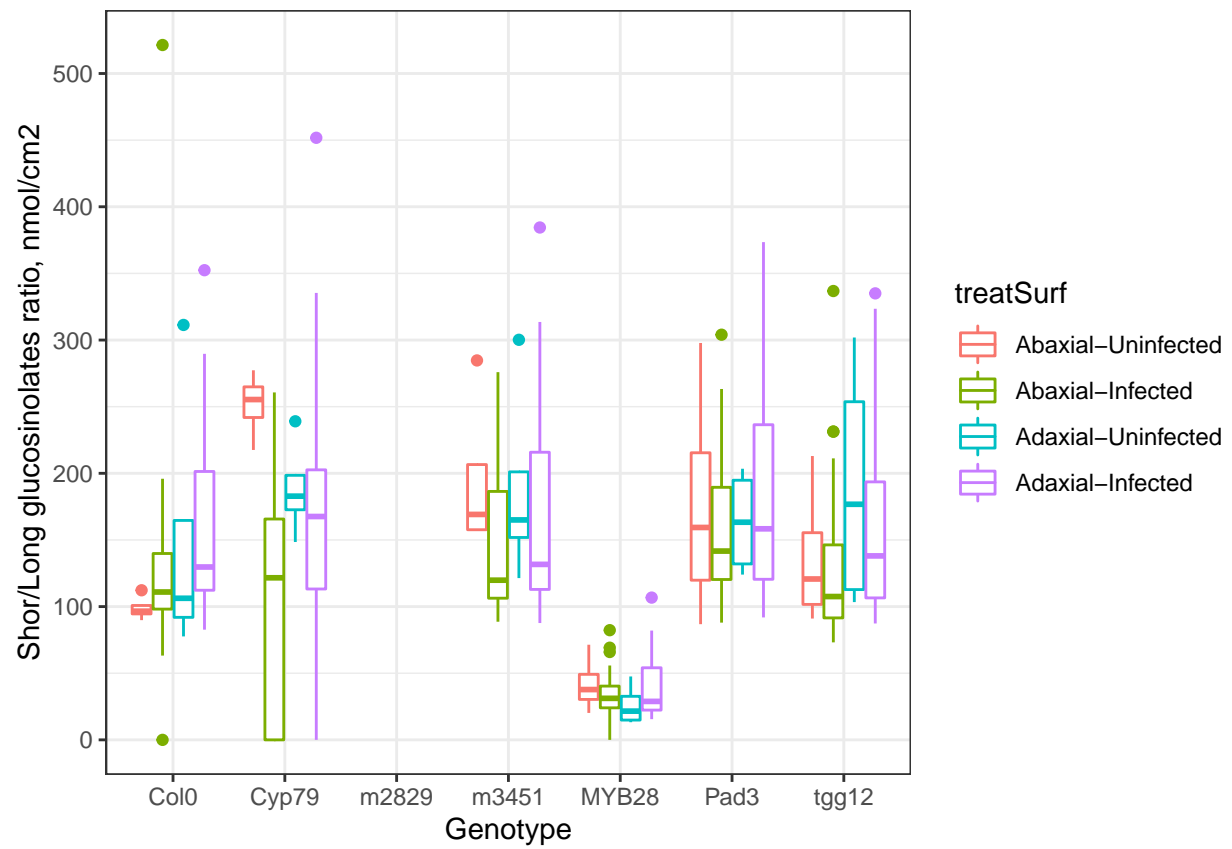
```
ggplot(GSL_Data, aes(Genotype, LC_GSL*1000, color=treatSurf))+
  geom_boxplot()+
  ylab("Long chain glucosinolates, nmol/cm2")+
  theme_bw()
```
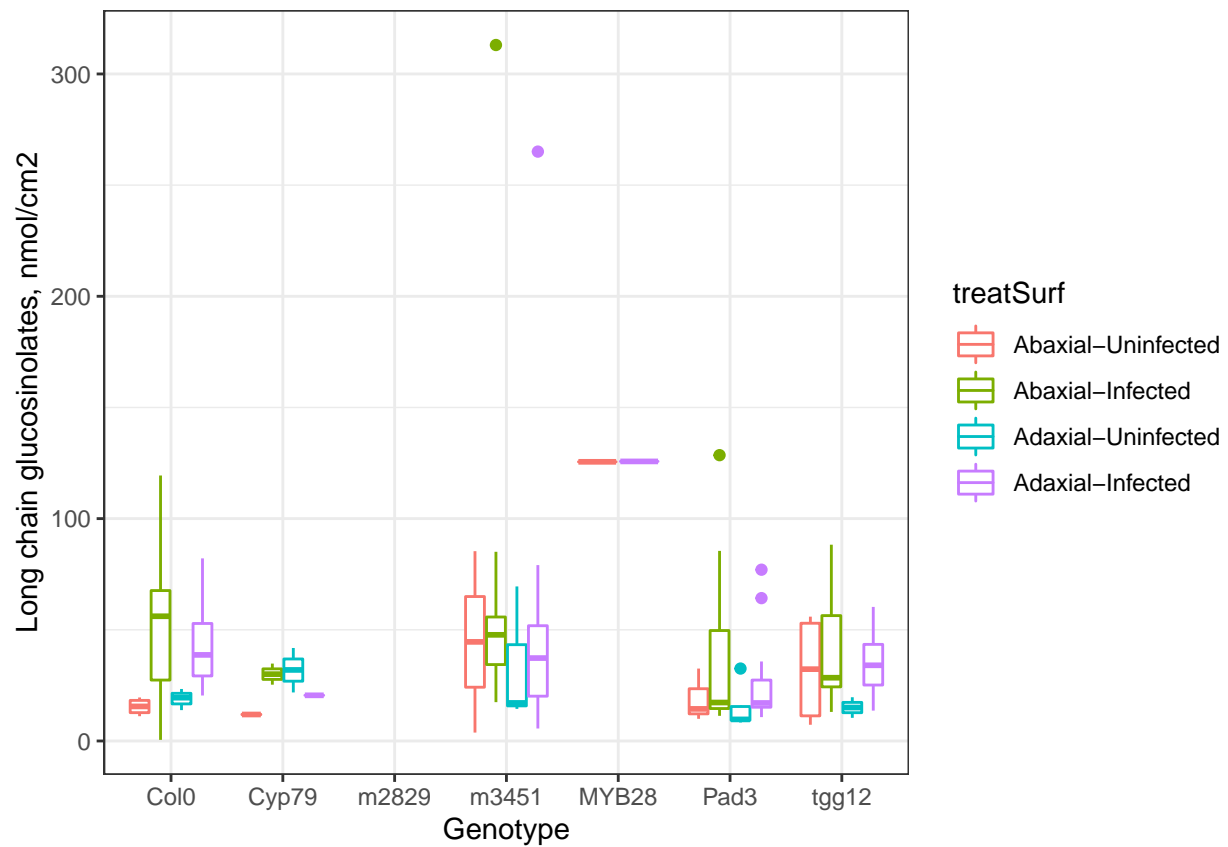
```
ggplot(GSL_Data, aes(Genotype, SCProp*1000, color=treatSurf))+
  geom_boxplot()+
  ylab("Shor/Long glucosinolates ratio, nmol/cm2")+
