

## A peculiar split-plot design

This is an example with an experimental design that may seem unproblematic at first glance, but is actually a not-so-standard experimental design. During consulting, I have come across multiple people who had versions of this “issue”. As a result, this chapter focusses a lot more on the experimental design discussion.

```
# packages
pacman::p_load(conflicted, # handle conflicting functions
               tidyverse,  # data import and handling
               glmmTMB,     # linear mixed model
               broom.mixed, car, DHARMa, # linear mixed model evaluation
               emmeans, modelbased,      # mean comparisons
               desplot, scales, see)     # plots

# function conflicts
conflict_prefer("select", "dplyr", quiet = TRUE) # set select() from dplyr as default
conflict_prefer("filter", "dplyr", quiet = TRUE) # set filter() from dplyr as default
```

## Data

This example considers a fictitious series of yield trials. There are 2 treatment factors:

- Str with levels Control and Street and
- Fru with levels Apple, Banana and Strawberry.

The trials were conducted

- at 2 locations (Loc with levels Berlin and Paris). Moreover, these trials were repeated
- across 2 years (Yea with levels 1989 and 1990).

Thus, there are 2 trials with repeated measures across 2 years, respectively. Similar experimental designs (with different randomizations) were used at each location and in each year.

```
# data (import via URL)
dataURL <- "https://raw.githubusercontent.com/SchmidtPaul/DSFAIR/master/data/StreetFruit.csv"
dat <- read_csv(dataURL)
```

dat

```
## # A tibble: 144 x 9
##   Yea Loc   Str   Fru   Blo   Yie   row   col Plot
##   <dbl> <chr> <chr> <chr> <chr> <dbl> <dbl> <dbl> <chr>
## 1 1989 Berlin Control Apple   B1    47.9     1     1 B1
## 2 1989 Berlin Control Strawberry B1    45.3     1     2 B2
## 3 1989 Berlin Control Banana   B1    44.7     1     3 B3
## 4 1989 Berlin Control Strawberry B2    45.6     1     4 B4
## 5 1989 Berlin Control Apple   B2    47.3     1     5 B5
## 6 1989 Berlin Control Banana   B2    45.0     1     6 B6
## 7 1989 Berlin Control Banana   B3    44.5     1     7 B7
## 8 1989 Berlin Control Apple   B3    47.2     1     8 B8
## 9 1989 Berlin Control Strawberry B3    45.8     1     9 B9
```

```
## 10 1989 Berlin Control Strawberry B4      47.1      1      10 B10
## # ... with 134 more rows
```

## Formatting

Before anything, the columns `Yea`, `Loc`, `Str`, `Fru`, `Blo` and `Plot` should be encoded as factors, since R by default encoded them as character.

```
dat <- dat %>%
  mutate_at(vars(Yea:Blo, Plot), as.factor)
```

## Preparations

In this example we will put some extra effort into the created outputs (graphs and tables). Therefore, we now set up some things before we continue.

```
# Colors for Fru levels
Fru_color <- c(Apple = "#9bc53d",
               Banana = "#fde74c",
               Strawberry = "#f25f5c")

# Colors for Str levels
Str_color <- c(Control = "#C3B299",
               Street = "#313E50")
```

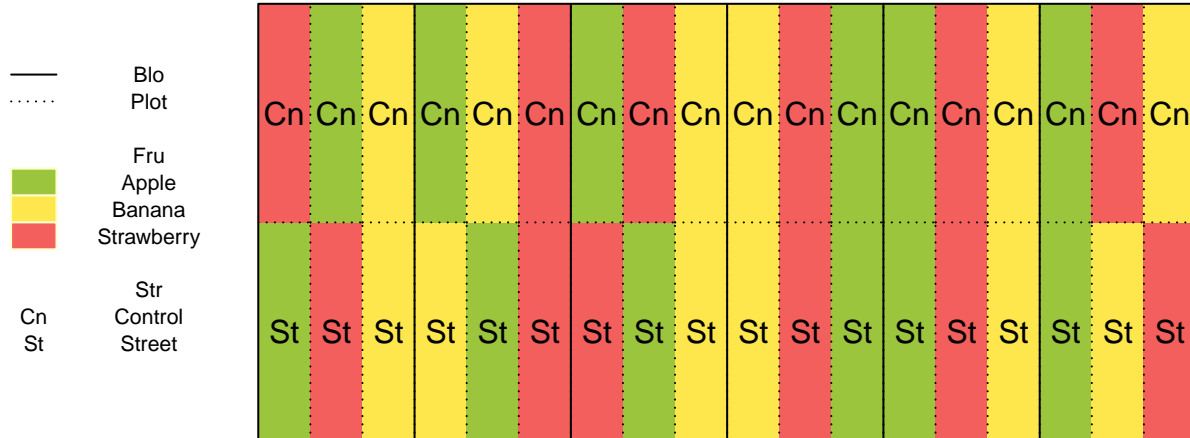
## Exploring

In order to obtain a field layout of the trial, we can use the `desplot()` function. Notice that for this we need two data columns that identify the `row` and `col` of each plot in the trial. Moreover, we need to use a subset of the data, since we have 2 trials with repeated measurements per plot, respectively.

```
Yea_i <- "1990"
Loc_j <- "Paris"

dat %>%
  filter(Yea == Yea_i & Loc == Loc_j) %>%
  desplot(data = .,
          form = Fru ~ col + row, # fill color per Fru
          col.regions = Fru_color, # use predefined colors
          text = Str, cex = 1, shorten = "abb", # show genotype names per plot
          out1 = Blo, out1.gpar = list(col = "black", lty = 1), # lines between blocks
          out2 = Plot, out2.gpar = list(col = "black", lty = 3), # lines between plots
          main = paste("Field layout:", Loc_j, Yea_i), # dynamic title
          show.key = TRUE, key.cex = 0.7) # formatting
```

## Field layout: Paris 1990



After playing around with `Yea_i` and `Loc_j` we can see how all trials are designed similarly and have different randomizations. However, what is peculiar about this trial design is that all plots that received the **Street** (or **Control**) treatment appear side-by-side in the same row. Moreover, it remains the same row across the two years. Note that per location the trials were laid out on exactly the same plots across the two years. Thus, the same 18 plots received the same **Street** (or **Control**) treatment in both years in **Berlin** and **Paris**, respectively.

