

Bioassays in fruits

2023-04-10

Preventive treatments

Disease severity analysis (spots per fruit)

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

Skipping install of 'drcData' from a github remote, the SHA1 (09f9da30) has not changed since last install.
Use `force = TRUE` to force installation

Skipping install of 'drc' from a github remote, the SHA1 (8719d43a) has not changed since last install.
Use `force = TRUE` to force installation

Skipping install of 'medrc' from a github remote, the SHA1 (bc36df51) has not changed since last install.
Use `force = TRUE` to force installation

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

```
library(lme4)  
library(glmmTMB)  
library(DHARMA)
```

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

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

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

```
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 ...
 $ day           : Factor w/ 3 levels "1","7","15": 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 ...
```

Data scheme

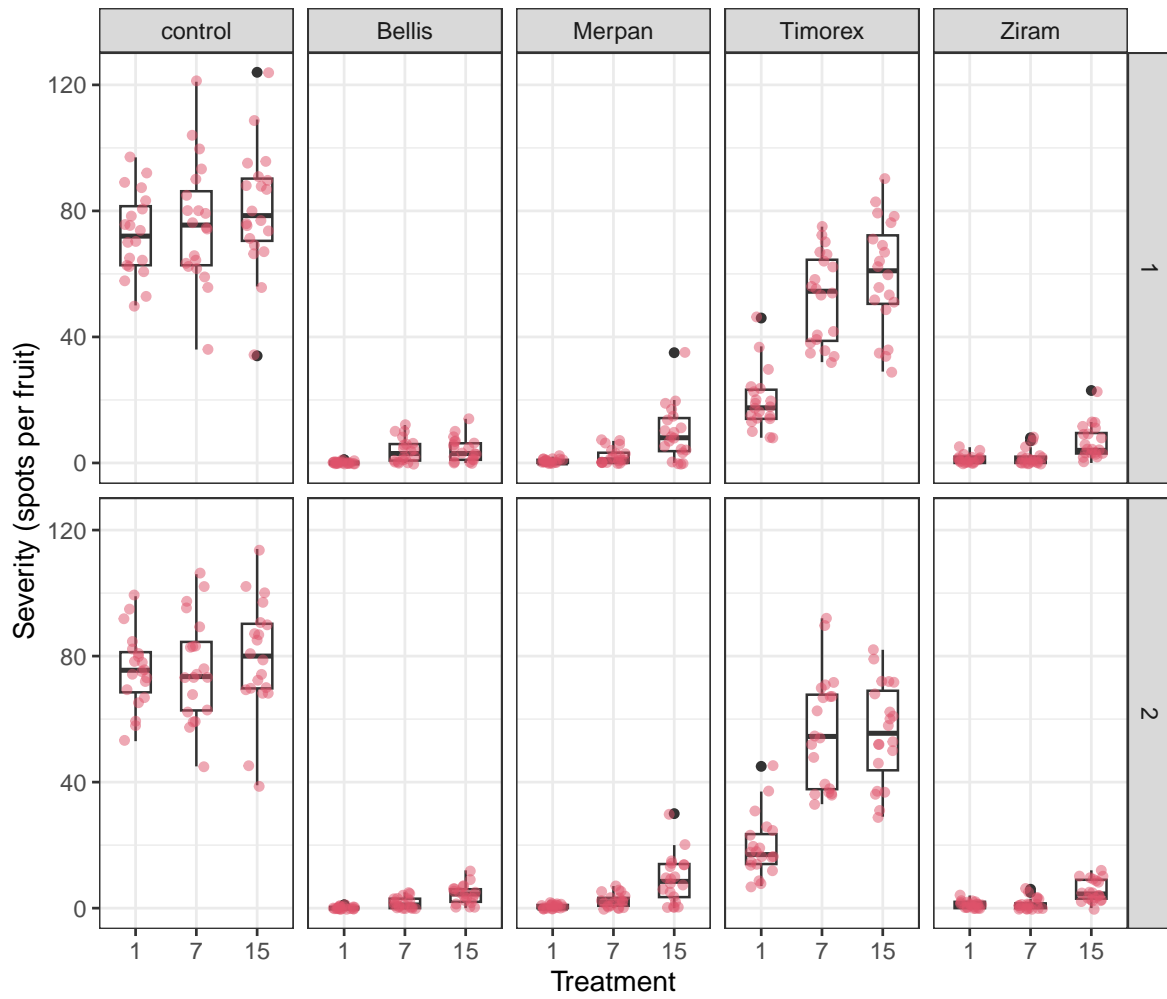
```
fctable(xtabs(~ fungicide + day + repetition + replicate, dat))
```