  theme_bw()
```
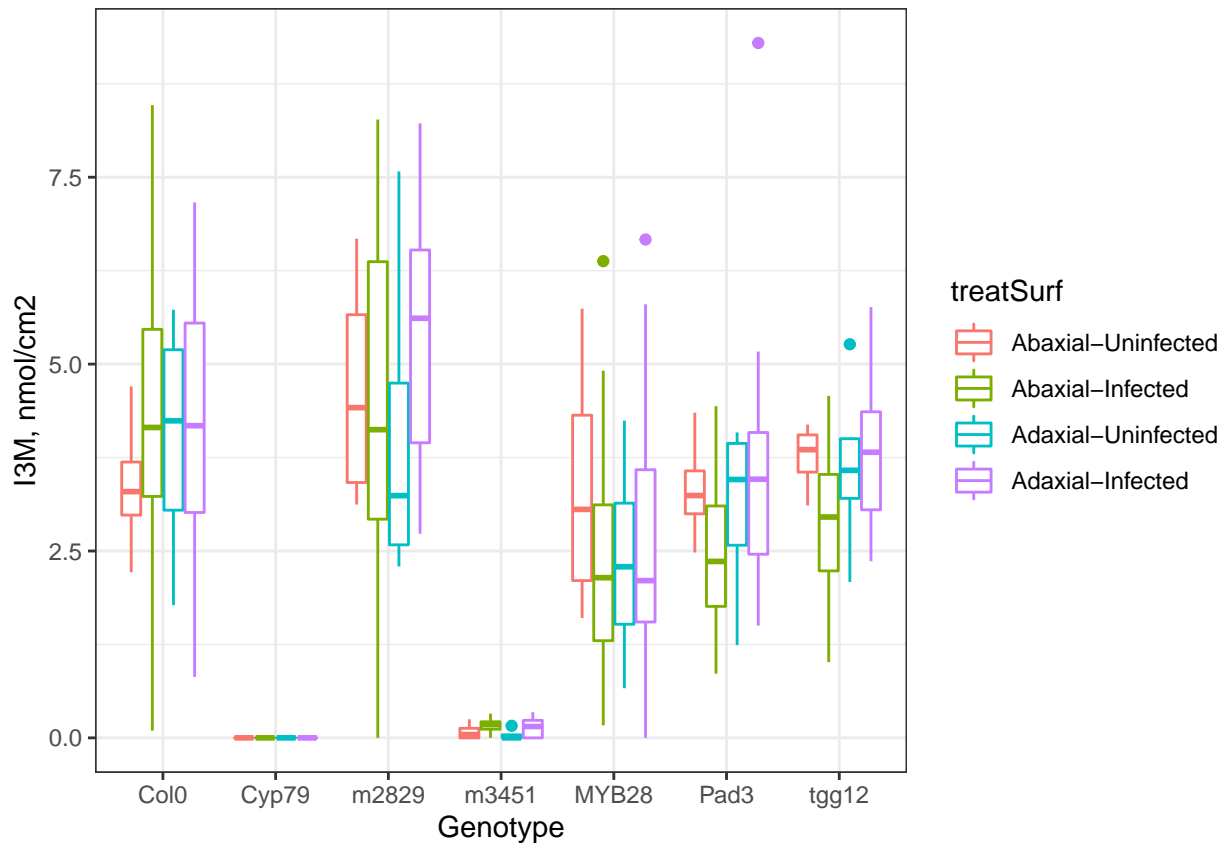
```
ggplot(GSL_Data, aes(Genotype, LCProp*1000, color=treatSurf))+
  geom_boxplot()+
  ylab("Shor/Long glucosinolates ratio, nmol/cm2")+
  theme_bw()
```

```
ggplot(GSL_Data, aes(Genotype, Stress, color=treatSurf))+
  geom_boxplot()+
  ylab("Long chain glucosinolates, nmol/cm2")+