## This experimental design...

### Why is it?

The goal of this trial was to find out if crops grown on soil where recently a street had been removed would perform differently compared to those grown on “normal” agricultural soil. Thus, multiple crops were chosen and aimed to grown on the two soil types under investigation.

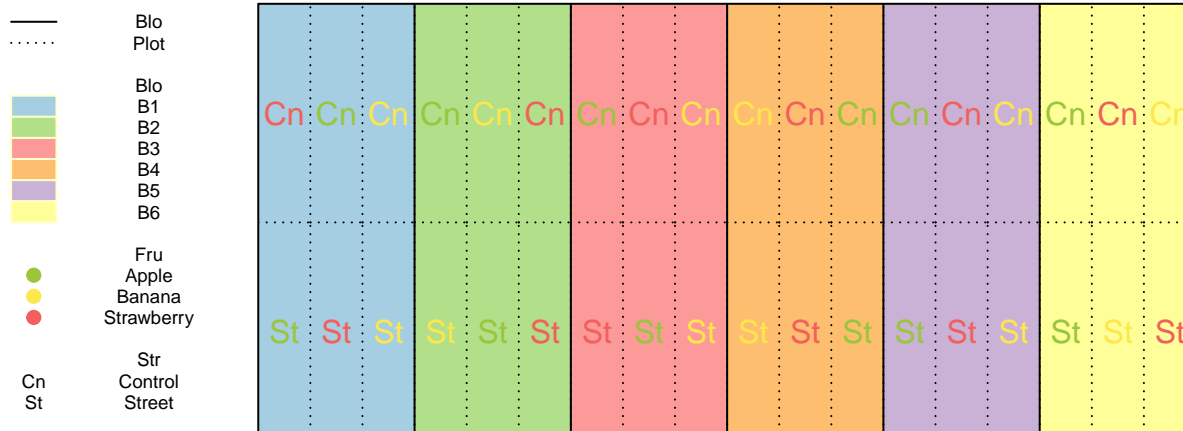
The reason for this layout lies in the nature of the **Str** treatment. These trials were laid out on agricultural fields where there used to be a street that was removed and turned into agricultural field again. Therefore, the plots with the **Street** treatment could obviously not be randomized.

### What is it?

Notice that there apparently are 6 blocks (**Blo**) and they are actually complete blocks, too, because all 6 factor level combinations appear in each one. However, this is not a *randomized complete block design* because of the restricted limitation of the **Str** treatment randomization (inside each block).

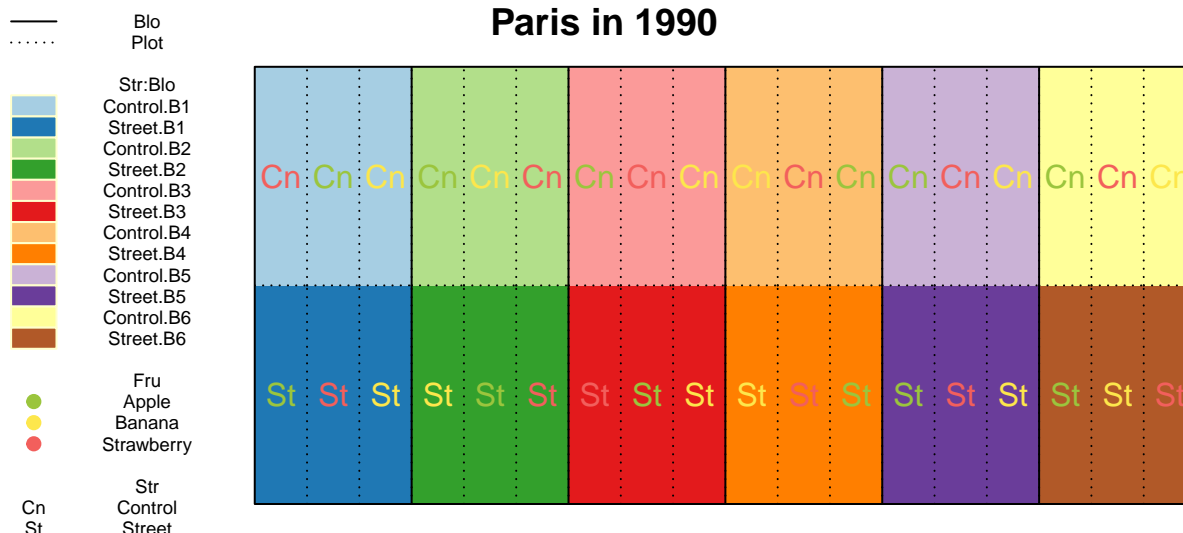
```
dat %>%
  filter(Yea == Yea_i & Loc == Loc_j) %>%
  desplot(data = .,
    form = Blo ~ col + row,
    col.regions = RColorBrewer::brewer.pal(12, "Paired")[c(1,3,5,7,9,11)],
    text = Str, cex = 1, shorten = "abb",
    col = Fru, col.text = Fru_color,
    out1 = Blo, out1.gpar = list(col = "black", lty = 1),
    out2 = Plot, out2.gpar = list(col = "black", lty = 3),
    show.key = TRUE, key.cex = 0.55,
    main = paste(Loc_j, "in", Yea_i))
```