			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

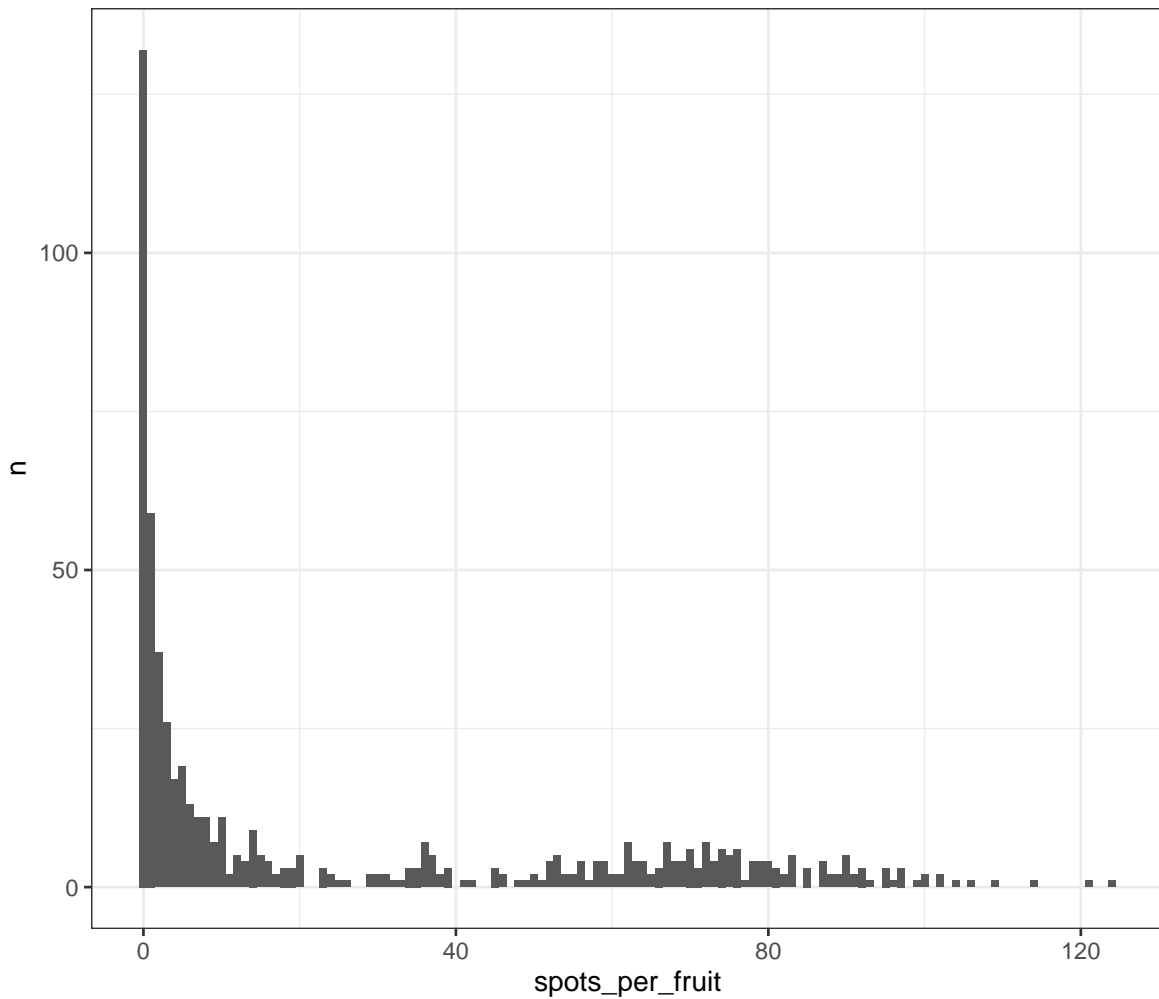
	7	1		5	5	5	5
		2		5	5	5	5
	15	1		5	5	5	5
		2		5	5	5	5
Ziram	1	1		5	5	5	5
		2		5	5	5	5
	7	1		5	5	5	5
		2		5	5	5	5
	15	1		5	5	5	5
		2		5	5	5	5

