

Fungicide efficacy In vitro

Actualizado en 2023-03-18

Mycelial growth

```
raw <- rio::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(dose_decimal = format(dose, scientific = FALSE) %>% str_squish()) %>%
  # mutate(dose = as.numeric(dose_decimal)) %>%
  mutate(curve_id = interaction(fungicide:strain:experiment))
```

Data scheme

```
dat %>% str
```

```
'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 ...
```

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

			dose	0	0.01	0.1	0.5	1	10	50	100	500	1000
fungicide	strain	experiment											
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	

```
# per fungicide / strain
dat %>%
```

```

    nest(data = c(dose, colony_diameter)) %>%
    mutate(mod = map(data, ~broom::tidy(drm(colony_diameter~dose, fct = LL.3(), data = .))))
    unnest(c(mod))%>%
    tibble()

# per fungicide
dat %>%
  nest(data = c(-fungicide)) %>%
  mutate(mod = map(data, ~broom::tidy(drm(colony_diameter~dose, fct = LL.3(), data = .))))
  unnest(c(mod)) %>%
  tibble()

```

Meta-analysis approach

```

mod_mg <- metadrm(colony_diameter ~ dose,
                  data=dat,
                  fct=LL.3(),
                  ind=curve_id,
                  cid2=fungicide,
                  struct="UN")
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	0.0477
tau ² .2	8.2157	2.8663
tau ² .3	0.0001	0.0075

	rho.b:(I	rho.d:(I	rho.e:(I
b:(Intercept)	1	-0.0775	0.9101
d:(Intercept)	-0.0775	1	-0.4838
e:(Intercept)	0.9101	-0.4838	1

Coefficients:

	Estimate	Std.Err	t value	Pr(> t)
b:Bellis	5.7967e-01	1.8145e-02	31.9477	< 2.2e-16 ***
b:Merpan	3.6099e-01	1.8237e-02	19.7947	< 2.2e-16 ***
b:Timorex	3.5094e-01	3.2622e-02	10.7578	< 2.2e-16 ***
b:Ziram	3.9329e-01	2.0151e-02	19.5166	< 2.2e-16 ***
d:Bellis	5.1373e+01	9.9046e-01	51.8674	< 2.2e-16 ***
d:Merpan	5.2517e+01	1.0685e+00	49.1484	< 2.2e-16 ***
d:Timorex	4.9914e+01	1.3982e+00	35.6973	< 2.2e-16 ***
d:Ziram	5.2997e+01	1.1231e+00	47.1867	< 2.2e-16 ***
e:Bellis	2.9753e-02	2.7103e-03	10.9778	< 2.2e-16 ***
e:Merpan	2.4802e+01	1.6697e+00	14.8543	< 2.2e-16 ***
e:Timorex	1.1510e+02	2.2515e+01	5.1119	1.624e-06 ***
e:Ziram	6.6219e+00	6.2870e-01	10.5328	< 2.2e-16 ***

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

Estimates

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

Estimated effective doses

	Estimate	Std. Error
e:Bellis:50	2.9753e-02	2.7103e-03
e:Merpan:50	2.4802e+01	1.6697e+00
e:Timorex:50	1.1510e+02	2.2515e+01
e:Ziram:50	6.6219e+00	6.2870e-01