## Paris in 1990



Ok, so our next guess could be that this is a split-plot design. We could argue that in split-plot designs, one of the treatment factors is harder to randomize than the other(s) and therefore grouped together in main plots. This does indeed seem to fit the setting here: In each of the 6 blocks, we have two main plots - one for each **Str** level and inside these 12 main plots we have three subplots respectively - one for each **Fru** level. So far, so good. What should also be done in a split-plot design is that first the main plots should be randomized and second the subplots within each main plot should be randomized. Again, the first step here did not occur. No randomization took place for the **Str** main plots, because it was simply not possible.

```
dat %>%
  filter(Yea == Yea_i & Loc == Loc_j) %>%
  mutate(`Str:Blo` = interaction(Str, Blo)) %>%
  desplot(data = .,
    form = `Str:Blo` ~ col + row,
    col.regions = RColorBrewer::brewer.pal(12, "Paired"),
    text = Str, cex = 1, shorten = "abb",
    col = Fru, col.text = Fru_color,
    out1 = Blo, out1.gpar = list(col = "black", lty = 1),
    out2 = Plot, out2.gpar = list(col = "black", lty = 3),
    show.key = TRUE, key.cex = 0.55,
    main = paste(Loc_j, "in", Yea_i))
```



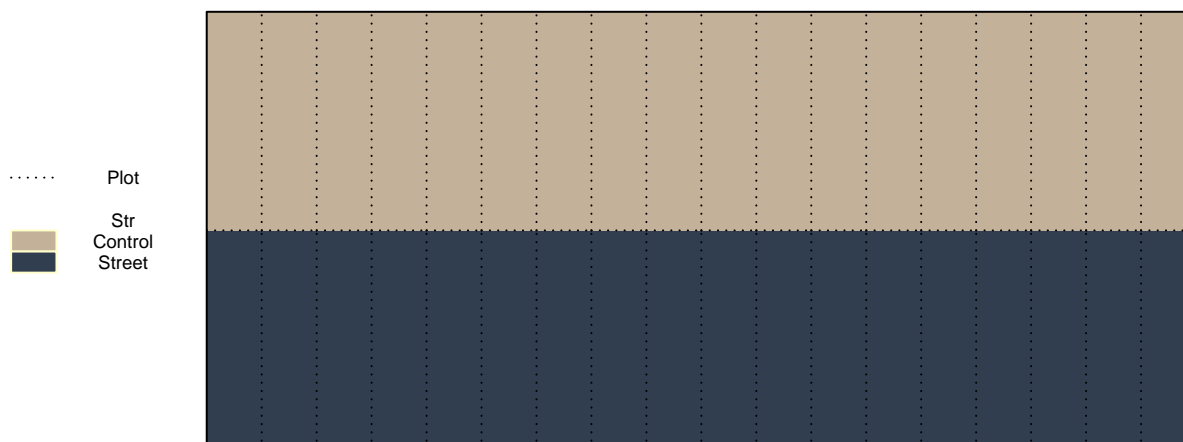
So is this a split-plot design with a certain lack of randomization? Can we simply acknowledge this minor inconvenience and go ahead with a model for a split-plot design at a single location with subplots nested inside mainplots nested inside 6 complete replicates in order to test both treatment main effects **Str** and **Fru** and compare their levels?

**Strictly speaking: No, we should not!**

The crucial and unfortunate detail here is that we do not really have *true replicates* for the **Str** treatment levels. Instead, we have a single large area/plot with **Street** and second one with **Control**. To make this as clear as possible, we could ignore the **Fru** factor for now, look at the trial layout via the following desplot and ask ourselves: *How is this different from having just 2 large plots where 18 measurements per plot from 2 large plots were taken?*

```
dat %>%
  filter(Yea == Yea_i & Loc == Loc_j) %>%
  desplot(data = .,
    form = Str ~ col + row,
    col.regions = Str_color,
    out1 = Plot, out1.gpar = list(col = "black", lty = 3),
    show.key = TRUE, key.cex = 0.55,
    main = paste(Loc_j, "in", Yea_i))
```

## Paris in 1990



The answer is: There is no difference. As a result of the nature of the **Str** treatment, its levels could not be randomized at all and we really only have 1 former street whose effect on the yield performance of our fruits could be investigated. Measuring this effect at different positions does not change the fact, that it was still the exact same street. Therefore, there is no true replicate of **Street** (or **Control**) at this trial. Regarding the **Str** factor, the number of true/independent replicates is 1 and when ignoring the second treatment factor **Fru**, the number of pseudo-replicates (*i.e.* replicated measurements on the same unit) is 18. In other words, we **do** have a sort of split-plot design, but it only has a single replicate and a total of 2 main plots.

### What does this mean for the analysis?

Having established that this is not a “standard split-plot” in the sense that we cannot conduct the same statistical analysis as in the previous GomezGomez1984 split-plot example, we should clarify what we can and cannot do to analyze this data properly.

What we can do is a descriptive statistical analysis *i.e.* calculate arithmetic means, standard deviations etc. and plot these values and/or the raw data. In other words: We can take analogous steps to those in the *Exploring* section of the GomezGomez1984 example (but note that the interpretation of these results should ultimately also be put in context of the limitations described below).

What we cannot do is to apply the same model and/or tests (*i.e.* statistical inference) as in the *Modelling* section of the GomezGomez1984 example. This is because in that example we did have a trial with multiple independent replicates for both our treatments, whereas here we do not.

Thus, we will take a step-by-step approach to investigate our options in terms of modelling the data.

#### A single location (1 year)

First, let us keep the focus on a single location in a single year - just like all the **desplot** graphs from above. Generally, one cannot make statistical inferences if only a single replicate is present.

