Efficacy of fungicides against brown spot of pear in Argentina

Tudela et al.

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Abstract

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Brown spot of pear (BSP), a fungal disease of importance in Europe, has been recently detected for the first time in pear orchards in the Alto Valle of Río Negro (Patagonia), Argentina, South America. The disease is caused by *Stemphylium vesicarium* and its main symptoms are lesions in fruits and leaves. To assess counteracting measures against BSP, the effects of four fungicides were tested to evaluate *in vitro* efficacy against mycelial growth and spore germination of native *S. vesicarium* strains, and preventive and curative control of lesion development in pear fruit 'D'Anjou' var. In addition, the activities of two selected fungicides were determined through in-field assays. The fungicides tested were chosen according to their commercial availability and registration in pear crops and included: pyraclostrobin + boscalid (Bellis®); ziram (Ziram®); captan (Merpan®); and *Melaleuca alternifolia* extract (Timorex®). Bellis® presented the lowest EC50 values in germination and mycelial growth tests. Ziram® and Merpan® were also very effective in inhibiting germination. The plant-based biofungicide Timorex® did not achieve satisfactory effectiveness in in vitro trials, nor in bioassays after preventive and curative treatments. Bioassay results showed that preventive measures using Bellis®, Ziram®, or Merpan® were effective in reducing disease severity and suggested that BSP could be controlled by adequate selection of treatment time.

Figure 1: Abate pear cultivar with symptoms of brown spot caused by *Stemphylium vesicarium*

Figure 2: Danjou pear cultivar with symptoms of brown spot caused by *Stemphylium vesicarium*

1 In vitro experiments

1.1 Mycelial growth

```
raw <- import("data/mycelial_growth.csv", dec=",")

dat <- raw %>%
  mutate_at(vars(dose, colony_diameter), as.numeric) %>%
  mutate_at(vars(fungicide, strain, experiment, plate), as.factor) %>%
  mutate(curve_id = interaction(fungicide:strain:experiment))
```

Data structure

```
ftable(xtabs(~ fungicide + strain + experiment + dose, dat))
```

fungicide	strain	experiment	dose 0	0.01	0.1	0.5	1	10	50	100	500	1000
Bellis	S20	1	3	3	3	0	3	3	3	3	0	0
		2	3	3	3	0	3	3	3	3	0	0
		3	3	3	3	0	3	3	3	3	0	0
	S23	1	3	3	3	0	3	3	3	3	0	0
		2	3	3	3	0	3	3	3	3	0	0
		3	3	3	3	0	3	3	3	3	0	0
	S8	1	3	3	3	0	3	3	3	3	0	0
		2	3	3	3	0	3	3	3	3	0	0
		3	3	3	3	0	3	3	3	3	0	0
Merpan	S20	1	3	0	3	0	3	3	0	3	3	3
		2	3	0	3	0	3	3	0	3	3	3
		3	3	0	3	0	3	3	0	3	3	3
	S23	1	3	0	3	0	3	3	0	3	3	3
		2	3	0	3	0	3	3	0	3	3	3
		3	3	0	3	0	3	3	0	3	3	3
	S8	1	3	0	3	0	3	3	0	3	3	3
		2	3	0	3	0	3	3	0	3	3	3
		3	3	0	3	0	3	3	0	3	3	3

```
Timorex    S20    1                    3    0    3    0 3   3   0    3    3    3
                  2                    3    0    3    0 3   3   0    3    3    3
                  3                    3    0    3    0 3   3   0    3    3    3
           S23    1                    3    0    3    0 3   3   0    3    3    3
                  2                    3    0    3    0 3   3   0    3    3    3
                  3                    3    0    3    0 3   3   0    3    3    3
           S8     1                    3    0    3    0 3   3   0    3    3    3
                  2                    3    0    3    0 3   3   0    3    3    3
                  3                    3    0    3    0 3   3   0    3    3    3
Ziram      S20    1                    3    0    3    3 3   3   3    3    0    0
                  2                    3    0    3    3 3   3   3    3    0    0
                  3                    3    0    3    3 3   3   3    3    0    0
           S23    1                    3    0    3    3 3   3   3    3    0    0
                  2                    3    0    3    3 3   3   3    3    0    0
                  3                    3    0    3    3 3   3   3    3    0    0
           S8     1                    3    0    3    3 3   3   3    3    0    0
                  2                    3    0    3    3 3   3   3    3    0    0
                  3                    3    0    3    3 3   3   3    3    0    0
```

```r
str(dat)
```

```
'data.frame':    756 obs. of  7 variables:
 $ fungicide      : Factor w/ 4 levels "Bellis","Merpan",..: 1 1 1 1 1 1 1 1 1 1 ...
 $ strain         : Factor w/ 3 levels "S20","S23","S8": 2 2 2 1 1 1 3 3 3 2 ...
 $ experiment     : Factor w/ 3 levels "1","2","3": 1 1 1 1 1 1 1 1 1 1 ...
 $ dose           : num  0 0 0 0 0 0 0 0 0 100 ...
 $ plate          : Factor w/ 3 levels "1","2","3": 1 2 3 1 2 3 1 2 3 1 ...
 $ colony_diameter: num  53 55 56 52 53 52 51 51 52 0 ...
 $ curve_id       : Factor w/ 36 levels "Bellis:S20:1",..: 4 4 4 1 1 1 7 7 7 4 ...
```

Dose-response curves fitting by meta-analysis approach

```r
# verify drc_per_strain.R
mod_mg <- metadrm(colony_diameter ~ dose,
             data=dat,
             fct=LL.3(),
             ind=curve_id,
             cid2=fungicide,
             struct="UN")
save(mod_mg, file= "models/invitro_mg.rds")
```

```
load("models/invitro_mg.rds")
summary(mod_mg)
```

Two-stage meta-analysis dose-response model
Model fitted: Log-logistic (ED50 as parameter) with lower limit at 0

Call:
metadrm(formula = colony_diameter ~ dose, fct = LL.3(), ind = curve_id,
    data = dat, cid2 = fungicide, struct = "UN")

Variance estimates:
          estim   sqrt
tau^2.1   0.0023 0.0481
tau^2.2   8.1930 2.8623
tau^2.3   0.0001 0.0076

               rho.b:(I rho.d:(I rho.e:(I
b:(Intercept)   1   -0.0949   0.9136
d:(Intercept)   -0.0949   1   -0.4915
e:(Intercept)   0.9136   -0.4915   1

Coefficients:
             Estimate   Std.Err t value   Pr(>|t|)
b:Bellis   0.5795367   0.0182490 31.7572 < 0.00000000000000022 ***
b:Merpan   0.3611793   0.0183369 19.6968 < 0.00000000000000022 ***
b:Timorex   0.3490411   0.0324495 10.7565 < 0.00000000000000022 ***
b:Ziram   0.3926960   0.0202379 19.4040 < 0.00000000000000022 ***
d:Bellis   51.3746461   0.9890286 51.9446 < 0.00000000000000022 ***
d:Merpan   52.5139150   1.0667613 49.2274 < 0.00000000000000022 ***
d:Timorex   49.9869522   1.3972223 35.7759 < 0.00000000000000022 ***
d:Ziram   53.0642566   1.1218557 47.3004 < 0.00000000000000022 ***
e:Bellis   0.0297542   0.0027299 10.8994 < 0.00000000000000022 ***
e:Merpan   24.5154698   1.6200305 15.1327 < 0.00000000000000022 ***
e:Timorex 112.8372474 22.1680049 5.0901   0.000001779 ***
e:Ziram   6.4555590   0.6162007 10.4764 < 0.00000000000000022 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