```
# 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 effective dose levels for meta analysis

```

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

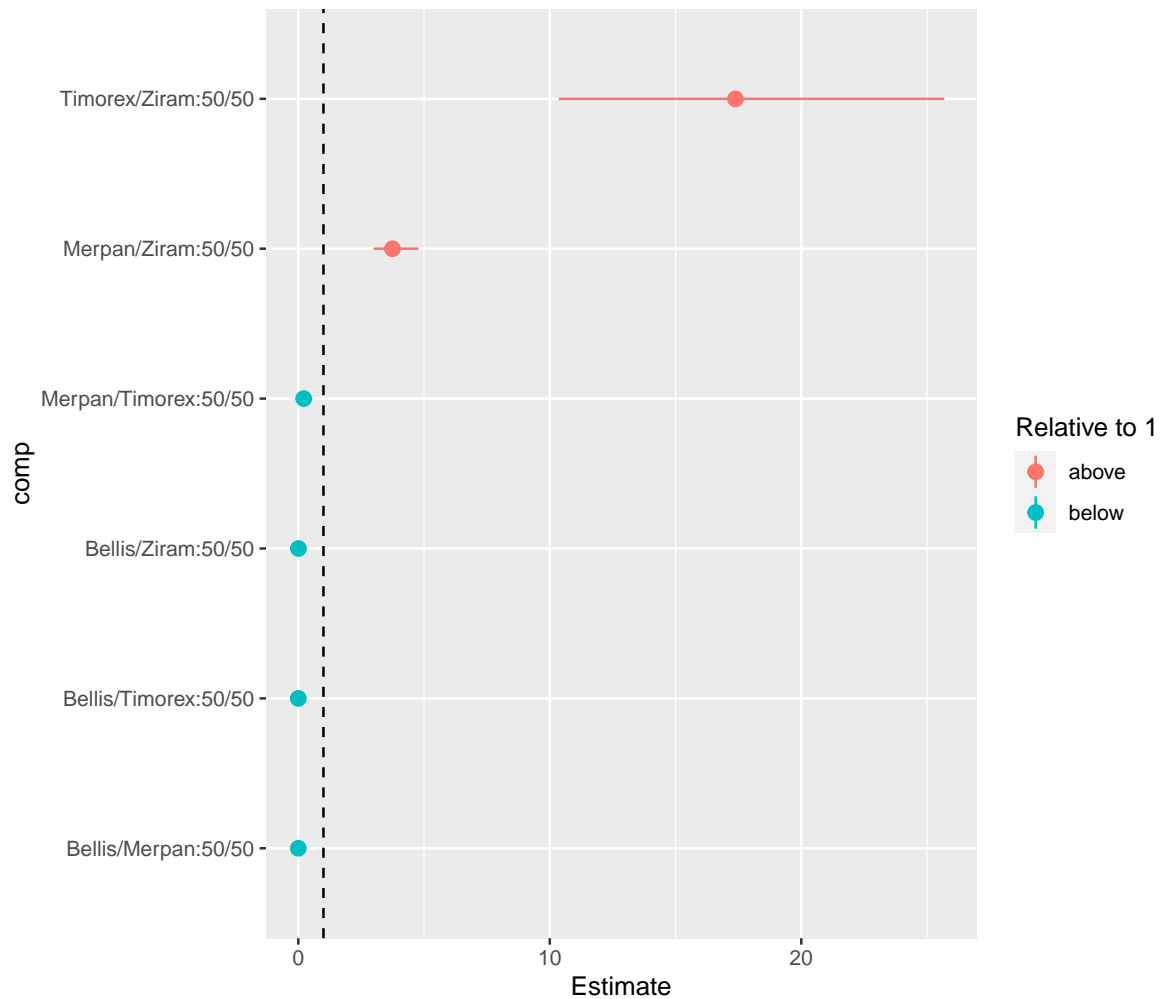
	Estimate	Lower	Upper
Bellis/Merpan:50/50	1.1996e-03	9.4841e-04	1.4945e-03
Bellis/Timorex:50/50	2.5851e-04	1.7578e-04	4.3303e-04
Bellis/Ziram:50/50	4.4931e-03	3.4523e-03	5.8649e-03
Merpan/Timorex:50/50	2.1549e-01	1.5038e-01	3.5711e-01
Merpan/Ziram:50/50	3.7454e+00	2.9915e+00	4.7751e+00
Timorex/Ziram:50/50	1.7381e+01	1.0355e+01	2.5687e+01

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