Yet, we only have a single replicate for our 2 **Str** levels (**Street** and **Control**). Again: Yes, we have multiple measurements for both of them, but these are not true (*i.e.* independent) replicates, but pseudo-replicates just like in the subsampling Piepho1997 example. As a consequence, we must not set up a model with a **Str** main effect, which means that we can neither test its significance in an ANOVA, nor can we compare its adjusted means.

We do, however, have multiple true replicates for the **Fru** treatment levels - that is **within** the 2 **Str** levels. In other words, we have 6 replicates per fruit in the **Street** row and the **Control**, respectively. Accordingly,

per fruit we have a total of 12 replicates (arranged in blocks) split up evenly between the two rows. This in turn would mean, that we could set up a model with a **Fru** main effect, which can be tested in an ANOVA and its adjusted means compared.

Accordingly, we would get the following model:

```
glmmTMB(
  Yie ~ Fru + Str:Fru + Str:Blo,
  REML = TRUE,
  data = dat %>% filter(Yea == Yea_i & Loc == Loc_j)
)
```

So our two treatment effects are affected differently, which makes sense as only the randomization for the **Str** treatment was limited. Unfortunately, we are actually much more interested in the **Str** treatment than we are in the **Fru** treatment: Nobody really wants to compare apple yields to banana yields (on **Street** plots and then again on **Control** plots). What we do want to compare is whether apple/banana/strawberry yields are different on **Street** plots than they are on **Control** plots.

Further notice that the model is similar to a model for one-factorial rcdb trials at two multiple locations if you replace **Str** with **Trial**. We would in a way treat the two rows as two separate trials. The difference is, however, that we also assume a **Str:Fru** interaction effect, which we would usually not do for design effects. Ultimately, this is because **Str** is neither just the effect of the street, nor just the effect of the row, but the **Str** effects and row effects are completely confounded.

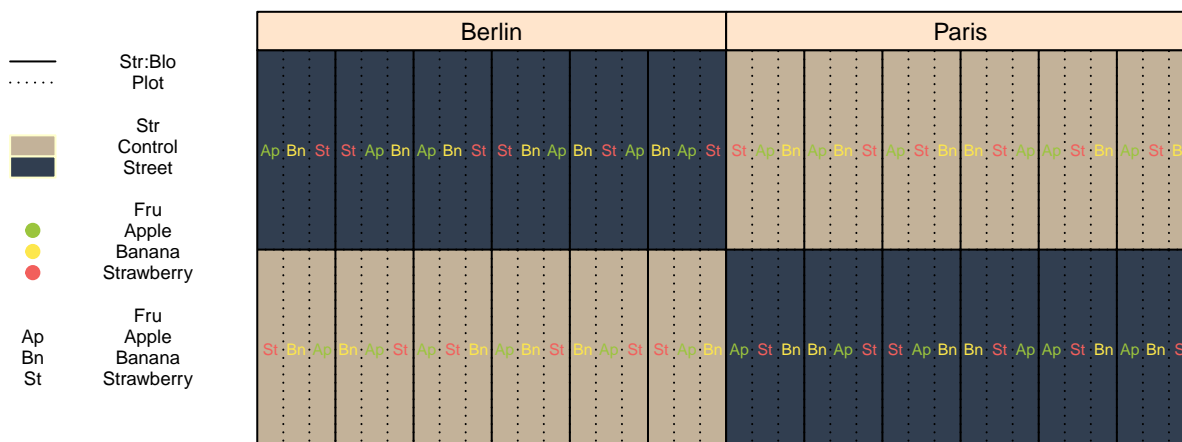
In conclusion, analyzing a single location will not allow us to make the statistical inferences we want on our main treatment effect **Str**.

### Both locations (1 year)

At this point it becomes clear our problem is mainly the lack of true replicates for **Str**. However, we actually do have an additional replicate for **Str** - it is simply not on the same location!

```
dat %>%
  filter(Yea == Yea_i) %>%
  mutate(`Str:Blo` = interaction(Str, Blo)) %>%
  desplot(data = .,
    form = Str ~ col + row|Loc,
    col.regions = Str_color,
    text = Fru, cex = 0.5, shorten = "abb",
    col = Fru, col.text = Fru_color,
    out1 = `Str:Blo`, out1.gpar = list(col = "black", lty = 1),
    out2 = Plot, out2.gpar = list(col = "black", lty = 3),
    show.key = TRUE, key.cex = 0.55,
    main = paste("Both locations in", Yea_i))
```

## Both locations in 1990



This is the game changer here. While everything that was said is indeed true for a single location, jointly modelling data from more than one location finally allows us to make desired statistical inferences about **Str**. Looking at the **desplot** above, it can be seen how we can take a step back and view the location as a true replicate, because the **Street** plots in Berlin are independent of those in Paris. Thus, we have 2 complete and true replicates for **Street** and **Control**, respectively. It is still true that those 4 rows (i.e. **Loc:Str** combinations) are grouped together and only within each one we have 6 true replicates for **Fru** arranged as blocks. Therefore, as design effects we have **plots nested in blocks nested in rows nested in locations**.