Disease severity

```
dat %>%
  ggplot() +
  aes(x=day, 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_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)
# performance::check_overdispersion(fit_zipoisson)
# simulateResiduals(fit_zipoisson) %>% testResiduals()
```

```

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
18	3.89e+03
19	3.49e+03
19	3.42e+03
20	3.41e+03

```

#           df      AIC
# fit_zipoisson 18 3895.200
# fit_zinbinom  19 3489.228
# fit_zinbinom1 19 3418.201
# fit_twediee   20 3405.606

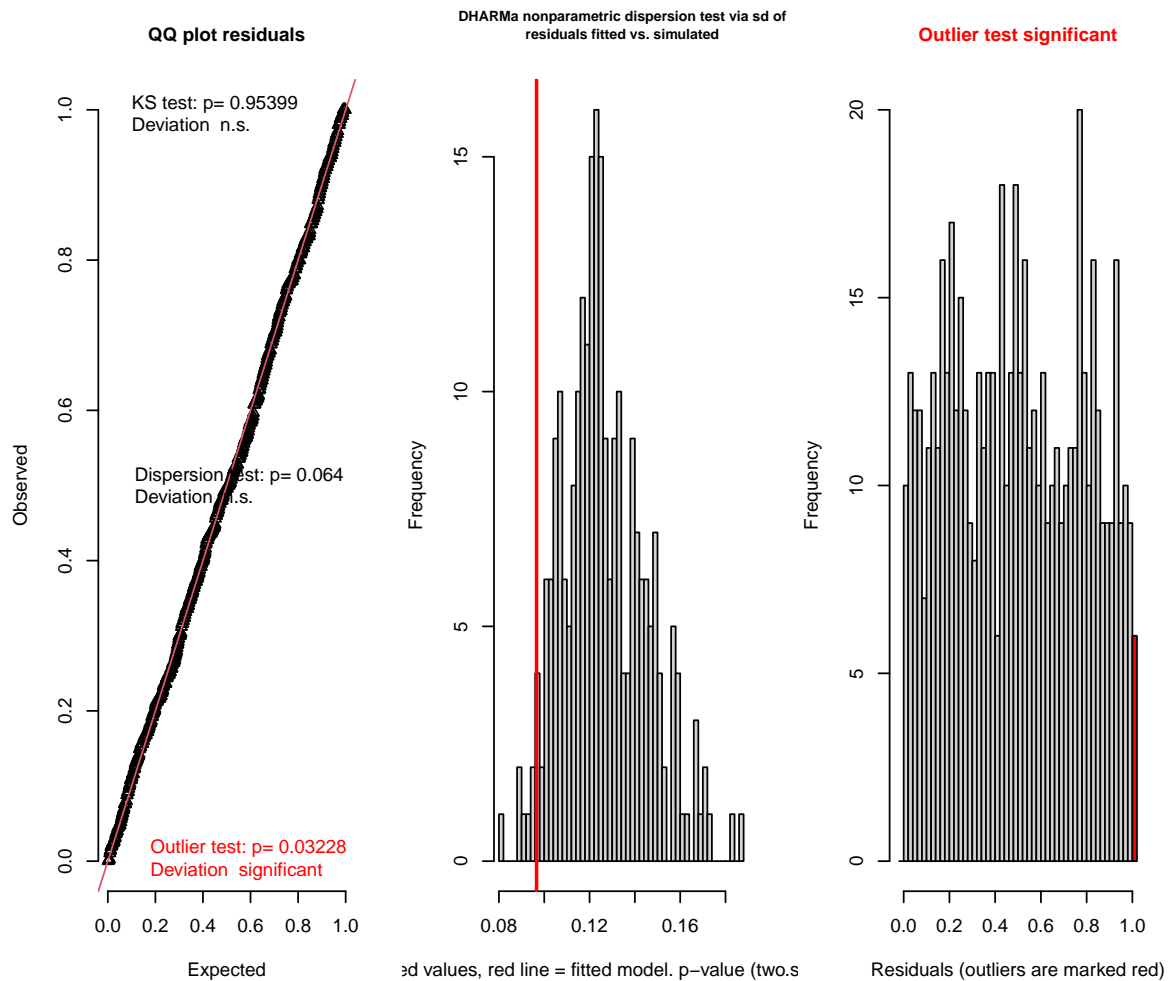
```

Goodness of fit

```

simulateResiduals(fit_twediee_prev) %>% testResiduals()

```



`$uniformity`

Asymptotic one-sample Kolmogorov-Smirnov test

```
data: simulationOutput$scaledResiduals
D = 0.021, p-value = 0.954
alternative hypothesis: two-sided
```

`$dispersion`

DHARMA nonparametric dispersion test via sd of residuals fitted vs. simulated

```
data: simulationOutput
dispersion = 0.76533, p-value = 0.064
alternative hypothesis: two.sided
```

```
$outliers
```

```
DHARMa outlier test based on exact binomial test with approximate
expectations
```

```
data: simulationOutput
outliers at both margin(s) = 10, observations = 600, p-value = 0.03228
alternative hypothesis: true probability of success is not equal to 0.007968127
95 percent confidence interval:
 0.008020507 0.030436459
sample estimates:
frequency of outliers (expected: 0.00796812749003984 )
                                0.01666667
```

```
$uniformity
```

```
Asymptotic one-sample Kolmogorov-Smirnov test
```

```
data: simulationOutput$scaledResiduals
D = 0.021, p-value = 0.954
alternative hypothesis: two-sided
```

```
$dispersion
```

```
DHARMa nonparametric dispersion test via sd of residuals fitted vs.
simulated
```

```
data: simulationOutput
dispersion = 0.76533, p-value = 0.064
alternative hypothesis: two.sided
```

```
$outliers
```

```
DHARMa outlier test based on exact binomial test with approximate
```


expectations