  theme_bw()
```

```
ggplot(GSL_Data, aes(Genotype, QLLf_I3M*1000, color=treatSurf))+
  geom_boxplot()+
  ylab("I3M, nmol/cm2")+
  theme_bw()
```

```
rm(list = ls())
```

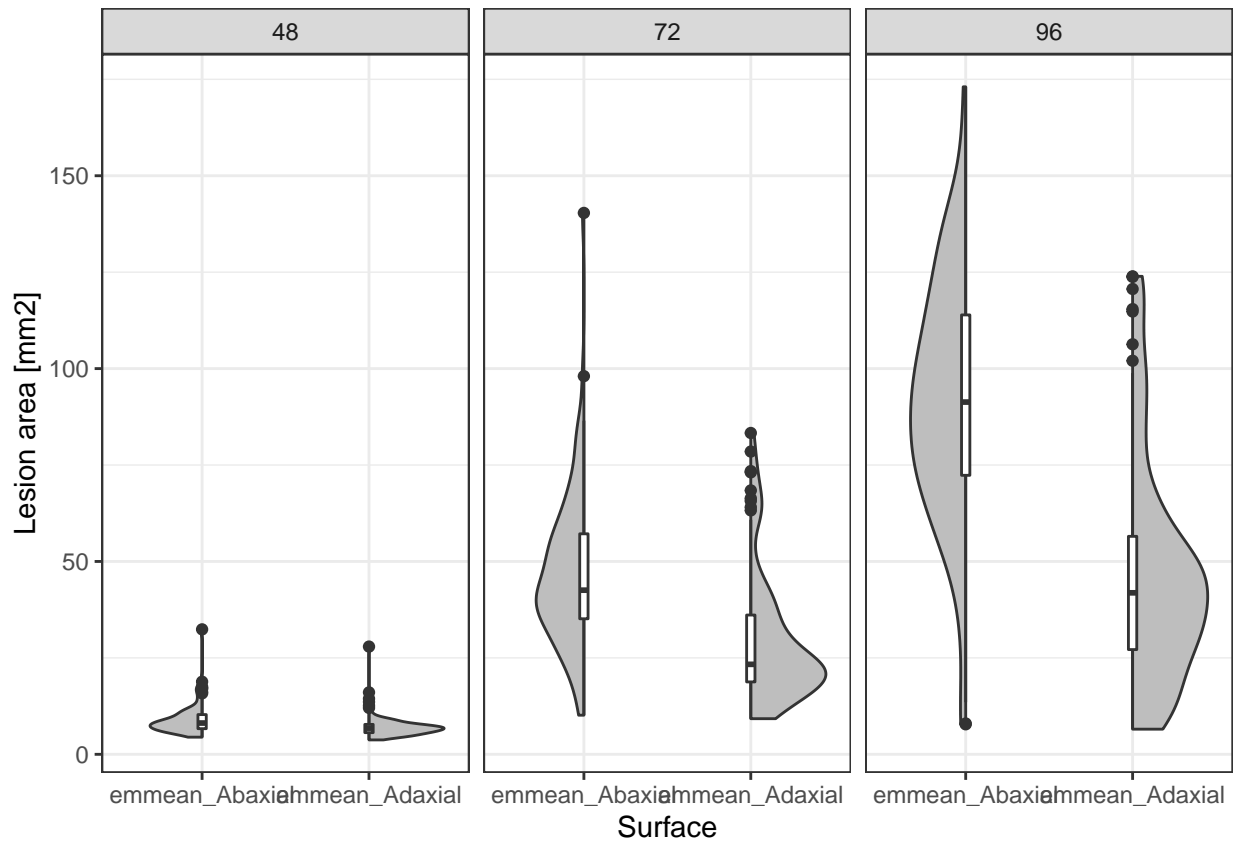# 4. Testing how the diverse Botrytis strains interact with the leaf surfaces

Associated files:

- Lesion__C0l0__48h.txt
- Col0__UpDown72h.txt
- UpDown__Exp1__Results96.txt
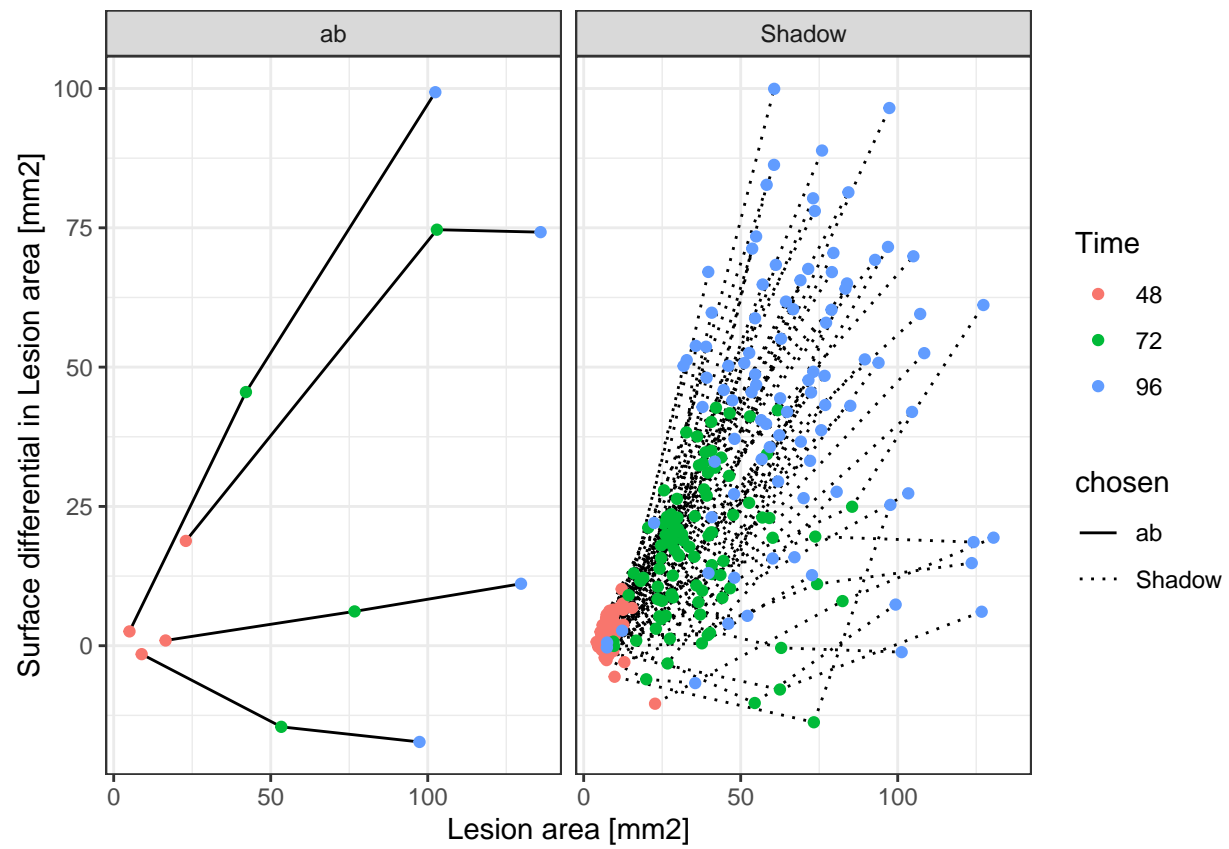
R Code:

- Bcin96.R

```
ggplot(data=S1L, aes(x=Surface, y=emmean)) +
  facet_grid(.~Time)+
  #geom_point()+
  geom_split_violin(fill="grey")+
  geom_boxplot(width=0.05)+
  ylab("Lesion area [mm2]")+
  theme_bw()
```

```