In order to avoid confusion in this complex model, we should create an additional factor variable in the dataset that denotes a trial's row, so that we do not actually write **Loc:Str** in the model. Furthermore, and albeit not strictly being necessary, we could reformat the block levels correctly:

```
dat <- dat %>%
  mutate(Row = as.factor(row)) %>%
  mutate(Blo = interaction(Row, Blo))
```

As treatment effects, we can now have both main effects and their interaction effect. Again, this is possible since we now also have true replicates for **Str** (and not only for **Fru**). Notice further that across multiple locations **Row** is no longer confounded with **Str**. Here is a summary table with all the effects:

Type	Label	Model term	Number of levels	Fixed/Random
Design	Location	<b>Loc</b>	2	fixed (complete replicates)
Design	Row	<b>Loc:Row</b>	4	random (main plot randomization units)
Design	Block	<b>Loc:Row:Blo</b>	24	random (sub plot randomization units)
Design	Plot	<b>Loc:Row:Blo:Plot</b>	48	random (sub sub plot randomization units / error)
Treatment	Street	<b>Str</b>	2	fixed (levels of interest)
Treatment	Fruit	<b>Fru</b>	3	fixed (levels of interest)
Treatment	Street-Fruit-interaction	<b>Str:Fru</b>	6	fixed (levels of interest)

Accordingly, we could set up this model for a single year:



```
glmmTMB(
  Yie ~
    Str + Fru + Str:Fru +                # Treatment
    Loc + (1 | Loc:Row) + (1 | Loc:Row:Blo), # Design
  REML = TRUE,
  data = dat %>% filter(Yea == Yea_i)
)
```

## Descriptive Statistics

Having established a general approach for modelling, we should probably first get a feeling for our data. Therefore, we explore it with several descriptive measures and plots.

```
dat %>%
  group_by(Fru, Str) %>% # or go all the way to group_by(Yea, Loc, Fru, Str)
  summarise(meanYield = mean(Yie),
            medYield  = median(Yie),
            maxYield  = max(Yie),
            minYield  = min(Yie),
            stddevYield = sd(Yie))
```

```
## # A tibble: 6 x 7
## # Groups:   Fru [3]
##   Fru      Str   meanYield medYield maxYield minYield stddevYield
##   <fct>    <fct>     <dbl>   <dbl>   <dbl>   <dbl>     <dbl>
## 1 Apple    Control    47.9    48.1    52.0    43.2      2.39
## 2 Apple    Street    46.6    46.7    51.0    42.3      2.40
## 3 Banana   Control    44.2    44.4    46.9    41.9      1.33
## 4 Banana   Street    42.8    43.0    44.8    41.2      1.05
## 5 Strawberry Control    44.5    44.6    47.7    40.0      1.86
## 6 Strawberry Street    43.5    43.4    46.5    40.7      1.58
```

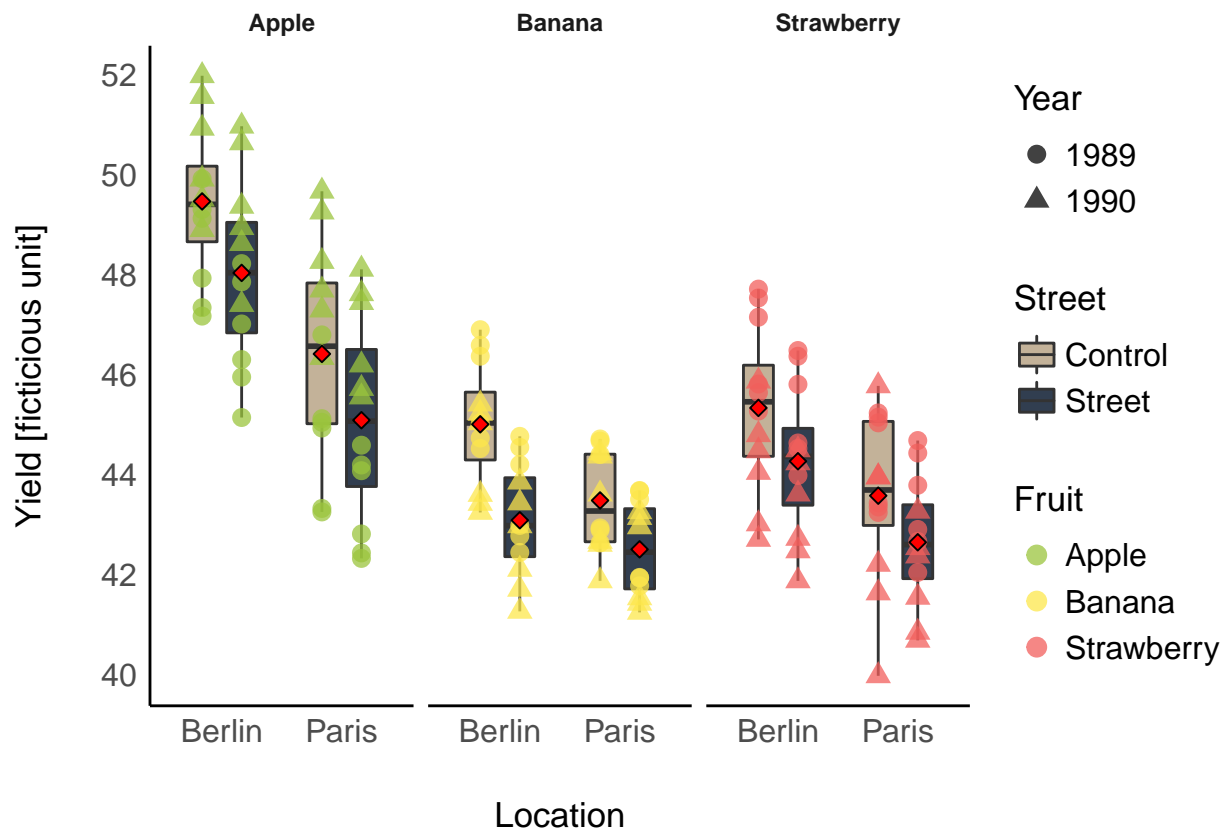
A ggplot packed with information could look like this:

```
ggplot(data = dat,
       aes(y = Yie, x = Loc)) + # y-axis: yield, x-axis: year
  facet_grid(cols = vars(Fru)) + # one facette per fruit
  geom_boxplot( # boxplot
    aes(fill = Str), # box color
    position = position_dodge(0.66), # box distance
    width = 0.5 # box width
  ) +
  geom_point( # scatter-plot
    aes(
      col = Fru, # point color
      shape = Yea, # point shape
      group = Str # point grouping
    ),
    position = position_dodge(0.66), # distance between groups
    size = 3, # dot size
    alpha = 0.75 # dot transparency
  ) +
  stat_summary( # add mean as red point
    fun = mean,
```

```

aes(group = Str), # per Str
geom = "point", # as scatter point
shape = 23,      # point shape
size = 2,        # point size
fill = "red",    # point color
show.legend = FALSE, # hide legend for this
position = position_dodge(0.66) # distance between groups
) +
scale_y_continuous(#limits = c(0, NA),      # y-axis must start at 0
                   breaks = pretty_breaks(),
                   name = "Yield [fictitious unit]") +
xlab("Location") +
scale_shape(name = "Year") +
scale_fill_manual(values = Str_color, name = "Street") +
scale_colour_manual(values = Fru_color, name = "Fruit") +
theme_modern()