EC50 Estimates

```
ec50s <- ED(mod_mg, respLev=c(50)) %>% data.frame
```

Estimated effective doses

```
               Estimate  Std. Error
e:Bellis:50    0.0297542   0.0027299
e:Merpan:50   24.5154698   1.6200305
e:Timorex:50 112.8372474  22.1680049
e:Ziram:50     6.4555590   0.6162007
```

```
# coef_mod_mg <- summary(mod_mg) %>% data.frame %>%
#    rownames_to_column("param")  %>%
#    separate(param, c("param", "fungicide"))
# ec50s <- coef_mod_mg %>% filter(param=="e")
# ec50s
```

Comparing fungicides EC50

As we compare EC50 ratios between fungicides, if the confidence interval does not contain 1, fungicides differ among them:

```
ed_comp <- EDcomp(mod_mg,
                  percVec=c(50),
                  percMat=rbind(c(1,1,1,1)),
                  interval="fieller") %>%
      data.frame %>%
   rownames_to_column("comp") %>%
   rowwise() %>%
   mutate(relative_to_one = f(Lower, Upper, 1)) # %>%
```

Estimated ratios of effect doses

```
                         Estimate        Lower        Upper
Bellis/Merpan:50/50    0.00121369   0.00095935   0.00151054
Bellis/Timorex:50/50   0.00026369   0.00017897   0.00044300
Bellis/Ziram:50/50     0.00460909   0.00353519   0.00602624
Merpan/Timorex:50/50   0.21726398   0.15162515   0.36083619
Merpan/Ziram:50/50     3.79757505   3.03655193   4.84141476
Timorex/Ziram:50/50   17.47908235  10.38447082  25.87541262
```

```
  # as_huxtable() %>%
  # set_number_format(1)
 ed_comp
```

```
# A tibble: 6 x 5
# Rowwise:
  comp                  Estimate     Lower      Upper relative_to_one
  <chr>                    <dbl>     <dbl>      <dbl> <chr>
1 Bellis/Merpan:50/50    0.00121  0.000959  0.00151  below
2 Bellis/Timorex:50/50   0.000264 0.000179  0.000443 below
3 Bellis/Ziram:50/50     0.00461  0.00354   0.00603  below
4 Merpan/Timorex:50/50   0.217    0.152     0.361    below
5 Merpan/Ziram:50/50     3.80     3.04      4.84     above
6 Timorex/Ziram:50/50   17.5     10.4      25.9      above
```

```
 ed_comp %>%
   ggplot()+
   aes(x=comp, y=Estimate) +
   geom_pointrange(aes(ymin = Lower, ymax = Upper, col=relative_to_one))+
   geom_hline(yintercept = 1, linetype=2)+
   # scale_y_log10()+
   coord_flip() +
   labs(col="Relative to 1")
```

```
 dat %>%
   ggplot()+
   aes(x=dose+0.0001, y=colony_diameter, col=fungicide) +
   geom_point(size=.5) +
   geom_smooth(method = drm,
             method.args = list(fct = L.3()), se = F) +
     geom_smooth(aes(group=curve_id), size=.3, linetype=2,
             method = drm,
             method.args = list(fct = L.3()), se = F) +
   labs(title= "", x = "Dose (ppm)",  y = "Colony diameter (cm)", col= "Fungicide") +
   scale_x_log10() +
   theme_bw(base_family=12)
```

```
 ggsave(last_plot(), file="plots/mg_curves.jpg", width = 8, height = 6, units = "cm", scale
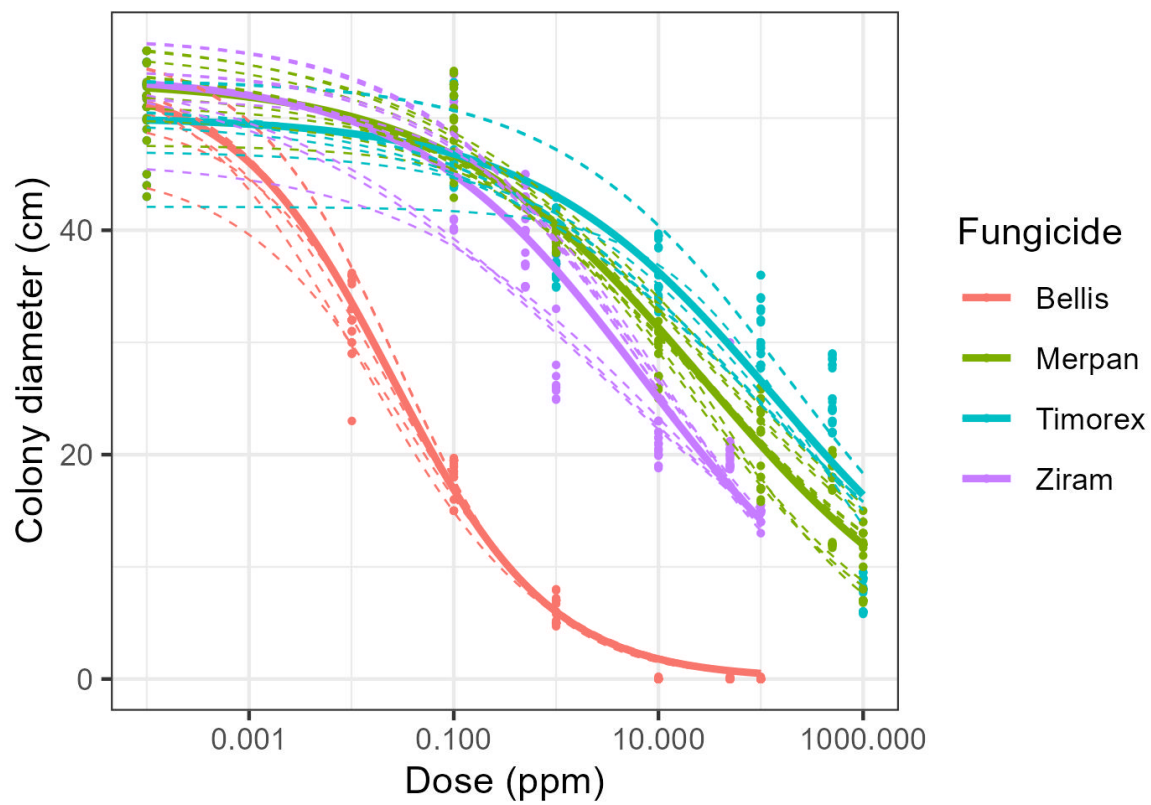```

Figure 1.1: Mycelial growth in function of increasing concentration of fungicides

## 1.2 Spore germination

```
germi_raw <- import("data/germination.csv", dec=",")
germi_dat <- germi_raw %>%
  mutate_at(vars(dose), as.numeric) %>%
  mutate_at(vars(fungicide, strain, repetition, replicate), as.factor) %>%
  mutate(curve_id = interaction(fungicide:strain:repetition)) %>%
  mutate(dose_ = dose+0.0001)
```

Data structure

```
ftable(xtabs(complete.cases(germination_percent)~fungicide+dose+strain , data=germi_dat))
```

fungicide	dose	strain	S20	S23	S8
Bellis	0		6	6	6
	0.1		6	6	6
	1		6	6	6
	10		6	6	6
	50		6	6	6
	100		6	6	6
	500		0	0	0
	1000		0	0	0
Merpan	0		6	6	6
	0.1		6	6	6
	1		6	6	6
	10		6	6	6
	50		0	0	0
	100		6	6	6
	500		6	6	6
	1000		6	6	6
Timorex	0		6	6	6
	0.1		6	6	6
	1		6	6	6
	10		6	6	6
	50		0	0	0
	100		6	6	6
	500		6	6	6
	1000		6	6	6
Ziram	0		6	6	6
	0.1		6	6	6