```

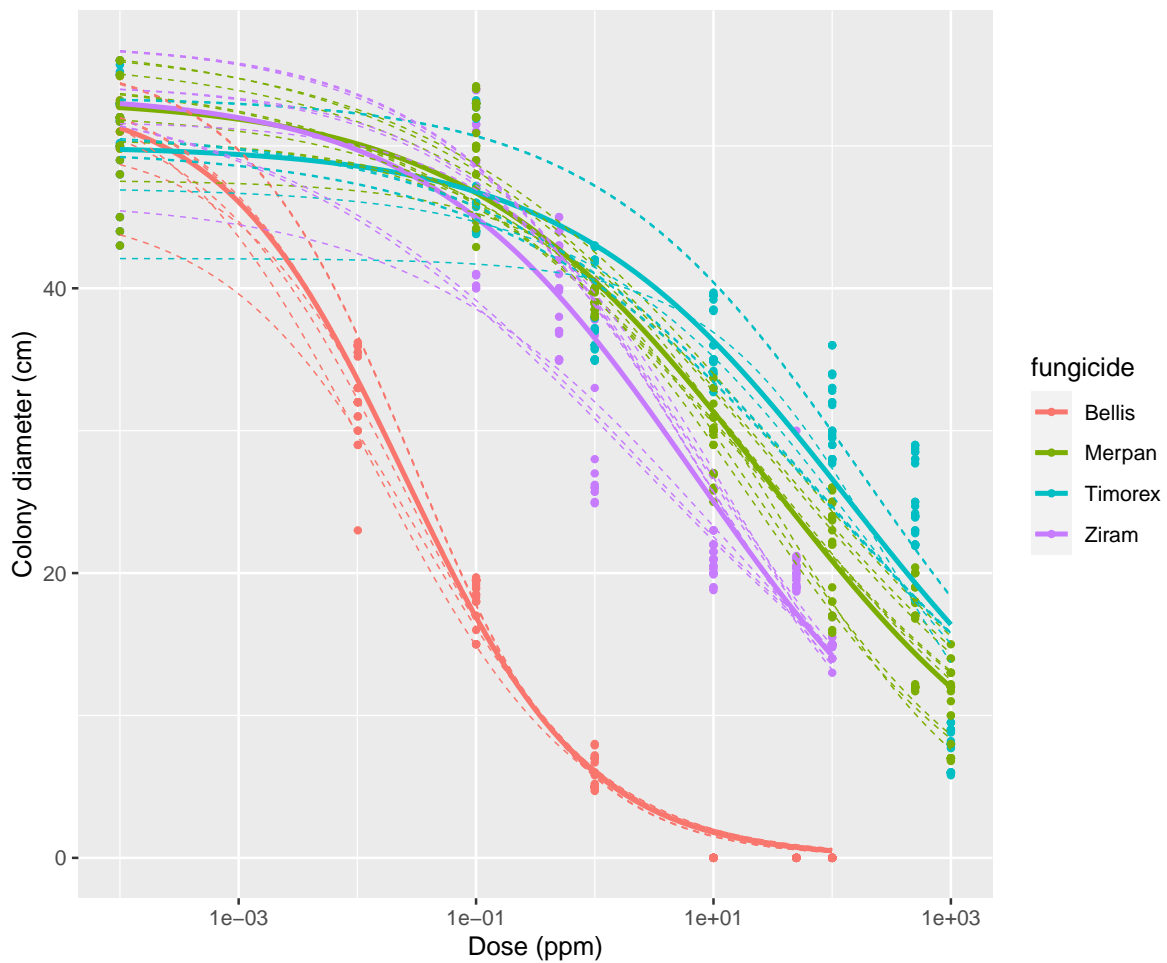
ed_comp %>%
  ggplot()+
  aes(x=comp, y=Estimate) +
  geom_pointrange(aes(ymin = Lower, ymax = Upper, col=relative_to_one))+
  geom_hline(yintercept = 1, linetype=2)+
  # scale_y_log10()+
  coord_flip() +
  labs(col="Relative to 1")

```



Bellis < Merpan < Timorex < Ziram

```
dat %>%
  ggplot()+
  aes(x=dose+0.0001, y=colony_diameter, col=fungicide) +
  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 = "Colony diameter (cm)") +
  scale_x_log10()
```



Spore germination

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

Data scheme