```



Additionally, it may also be informative to visualize the data using desplot. Obviously, we could simply create a desplot and fill the plots according to the raw data:

```

dat %>%
  filter(Yea == Yea_i) %>%
  desplot(data = .,
    form = Yie ~ col + row|Loc,
    text = Str, cex = 0.5, shorten = "abb",
    col = Fru, col.text = Fru_color,

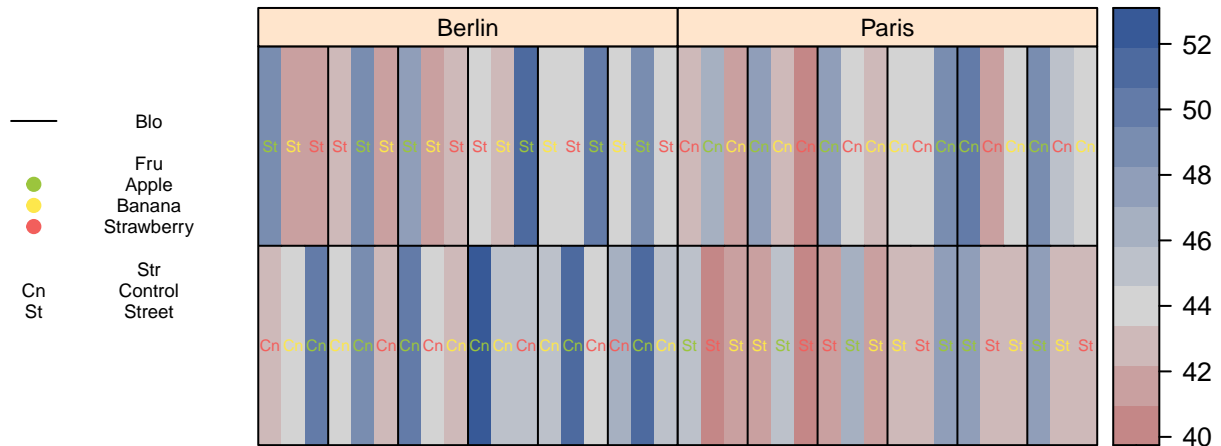
```

```

out1 = Blo, out1.gpar = list(col = "black", lty = 1),
# out2 = Plot, out2.gpar = list(col = "black", lty = 3),
show.key = TRUE, key.cex = 0.55,
main = paste("Absolute yield for both locations in", Yea_i))

```

## Absolute yield for both locations in 1990



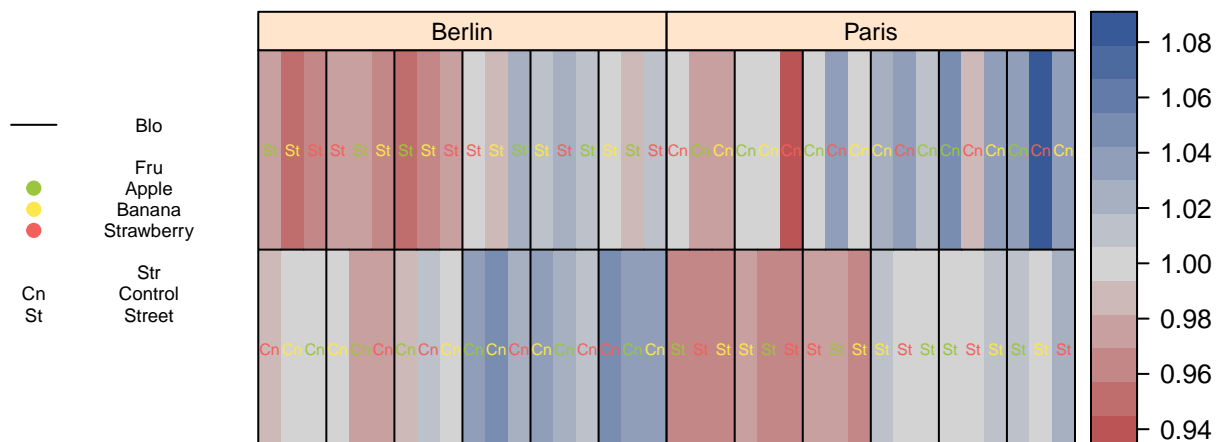
However, this is not very helpful as the values vary too much for the different Loc and Fru. We can, however, express the raw data per plot as the deviation from the average value per Location and Fruit:

```

dat %>%
  filter(Yea == Yea_i) %>%
  group_by(Loc, Fru) %>%
  mutate(
    meanYield = mean(Yie),
    ratioToMeanYield = Yie / mean(Yie)
  ) %>%
  ungroup() %>%
  desplot(data = .,
    form = ratioToMeanYield ~ col + row|Loc,
    text = Str, cex = 0.5, shorten = "abb",
    col = Fru, col.text = Fru_color,
    out1 = Blo, out1.gpar = list(col = "black", lty = 1),
    # out2 = Plot, out2.gpar = list(col = "black", lty = 3),
    show.key = TRUE, key.cex = 0.55,
    main = paste("Relative yield ratio to fruit-location-mean for both locations in", Yea_i))

```

## Relative yield ratio to fruit–location–mean for both locations in 1990



Roughly summarizing, we can say that yield measurements on **Street** plots have generally been lower than those on **Control** plots.