```
        1              6   6   6
        10             6   6   6
        50             6   6   6
        100            6   6   6
        500            0   0   0
        1000           0   0   0
```

```r
str(germi_dat)
```

```
'data.frame':    468 obs. of  12 variables:
 $ fungicide            : Factor w/ 4 levels "Bellis","Merpan",..: 1 1 1 1 1 1 1 1 1 1 ...
 $ strain               : Factor w/ 3 levels "S20","S23","S8": 2 2 2 1 1 1 3 3 3 2 ...
 $ repetition           : Factor w/ 2 levels "1","2": 1 1 1 1 1 1 1 1 1 1 ...
 $ replicate            : Factor w/ 3 levels "1","2","3": 1 2 3 1 2 3 1 2 3 1 ...
 $ dose                 : num  100 100 100 100 100 100 100 100 100 50 ...
 $ germinated_conidia   : int  0 0 0 0 0 0 0 0 0 0 ...
 $ total_conida         : int  100 100 100 100 100 100 100 100 100 100 ...
 $ non_germinated_conidia: int  100 100 100 100 100 100 100 100 100 100 ...
 $ inhibition_perc      : int  100 100 100 100 100 100 100 100 100 100 ...
 $ germination_percent  : int  0 0 0 0 0 0 0 0 0 0 ...
 $ curve_id             : Factor w/ 24 levels "Bellis:S20:1",..: 3 3 3 1 1 1 5 5 5 3 ...
 $ dose_                : num  100 100 100 100 100 ...
```

Dose-response curves fitting by meta-analysis approach

```r
mod_germ <- metadrm(germination_percent ~ dose_,
              data=germi_dat,
              fct=LL.3(),
              ind=curve_id,
              cid2=fungicide,
              struct="UN")
save(mod_germ, file= "models/invitro_germ.rds")
```

```r
load("models/invitro_germ.rds")
summary(mod_germ)
```

```
Two-stage meta-analysis dose-response model
Model fitted: Log-logistic (ED50 as parameter) with lower limit at 0
```

```
Call:
metadrm(formula = germination_percent ~ dose_, fct = LL.3(),
    ind = curve_id, data = germi_dat, cid2 = fungicide, struct = "UN")

Variance estimates:
          estim    sqrt
tau^2.1   0.0024   0.0494
tau^2.2   0.0063   0.0792
tau^2.3   0.0001   0.0090


              rho.b:(I  rho.d:(I  rho.e:(I
b:(Intercept)        1    1.0000    1.0000
d:(Intercept)   1.0000         1    1.0000
e:(Intercept)   1.0000    1.0000         1


Coefficients:
              Estimate      Std.Err   t value              Pr(>|t|)
b:Bellis     1.0898626    0.0854167   12.7594 < 0.00000000000000022 ***
b:Merpan     1.3070417    0.0560275   23.3286 < 0.00000000000000022 ***
b:Timorex    1.0749724    0.1249763    8.6014     0.000000000004650 ***
b:Ziram      0.6145974    0.0415450   14.7935 < 0.00000000000000022 ***
d:Bellis    99.8992681    0.2360299  423.2484 < 0.00000000000000022 ***
d:Merpan    97.9883814    0.5305522  184.6913 < 0.00000000000000022 ***
d:Timorex   97.6597656    1.7236343   56.6592 < 0.00000000000000022 ***
d:Ziram     99.0543330    2.0387322   48.5862 < 0.00000000000000022 ***
e:Bellis     0.0231700    0.0051014    4.5419     0.000027438625743 ***
e:Merpan     1.1355696    0.0241592   47.0036 < 0.00000000000000022 ***
e:Timorex  289.0283519   34.0651922    8.4846     0.000000000007338 ***
e:Ziram      0.1557700    0.0270433    5.7600     0.000000308016186 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

EC50´s Estimates

```
  ec50s_g <- ED(mod_germ, respLev=c(50)) %>% as.data.frame()
```