```
germi_dat %>% str
```

```
'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 ...
 $ experiment     : Factor w/ 2 levels "1","2": 1 1 1 1 1 1 1 1 1 1 ...
 $ plate         : 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     : 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 : 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_perc: 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 ...
```

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

	dose	0	0.1	1	10	50	100	500	1000
fungicide									
Bellis		18	18	18	18	18	18	0	0
Merpan		18	18	18	18	0	18	18	18
Timorex		18	18	18	18	0	18	18	18
Ziram		18	18	18	18	18	18	0	0

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

		strain	S20	S23	S8
fungicide	dose				
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

Meta-analysis

```
mod_germ <- metadrm(germination_perc ~ dose_,
  data=germi_dat,
  fct=LL.3(),
  ind=curve_id,
  cid2=fungicide,
  struct="UN")
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_perc ~ 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	1.0899e+00	8.5417e-02	12.7594	< 2.2e-16 ***
b:Merpan	1.3070e+00	5.6028e-02	23.3286	< 2.2e-16 ***
b:Timorex	1.0750e+00	1.2498e-01	8.6014	4.650e-12 ***
b:Ziram	6.1460e-01	4.1545e-02	14.7935	< 2.2e-16 ***
d:Bellis	9.9899e+01	2.3603e-01	423.2484	< 2.2e-16 ***
d:Merpan	9.7988e+01	5.3055e-01	184.6913	< 2.2e-16 ***
d:Timorex	9.7660e+01	1.7236e+00	56.6592	< 2.2e-16 ***
d:Ziram	9.9054e+01	2.0387e+00	48.5862	< 2.2e-16 ***
e:Bellis	2.3170e-02	5.1014e-03	4.5419	2.744e-05 ***
e:Merpan	1.1356e+00	2.4159e-02	47.0036	< 2.2e-16 ***
e:Timorex	2.8903e+02	3.4065e+01	8.4846	7.338e-12 ***
e:Ziram	1.5577e-01	2.7043e-02	5.7600	3.080e-07 ***

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

```
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	2.0404e-02	1.1405e-02	2.9477e-02
Bellis/Timorex:50/50	8.0165e-05	4.3407e-05	1.2636e-04
Bellis/Ziram:50/50	1.4875e-01	7.7874e-02	2.6041e-01
Merpan/Timorex:50/50	3.9289e-03	3.1644e-03	5.1559e-03
Merpan/Ziram:50/50	7.2900e+00	5.3920e+00	1.1188e+01
Timorex/Ziram:50/50	1.8555e+03	1.2413e+03	2.9785e+03

```
# germ_comp
```

```
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_perc ~ 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	1.0899e+00	8.5417e-02	12.7594	< 2.2e-16 ***
b:Merpan	1.3070e+00	5.6028e-02	23.3286	< 2.2e-16 ***

```

b:Timorex 1.0750e+00 1.2498e-01 8.6014 4.650e-12 ***
b:Ziram 6.1460e-01 4.1545e-02 14.7935 < 2.2e-16 ***
d:Bellis 9.9899e+01 2.3603e-01 423.2484 < 2.2e-16 ***
d:Merpan 9.7988e+01 5.3055e-01 184.6913 < 2.2e-16 ***
d:Timorex 9.7660e+01 1.7236e+00 56.6592 < 2.2e-16 ***
d:Ziram 9.9054e+01 2.0387e+00 48.5862 < 2.2e-16 ***
e:Bellis 2.3170e-02 5.1014e-03 4.5419 2.744e-05 ***
e:Merpan 1.1356e+00 2.4159e-02 47.0036 < 2.2e-16 ***
e:Timorex 2.8903e+02 3.4065e+01 8.4846 7.338e-12 ***
e:Ziram 1.5577e-01 2.7043e-02 5.7600 3.080e-07 ***