## Inferential Statistics (Modelling)

### 1 Year

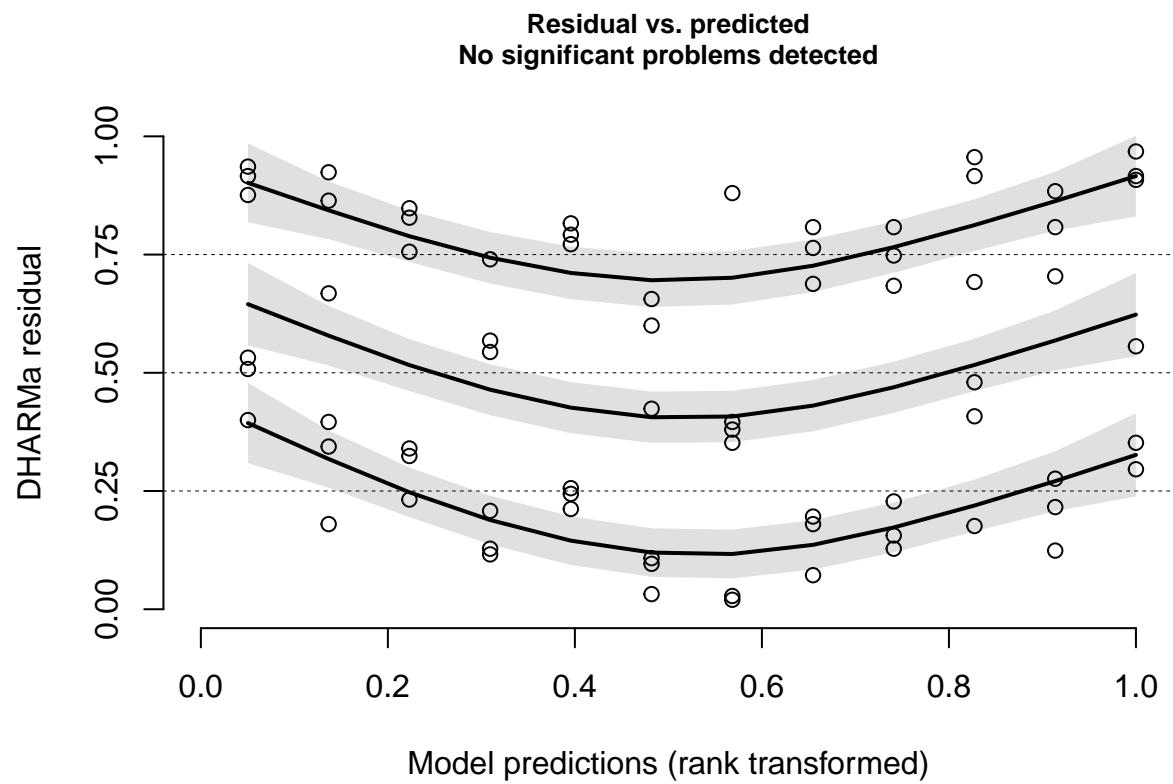
Finally, we can fit the model we suggested above. As a reminder: This is only a model for data from a single year. We will explicitly go with 1989 for now.

```
mod1989 <- glmmTMB(
  Yie ~
    Str + Fru + Str:Fru +                      # Treatment
    Loc + (1 | Loc:Row) + (1 | Loc:Row:Blo), # Design
  REML = TRUE,
  data = dat %>% filter(Yea == "1989")
)
```