Estimated effective doses

```
             Estimate  Std. Error
e:Bellis:50    0.0231700   0.0051014
```

```
e:Merpan:50     1.1355696    0.0241592
e:Timorex:50 289.0283519   34.0651922
e:Ziram:50      0.1557700    0.0270433
```

```
ec50s_g
```

```
                 Estimate    Std. Error
e:Bellis:50     0.02317004   0.005101351
e:Merpan:50     1.13556964   0.024159210
e:Timorex:50  289.02835194  34.065192159
e:Ziram:50      0.15577000   0.027043327
```

```
germ_comp <- EDcomp(mod_germ,
        percVec=c(50),
        percMat=rbind(c(1,1,1,1)),
        interval="fieller")  %>%
    data.frame %>%
  rownames_to_column("comp") %>%
  rowwise() %>%
  mutate(relative_to_one = f(Lower, Upper, 1))
```

```
Estimated ratios of effect doses

                            Estimate             Lower              Upper
Bellis/Merpan:50/50        0.020403889        0.011404798        0.029477018
Bellis/Timorex:50/50       0.000080165        0.000043407        0.000126360
Bellis/Ziram:50/50         0.148745178        0.077874246        0.260413050
Merpan/Timorex:50/50       0.003928921        0.003164387        0.005155910
Merpan/Ziram:50/50         7.290040642        5.392013944       11.187536191
Timorex/Ziram:50/50     1855.481470394     1241.326870777     2978.546457196
```

```
germ_comp
```

```
# A tibble: 6 x 5
# Rowwise:
  comp                   Estimate        Lower        Upper relative_to_one
  <chr>                     <dbl>        <dbl>        <dbl> <chr>
1 Bellis/Merpan:50/50      0.0204       0.0114       0.0295 below
```

```
2 Bellis/Timorex:50/50    0.0000802    0.0000434    0.000126 below
3 Bellis/Ziram:50/50      0.149        0.0779       0.260    below
4 Merpan/Timorex:50/50    0.00393      0.00316      0.00516  below
5 Merpan/Ziram:50/50      7.29         5.39         11.2     above
6 Timorex/Ziram:50/50  1855.        1241.        2979.       above
```

```r
  coef_mod_germ <- summary(mod_germ) %>% data.frame %>%
      rownames_to_column("param")  %>%
    separate(param, c("param", "fungicide"))
```

```
Two-stage meta-analysis dose-response model
Model fitted: Log-logistic (ED50 as parameter) with lower limit at 0

Call:
metadrm(formula = germination_percent ~ dose_, fct = LL.3(),
    ind = curve_id, data = germi_dat, cid2 = fungicide, struct = "UN")

Variance estimates:
            estim    sqrt
tau^2.1    0.0024  0.0494
tau^2.2    0.0063  0.0792
tau^2.3    0.0001  0.0090

               rho.b:(I  rho.d:(I  rho.e:(I
b:(Intercept)         1    1.0000    1.0000
d:(Intercept)    1.0000         1    1.0000
e:(Intercept)    1.0000    1.0000         1


Coefficients:
              Estimate     Std.Err   t value             Pr(>|t|)
b:Bellis     1.0898626   0.0854167   12.7594 < 0.00000000000000022 ***
b:Merpan     1.3070417   0.0560275   23.3286 < 0.00000000000000022 ***
b:Timorex    1.0749724   0.1249763    8.6014     0.000000000004650 ***
b:Ziram      0.6145974   0.0415450   14.7935 < 0.00000000000000022 ***
d:Bellis    99.8992681   0.2360299  423.2484 < 0.00000000000000022 ***
d:Merpan    97.9883814   0.5305522  184.6913 < 0.00000000000000022 ***
d:Timorex   97.6597656   1.7236343   56.6592 < 0.00000000000000022 ***
d:Ziram     99.0543330   2.0387322   48.5862 < 0.00000000000000022 ***
e:Bellis     0.0231700   0.0051014    4.5419     0.000027438625743 ***
```

```
e:Merpan      1.1355696    0.0241592   47.0036 < 0.00000000000000022 ***
e:Timorex 289.0283519   34.0651922    8.4846      0.000000000007338 ***
e:Ziram       0.1557700    0.0270433    5.7600      0.000000308016186 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
# ec50_germ <- coef_mod_germ %>% filter(param=="e")
# ec50_germ
```

```r
germ_comp %>%
  ggplot()+
  aes(x=comp, y=Estimate) +
  geom_pointrange(aes(ymin = Lower, ymax = Upper, col=relative_to_one))+
  geom_hline(yintercept = 1, linetype=2)+
  coord_flip() +
  labs(col="Relative to 1")
```

```r
germi_dat %>%
  ggplot()+
  aes(x=dose+0.0001, y=germination_percent, col=fungicide) +
  scale_x_log10() +
  geom_point(size=1) +
  geom_smooth(method = drm,
              method.args = list(fct = L.3()), se = F) +
  geom_smooth(aes(group=curve_id), size=.3, linetype=2,
              method = drm,
              method.args = list(fct = L.3()), se = F) +
  labs(title= "", x = "Dose (ppm)",  y = "Germination (%)", col= "Fungicide")  +
  theme_bw(base_family=12)
```

```r
ggsave(last_plot(), file="plots/germi_curves.jpg", width = 8, height = 6, units = "cm", sc
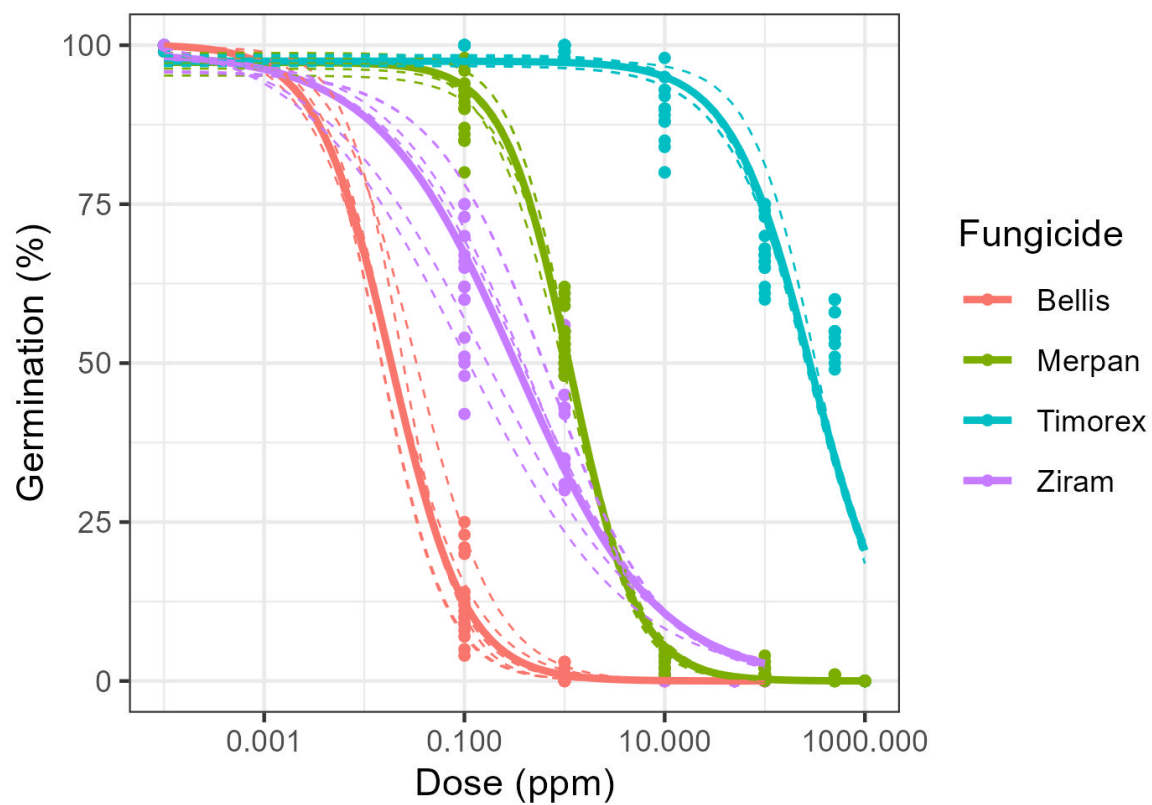```

Figure 1.2: Inhibition of conidial germination

# 2 Bioassays in fruit

```r
source(here::here("setup.R"))
```

```
[conflicted] Will prefer dplyr::filter over any other package.
[conflicted] Will prefer dplyr::select over any other package.
```

```r
library(lme4)
library(glmmTMB)
library(DHARMa)
```

```
This is DHARMa 0.4.6. For overview type '?DHARMa'. For recent changes, type news(package = '
```

```r
theme_set(theme_bw(base_size=12))
```

```r
dat <- rio::import("data/bioassay_data.csv") %>%
  mutate_at(vars(fungicide, repetition, day, replicate, fruit), as.factor) %>%
  mutate(fungicide=fct_relevel(fungicide, "control"))
```

Data structure

```r
ftable(xtabs(~ fungicide + day + repetition + replicate, dat))
```

```
                        replicate 1 2 3 4
fungicide day repetition
control    1   1                  5 5 5 5
               2                  5 5 5 5
           7   1                  5 5 5 5
               2                  5 5 5 5
           15  1                  5 5 5 5
               2                  5 5 5 5
Bellis     1   1                  5 5 5 5
```

```
                    2                    5 5 5 5
            7    1                        5 5 5 5
                 2                        5 5 5 5
           15    1                        5 5 5 5
                 2                        5 5 5 5
Merpan      1    1                        5 5 5 5
                 2                        5 5 5 5
            7    1                        5 5 5 5
                 2                        5 5 5 5
           15    1                        5 5 5 5
                 2                        5 5 5 5
Timorex     1    1                        5 5 5 5
                 2                        5 5 5 5
            7    1                        5 5 5 5
                 2                        5 5 5 5
           15    1                        5 5 5 5
                 2                        5 5 5 5
Ziram       1    1                        5 5 5 5
                 2                        5 5 5 5
            7    1                        5 5 5 5
                 2                        5 5 5 5
           15    1                        5 5 5 5
                 2                        5 5 5 5
```