```

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

```

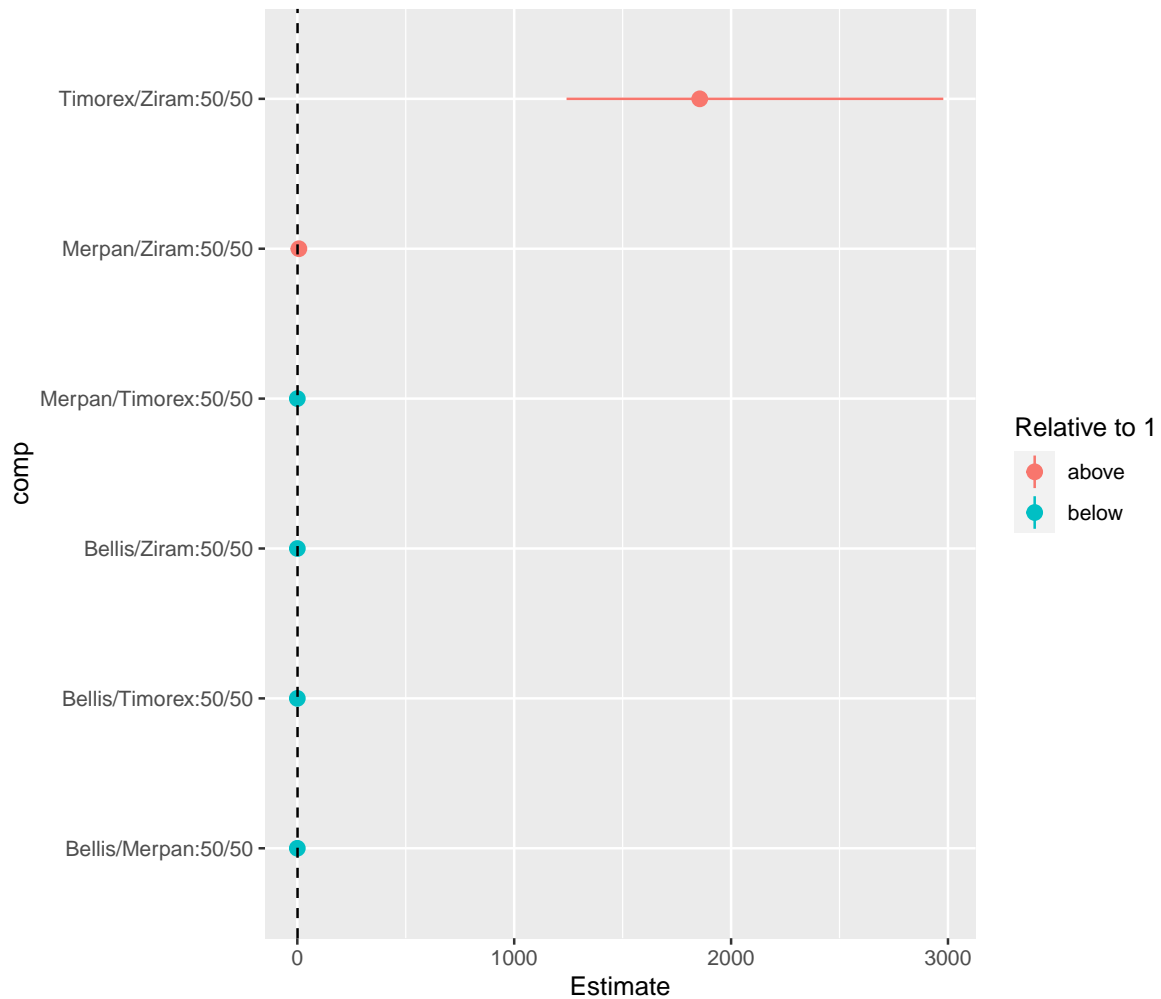
# ec50_germ <- coef_mod_germ %>% filter(param=="e")
# ec50_germ

```

```

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")

```



Bellis < Ziram < Merpan < Timorex

```
germi_dat %>%
  ggplot()+
  aes(x=dose+0.0001, y=germination_perc, 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")
```