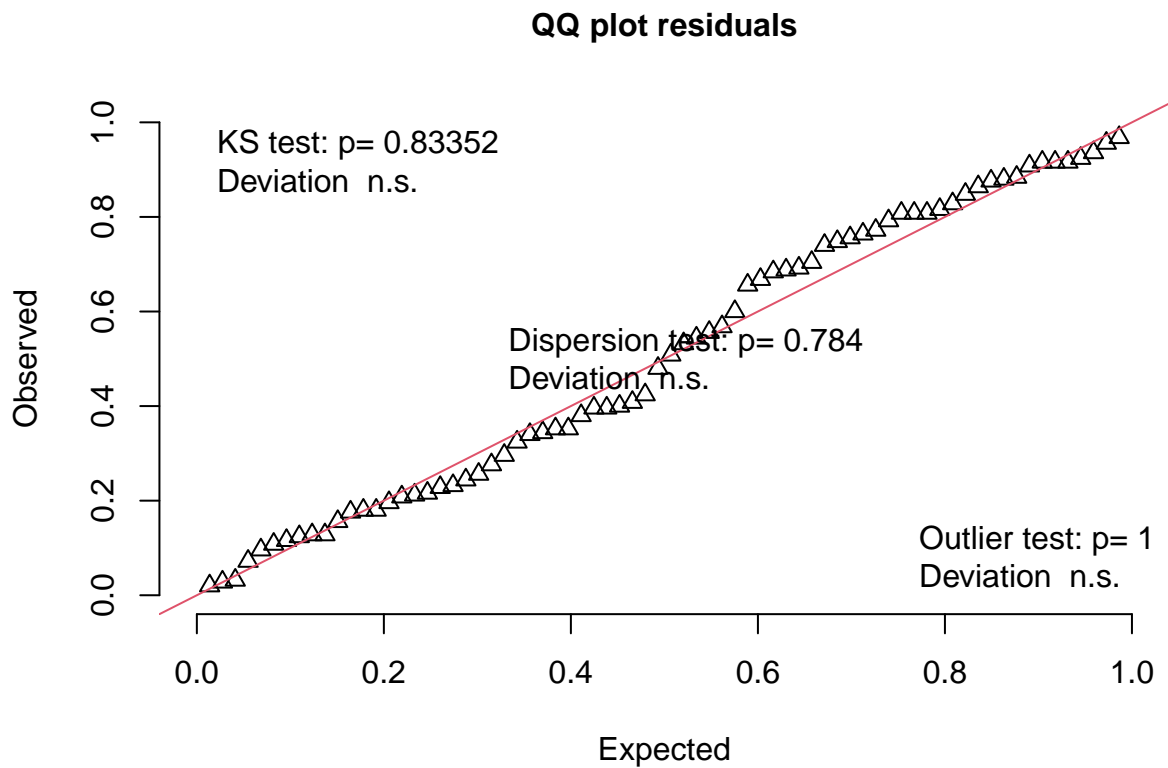
### Check model assumptions

We can very efficiently check the residual plots using the **DHARMA** package. No problem is suggested.

```
DHARMA::plotResiduals(mod1989)
```



```
DHARMA::plotQQunif(mod1989)
```



### Variance components

We can check the variance components and see that as expected a considerably large portion of the variance is attributed to the blocks.

```
mod1989 %>%
  broom.mixed::tidy(effects = "ran_pars", scales = "vcov") %>%
  select(group, estimate)
```

```
## # A tibble: 3 x 2
##   group      estimate
##   <chr>      <dbl>
## 1 Loc:Row    0.00000000723
## 2 Loc:Row:Blo 0.866
## 3 Residual   0.466
```

### ANOVA

Looking at the ANOVA, we see that both treatment main effects, but not their interaction effects are statistically significant.

```
Anova(mod1989)

## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: Yie
##           Chisq Df Pr(>Chisq)
## Str       10.7919  1  0.001019 **
```

```
## Fru      92.9458  2  < 2.2e-16 ***
## Loc      32.3506  1  1.287e-08 ***
## Str:Fru  2.1421  2   0.342650
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

## Mean comparisons

```
# emmeans:
mod1989 %>%
  emmeans(pairwise ~ Str | Fru,
           adjust = "tukey",
           lmer.df = "kenward-roger") %>%
  pluck("emmeans") %>%
  multcomp::cld(details = TRUE, Letters = letters)
```

```
## $emmeans
## Fru = Apple:
##   Str      emmean    SE df lower.CL upper.CL .group
##   Street    45.1 0.333 69     44.4     45.7    a
##   Control    46.6 0.333 69     45.9     47.3    b
##
## Fru = Banana:
##   Str      emmean    SE df lower.CL upper.CL .group
##   Street    43.2 0.333 69     42.5     43.8    a
##   Control    44.7 0.333 69     44.0     45.4    b
##
## Fru = Strawberry:
##   Str      emmean    SE df lower.CL upper.CL .group
##   Street    44.4 0.333 69     43.7     45.0    a
##   Control    45.4 0.333 69     44.7     46.0    b
##
## Results are averaged over the levels of: Loc
## Confidence level used: 0.95
## significance level used: alpha = 0.05
## NOTE: Compact letter displays can be misleading
##       because they show NON-findings rather than findings.
##       Consider using 'pairs()', 'pwpp()', or 'pwpm()' instead.
##
## $comparisons
## Fru = Apple:
##   contrast      estimate    SE df t.ratio p.value
##   Control - Street    1.53 0.471 69   3.246  0.0018
##
## Fru = Banana:
##   contrast      estimate    SE df t.ratio p.value
##   Control - Street    1.51 0.471 69   3.214  0.0020
##
## Fru = Strawberry:
##   contrast      estimate    SE df t.ratio p.value
##   Control - Street    1.02 0.471 69   2.170  0.0334
##
## Results are averaged over the levels of: Loc
```

```
# modelbased:
mod1989 %>%
  estimate_means(
    levels = "Str",
    modulate = "Fru",
    lmer.df = "kenward-roger",
    adjust = "holm"
  )
```

## Estimated Marginal Means

```
##
## Str      |      Fru | Mean | SE |      95% CI
## -----|-----|-----|-----|-----
## Control | Apple | 46.60 | 0.33 | [45.70, 47.51]
## Street  | Apple | 45.07 | 0.33 | [44.17, 45.98]
## Control | Banana | 44.70 | 0.33 | [43.79, 45.60]
## Street  | Banana | 43.18 | 0.33 | [42.28, 44.09]
## Control | Strawberry | 45.38 | 0.33 | [44.47, 46.28]
## Street  | Strawberry | 44.36 | 0.33 | [43.45, 45.26]
##
```

## Marginal means estimated at Str, Fru

```
mod1989 %>%
  estimate_contrasts(
    levels = "Str",
    modulate = "Fru",
    lmer.df = "kenward-roger",
    adjust = "holm",
    standardize = FALSE
  )
```

## Marginal Contrasts Analysis

```
##
## Level1 | Level2 | Difference |      95% CI | SE | t(69) |      p
## -----|-----|-----|-----|-----|-----|-----
## Control | Street |      1.36 | [0.53, 2.18] | 0.41 |  3.29 | 0.002
##
```

## Marginal contrasts estimated at Str

## p-value adjustment method: Holm (1979)