```r
dat %>% str
```

```
'data.frame':    600 obs. of  6 variables:
 $ fungicide     : Factor w/ 5 levels "control","Bellis",..: 2 2 2 2 2 2 2 2 2 2 ...
 $ repetition    : Factor w/ 2 levels "1","2": 1 1 1 1 1 1 1 1 1 1 1 1 ...
 $ day           : Factor w/ 3 levels "1","7","15": 1 1 1 1 1 1 1 1 1 1 1 ...
 $ replicate     : Factor w/ 4 levels "1","2","3","4": 1 1 1 1 1 2 2 2 2 2 ...
 $ fruit         : Factor w/ 5 levels "1","2","3","4",..: 1 2 3 4 5 1 2 3 4 5 ...
 $ spots_per_fruit: int  1 0 0 0 0 0 0 0 0 0 ...
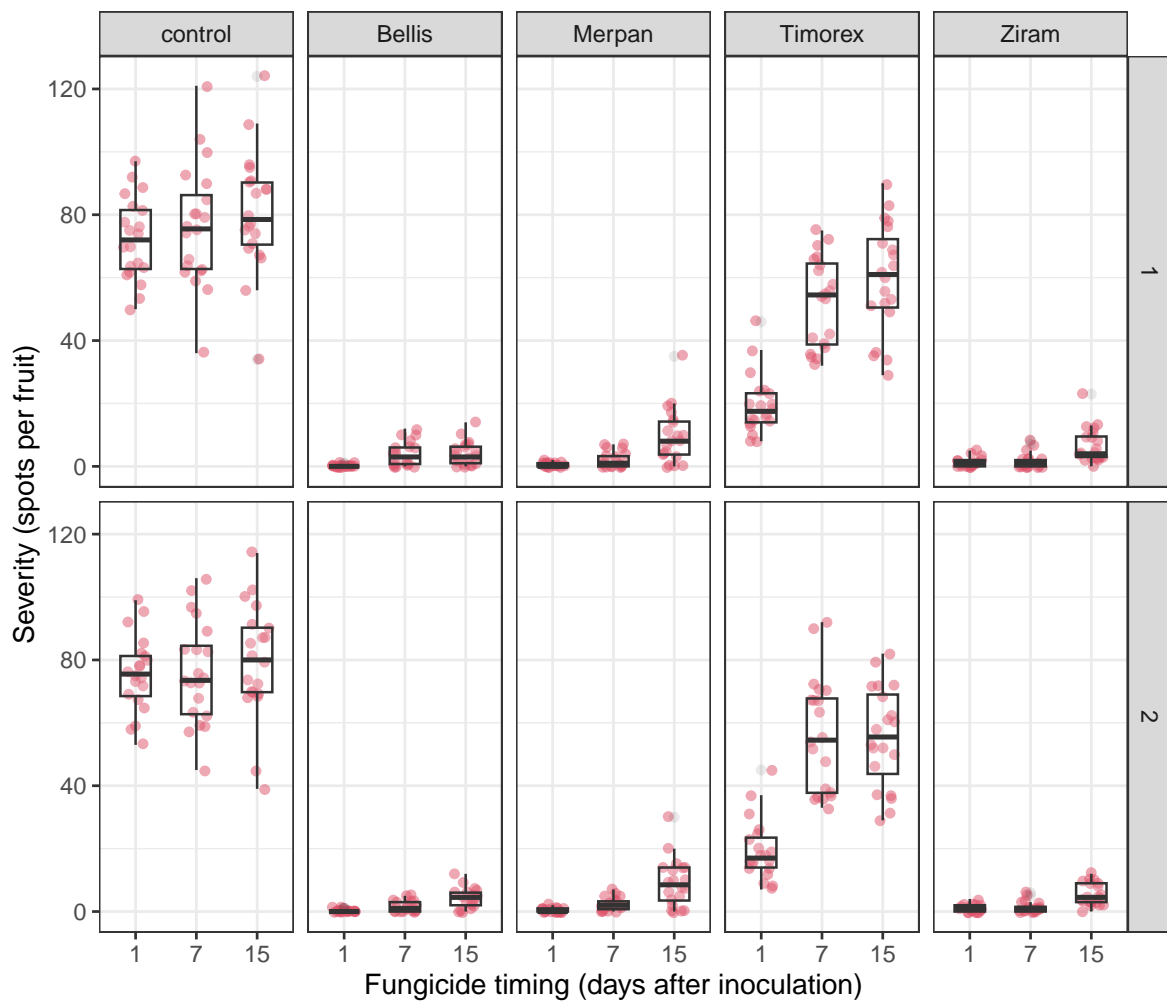```

## 2.1 Preventive treatments

Disease severity (spots per fruit)

```r
dat %>%
  ggplot() +
```

```
aes(x=day, y=spots_per_fruit) +
geom_jitter(width=.2, col=2, alpha=.5) +
geom_boxplot(width=.5, alpha=.1) +
labs(x="Fungicide timing (days after inoculation)",
     y="Severity (spots per fruit)") +
facet_grid(repetition~fungicide)
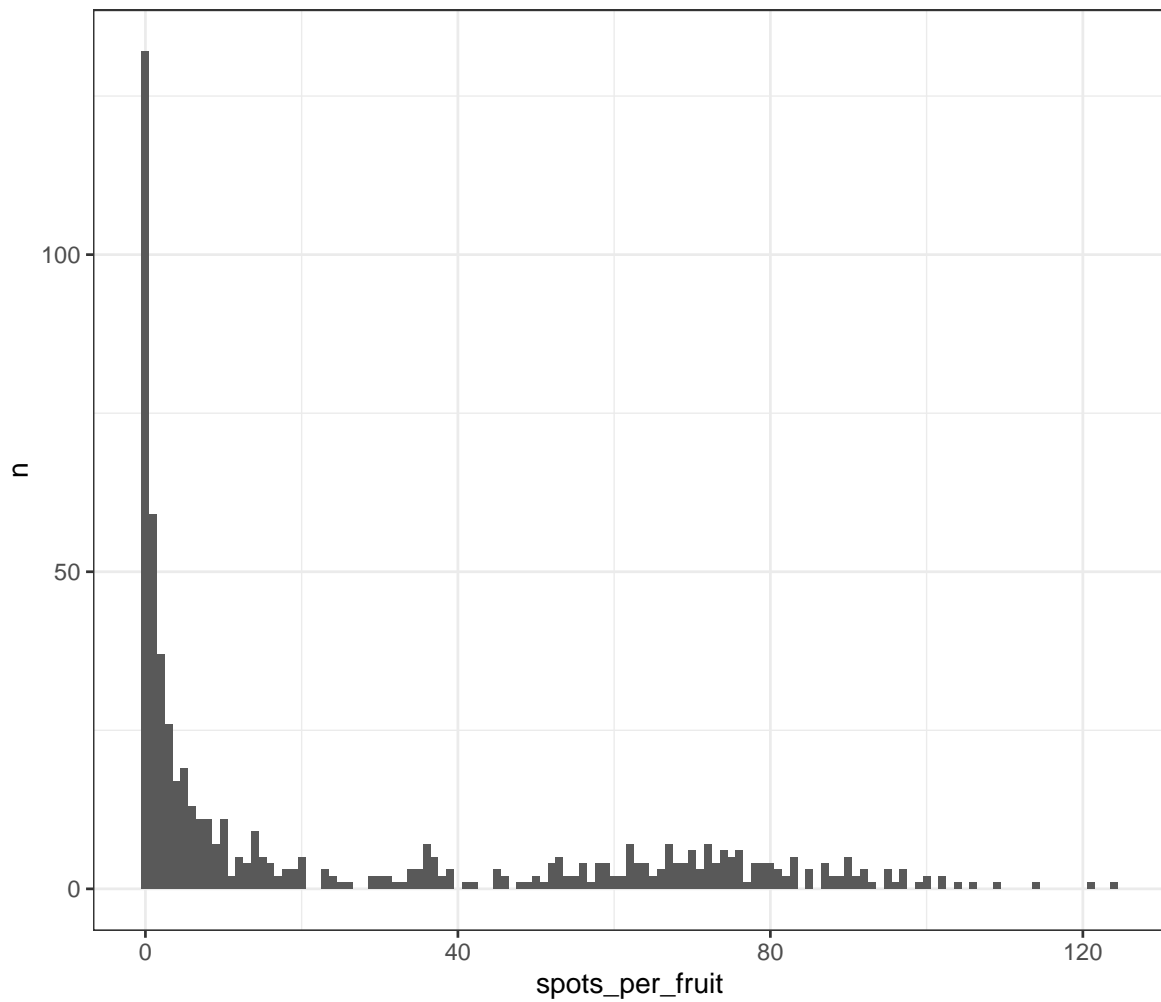```