ggplot(data=Heritability, aes(Time, heritabilityPerc, color=term)) +
  geom_line(aes(linetype=Cat))+
  geom_point() +
  scale_color_manual(values=PalCol)+
  ylab("Percentage of the variance in lesion area")+
  xlab("Hours post inoculation")+
  theme_bw()
```
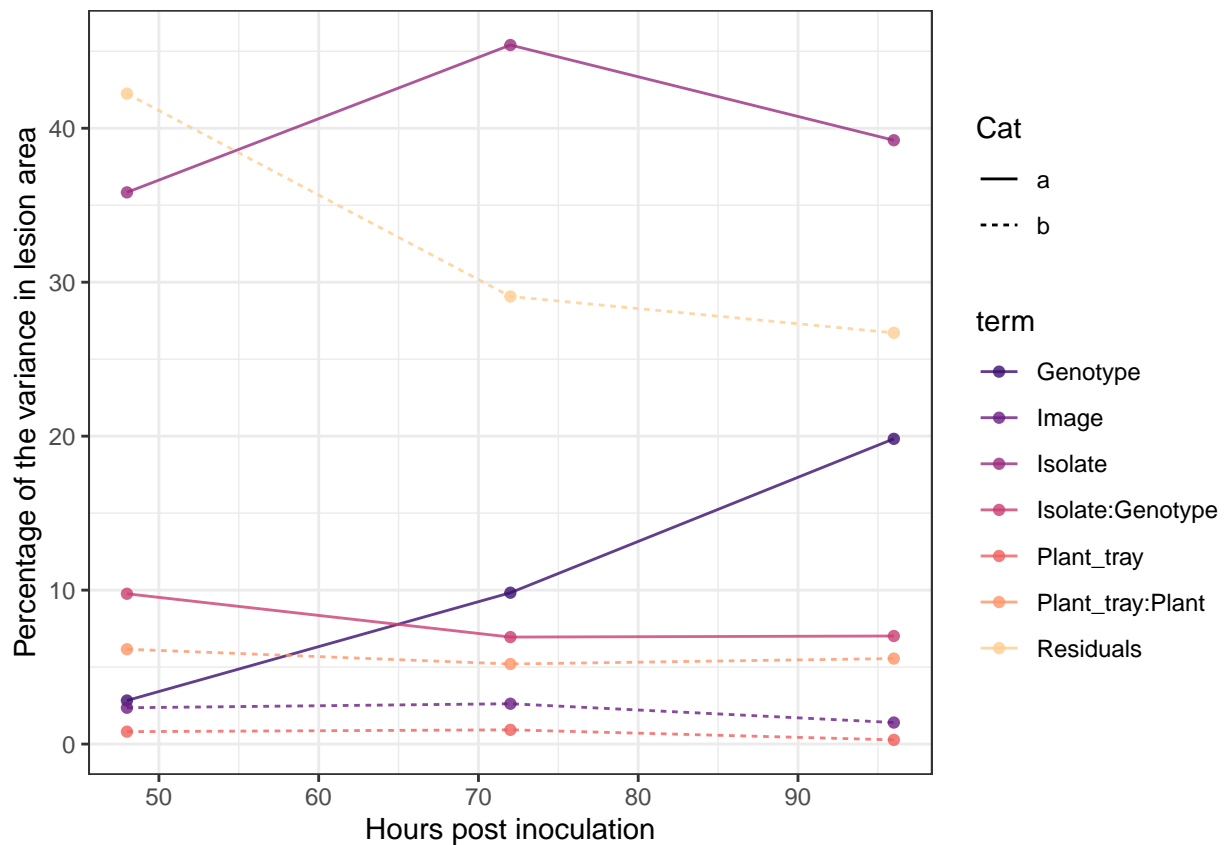
```
ggplot(data=All_lsm_497296, aes(emmean, Lsm_diff)) +
  facet_grid(.~chosen)+
  #facet_wrap(Isolate~.)+
  #geom_line(aes(group= Isolate))+
  geom_line(aes( group= Isolate,linetype=chosen))+
  scale_linetype_manual(values=c("solid", "dotted"))+
  geom_point(aes(color=Time)) +
  ylab("Surface differential in Lesion area [mm2]")+
  xlab("Lesion area [mm2]")+
  theme_bw()
```

```r
rm(list = ls())
```