```
dat %>%
  count(spots_per_fruit) %>%
  ggplot() +
  aes(x=spots_per_fruit, y=n) +
```

```
geom_col()
```


Model fitting

```
# https://glmmtmb.github.io/glmmTMB/articles/glmmTMB.pdf
fit_zipoisson_prev <- glmmTMB(spots_per_fruit~fungicide*day +
                          (1|repetition/replicate),
             ziformula = ~1, family = poisson,
          dat)
```

```
fit_zinbinom_prev <- update(fit_zipoisson_prev,family=nbinom2)
fit_zinbinom1_prev <- update(fit_zipoisson_prev,family=nbinom1)
fit_twediee_prev <- update(fit_zipoisson_prev,family=tweedie)
AIC(fit_zipoisson_prev,fit_zinbinom_prev,fit_zinbinom1_prev, fit_twediee_prev)
```

```
                   df      AIC
fit_zipoisson_prev 18 3890.310
fit_zinbinom_prev  19 3486.965
fit_zinbinom1_prev 19 3419.425
fit_twediee_prev   20 3405.244
```

Goodness of fit

```
DHARMa::testOverdispersion(fit_twediee_prev)
DHARMa::testSimulatedResiduals(fit_twediee_prev)
# simulateResiduals(fit_twediee_prev) %>% testResiduals()
```

```
car::Anova(fit_twediee_prev)
```

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

Response: spots_per_fruit
                Chisq Df          Pr(>Chisq)
fungicide     2826.15  4 < 0.00000000000000022 ***
day            148.41  2 < 0.00000000000000022 ***
fungicide:day  327.88  8 < 0.00000000000000022 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Treatment means comparison

```
emm_prev <- emmeans(fit_twediee_prev, ~ fungicide|day, type="response")
res_prev <- cld(emm_prev, alpha=0.05, Letters=letters,  type="response")
res_prev %>%
   mutate(`%Control`=abs((response/filter(.,fungicide=="control")%>% pull(response)-1)*100
```

```
  fungicide day  response        SE  df  asymp.LCL asymp.UCL .group
2    Bellis   1 0.1266087 0.06435246 Inf 0.04675374 0.3428553     a
3    Merpan   1 0.5055717 0.14652770 Inf 0.28647233 0.8922425    ab
```

```
5       Ziram   1  1.1179829 0.23580319 Inf  0.73943685  1.6903213    b
4      Timorex  1 19.4414909 1.33246605 Inf 16.99771586 22.2366094     c
1      control  1 73.8540017 3.22802344 Inf 67.79061416 80.4597161      d
10      Ziram   7  1.4931835 0.28182958 Inf  1.03146361  2.1615857   a
8      Merpan   7  2.3203471 0.36915475 Inf  1.69875745  3.1693817   a
7      Bellis   7  2.7964637 0.41667960 Inf  2.08822925  3.7448997   a
9      Timorex  7 54.1858706 2.60846796 Inf 49.30714307 59.5473271    b
6      control  7 75.9541403 3.29232755 Inf 69.76780397 82.6890213     c
12      Bellis  15  4.3163835 0.54531743 Inf  3.36962889  5.5291449  a
15      Ziram   15  6.1277829 0.66068554 Inf  4.96053467  7.5696928  a
13      Merpan  15 10.2948040 1.26533588 Inf  8.09089887 13.0990376   b
14     Timorex  15 57.5035175 2.71593663 Inf 52.41933283 63.0808205    c
11     control  15 79.8718272 3.41023449 Inf 73.45991670 86.8433980     d
    %Control
2  99.828569
3  99.334372
5  98.600279
4  73.675779
1   2.765009
10 98.130525
8  96.858197
7  96.318221
9  32.158970
6   2.843635
12 94.317119
15 92.327980
13 86.060601
14 24.291793
11  0.000000
```

```
res_prev %>%
  ggplot()+
  aes(x=day, y =response)+
  geom_pointrange(aes(ymin=asymp.LCL , ymax=asymp.UCL))+
  geom_col(alpha=.2, width=.2)+
  facet_wrap("fungicide")+
    labs(x="Fungicide timing (days before inoculation)",
         y="Spots per fruit (counts)")
```
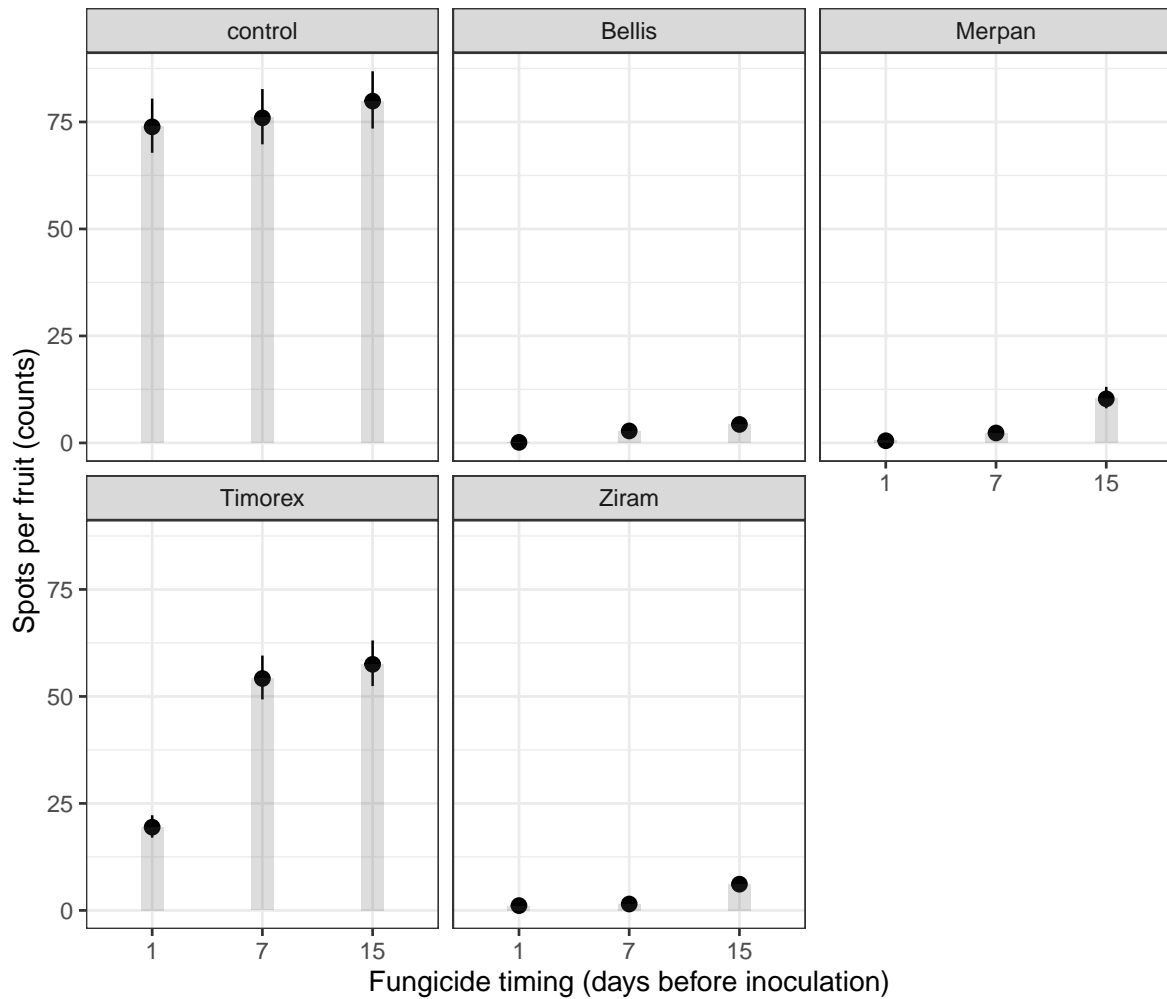
2.2 Curative treatments

```
cura <- rio::import("data/curative.csv") %>%
    mutate_at(vars(fungicide, repetition, replicate, fruit), as.factor) %>%
  mutate(fungicide=fct_relevel(fungicide, "Control"))
```

Data structure

```
ftable(xtabs(~ fungicide + repetition + replicate, cura))
```

```
                replicate 1 2 3 4
```

```
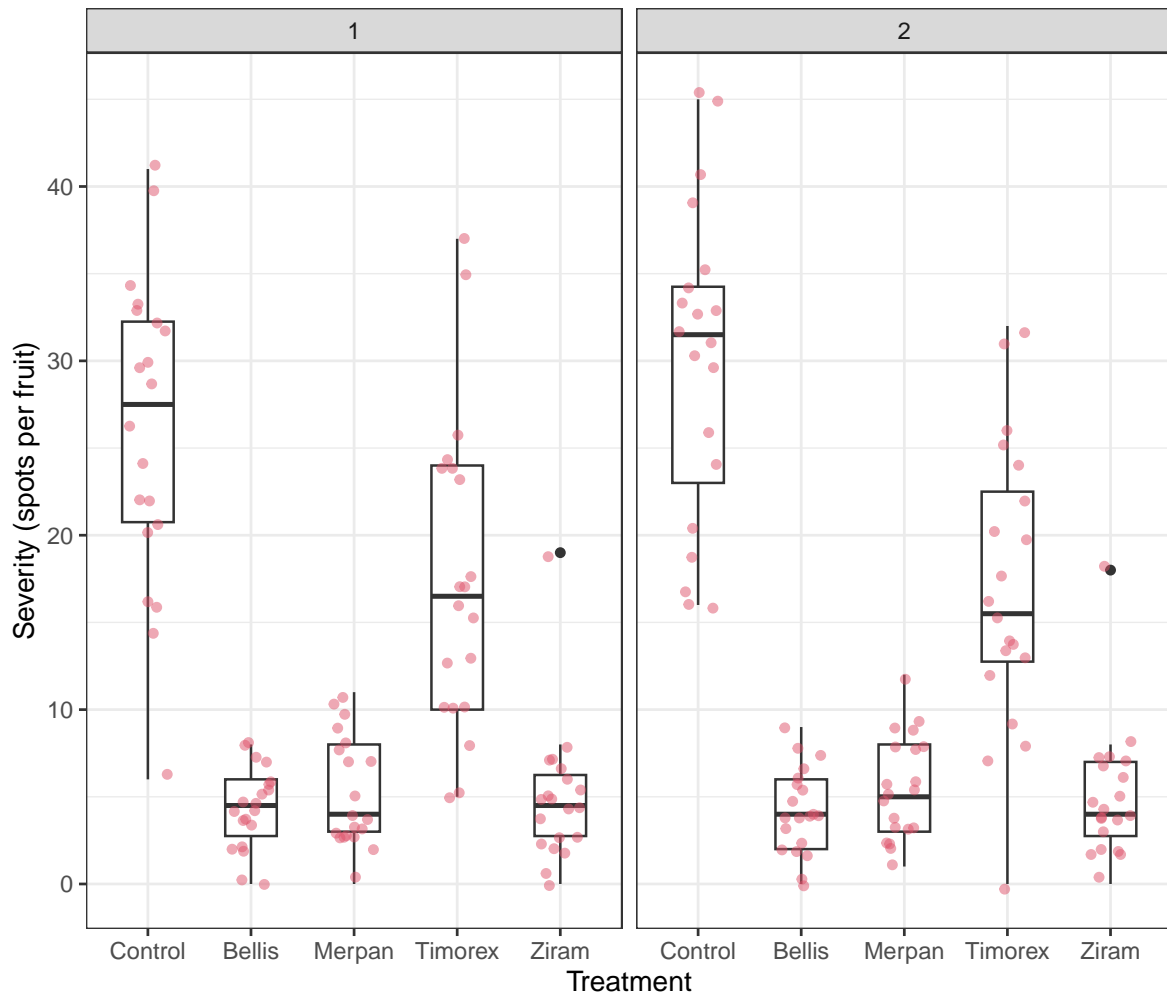fungicide repetition
Control   1                    5 5 5 5
          2                    5 5 5 5
Bellis    1                    5 5 5 5
          2                    5 5 5 5
Merpan    1                    5 5 5 5
          2                    5 5 5 5
Timorex   1                    5 5 5 5
          2                    5 5 5 5
Ziram     1                    5 5 5 5
          2                    5 5 5 5
```

```
cura %>% str
```

```
'data.frame':   200 obs. of  5 variables:
 $ fungicide     : Factor w/ 5 levels "Control","Bellis",..: 1 1 1 1 1 1 1 1 1 1 ...
 $ repetition    : Factor w/ 2 levels "1","2": 1 1 1 1 1 1 1 1 1 1 ...
 $ replicate     : Factor w/ 4 levels "1","2","3","4": 1 1 1 1 1 2 2 2 2 2 ...
 $ fruit         : Factor w/ 5 levels "1","2","3","4",..: 1 2 3 4 5 1 2 3 4 5 ...
 $ spots_per_fruit: int  33 32 40 22 16 16 6 14 41 30 ...
```

Disease severity (spots per fruit)

```
cura %>%
  ggplot() +
  aes(x=fungicide, y=spots_per_fruit) +
  geom_boxplot(width=.5) +
  geom_jitter(width=.2, col=2, alpha=.5) +
  labs(x="Treatment", y="Severity (spots per fruit)") +
  facet_wrap("repetition")
```

Model fitting

```
# https://glmmtmb.github.io/glmmTMB/articles/glmmTMB.pdf
fit_zipoisson_cur <- glmmTMB(spots_per_fruit~fungicide +
                                (1|repetition/replicate),
                             ziformula = ~1,
                             family = poisson,data = cura)


fit_zinbinom_cur <- update(fit_zipoisson_cur,family=nbinom2)
fit_zinbinom1_cur <- update(fit_zipoisson_cur,family=nbinom1)
fit_twediee_cur <- update(fit_zipoisson_cur,family=tweedie)
AIC(fit_zipoisson_cur,fit_zinbinom_cur,fit_zinbinom1_cur, fit_twediee_cur)
```

```
                df      AIC
fit_zipoisson_cur   8 1264.879
fit_zinbinom_cur    9 1176.775
fit_zinbinom1_cur   9 1184.694
fit_twediee_cur    10 1179.372
```

```
  #              df      AIC
  # fit_zipoisson  8 1270.421
  # fit_zinbinom   9 1176.582
  # fit_zinbinom1  9 1182.421
  # fit_twediee   10 1180.572
```

Goodness of fit

```
  simulateResiduals(fit_twediee_cur) %>% testResiduals()
```

```
  car::Anova(fit_twediee_cur)
```

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

Response: spots_per_fruit
          Chisq Df          Pr(>Chisq)
fungicide 533.4  4 < 0.00000000000000022 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Treatment means comparison

```
  emm_cura <- emmeans(fit_twediee_cur, ~ fungicide, type="response")
  res_cura <- cld(emm_cura, alpha=0.05, Letters=letters,  type="response")
  res_cura %>%
      mutate(`%Control`=abs((response/filter(.,fungicide=="Control")%>% pull(response)-1)*10
```

```
  fungicide  response        SE  df asymp.LCL asymp.UCL .group %Control
2    Bellis  4.803382 0.4445747 Inf  4.006495  5.758770      a 82.81883
5     Ziram  5.180715 0.4648248 Inf  4.345283  6.176768      a 81.46916
3    Merpan  5.478331 0.4804890 Inf  4.613089  6.505861      a 80.40462
4   Timorex 17.539752 1.3205836 Inf 15.133372 20.328773      b 37.26226
1   Control 27.957257 1.9647959 Inf 24.359778 32.086015      c  0.00000
```

```
res_cura %>%
  ggplot()+
  aes(x=fungicide, y =response)+
  geom_pointrange(aes(ymin=asymp.LCL , ymax=asymp.UCL))+
  geom_col(alpha=.2, width=.2)
```
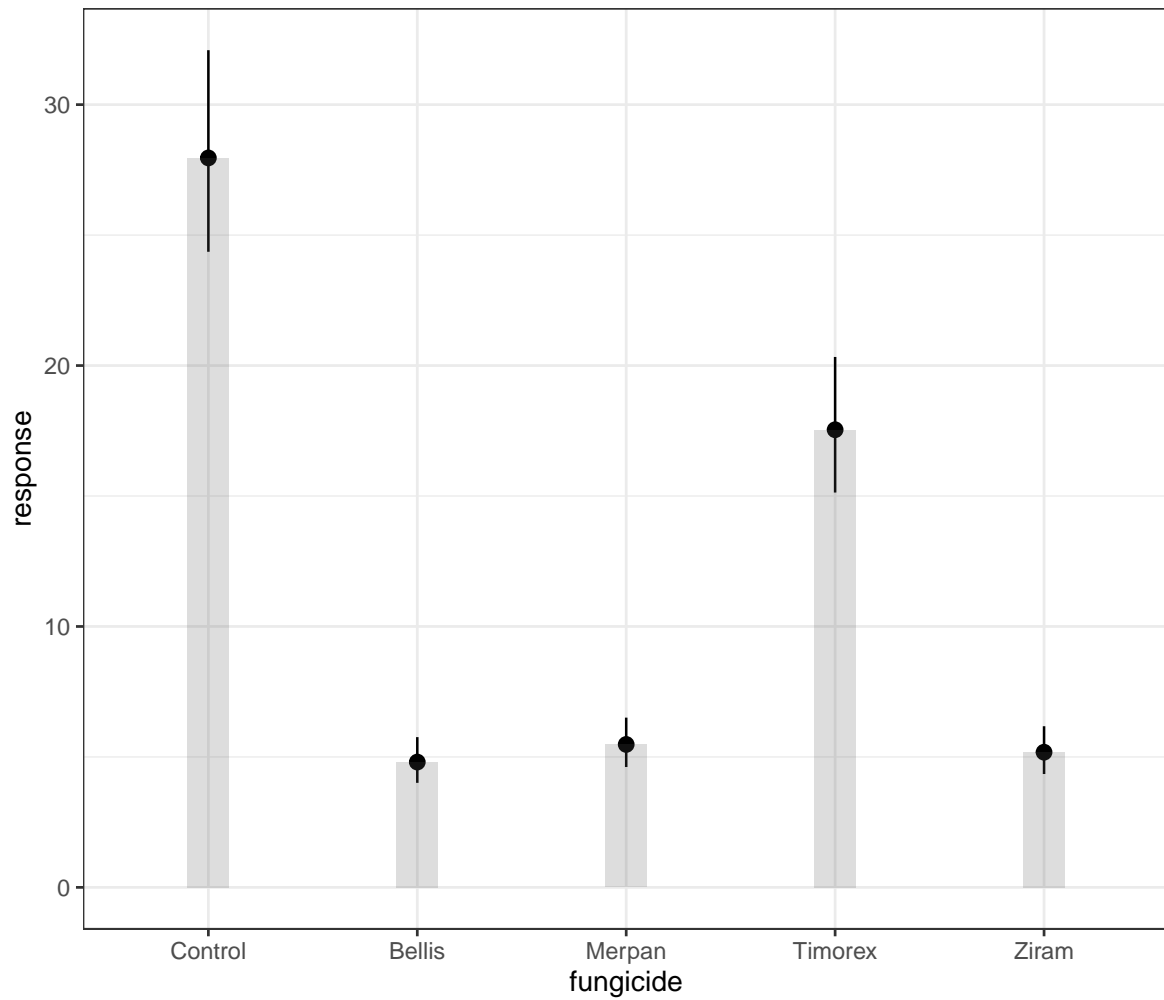
3 Field experiments

```r
source(here::here("setup.R"))
```

[conflicted] Will prefer dplyr::filter over any other package.
[conflicted] Will prefer dplyr::select over any other package.

```r
library(lme4)
theme_set(theme_bw(base_size=12))
```

```r
dat <- rio::import("data/field.csv") %>%
  mutate_at(vars(fungicide, season, tree), as.factor) %>%
  mutate(fungicide=fct_relevel(fungicide, "Control"))
str(dat)
```

```
'data.frame':    60 obs. of  5 variables:
 $ fungicide: Factor w/ 3 levels "Control","Bellis",..: 1 1 1 1 1 1 1 1 1 1 ...
 $ season   : Factor w/ 2 levels "1","2": 2 2 2 2 2 2 2 2 2 2 ...
 $ tree     : Factor w/ 10 levels "1","2","3","4",..: 1 2 3 4 5 6 7 8 9 10 ...
 $ leaves   : int  250 250 250 250 250 250 250 250 250 250 ...
 $ diseased : int  27 19 26 30 16 40 27 22 19 8 ...
```

Data structure

```r
ftable(xtabs(~ fungicide + tree + season, dat))
```

```
              season 1 2
fungicide tree
Control   1          1 1
          2          1 1
          3          1 1
          4          1 1
```

```
           5           1 1
           6           1 1
           7           1 1
           8           1 1
           9           1 1
          10           1 1
Bellis     1           1 1
           2           1 1
           3           1 1
           4           1 1
           5           1 1
           6           1 1
           7           1 1
           8           1 1
           9           1 1
          10           1 1
Ziram      1           1 1
           2           1 1
           3           1 1
           4           1 1
           5           1 1
           6           1 1
           7           1 1
           8           1 1
           9           1 1
          10           1 1
```

Model fitting

```r
dat %>%
  ggplot() +
  aes(x=fungicide, y=diseased/leaves) +
  # geom_boxplot(width=.5) +
  geom_text(aes(label=tree))+
  geom_jitter(width=.2, col=2, alpha=.5) +
  labs(x="Treatment", y="Disease incidence") +
  facet_wrap("season")
```