```
data: simulationOutput
outliers at both margin(s) = 10, observations = 600, p-value = 0.03228
alternative hypothesis: true probability of success is not equal to 0.007968127
95 percent confidence interval:
 0.008020507 0.030436459
sample estimates:
frequency of outliers (expected: 0.00796812749003984 )
                                0.01666667
```

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

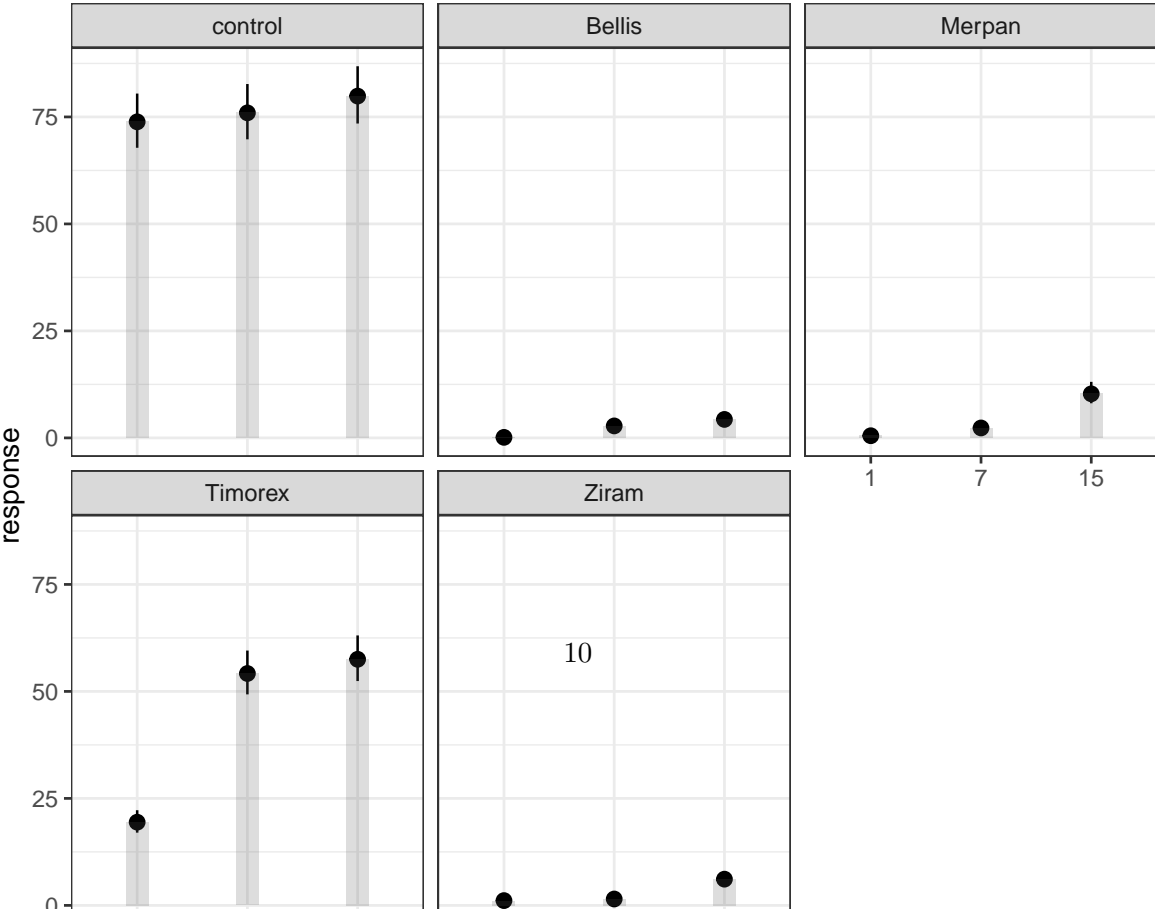
Chisq	Df	Pr(>Chisq)
2.83e+03	4	0
148	2	5.92e-33
328	8	4.73e-66

Means comparison test

```
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)

res_prev %>%
  ggplot()+
  aes(x=day, y =response)+
  geom_pointrange(aes(ymin=asympt.LCL , ymax=asympt.UCL))+
  geom_col(alpha=.2, width=.2)+
  facet_wrap("fungicide")
```

fungicide	day	response	SE	df	asympt.LCL	asympt.UCL	.group	%Control
Bellis	1	0.127	0.0644	Inf	0.0468	0.343	a	99.8
Merpan	1	0.506	0.147	Inf	0.286	0.892	ab	99.3
Ziram	1	1.12	0.236	Inf	0.739	1.69	b	98.6
Timorex	1	19.4	1.33	Inf	17	22.2	c	73.7
control	1	73.9	3.23	Inf	67.8	80.5	d	2.77
Ziram	7	1.49	0.282	Inf	1.03	2.16	a	98.1
Merpan	7	2.32	0.369	Inf	1.7	3.17	a	96.9
Bellis	7	2.8	0.417	Inf	2.09	3.74	a	96.3
Timorex	7	54.2	2.61	Inf	49.3	59.5	b	32.2
control	7	76	3.29	Inf	69.8	82.7	c	2.84
Bellis	15	4.32	0.545	Inf	3.37	5.53	a	94.3
Ziram	15	6.13	0.661	Inf	4.96	7.57	a	92.3
Merpan	15	10.3	1.27	Inf	8.09	13.1	b	86.1
Timorex	15	57.5	2.72	Inf	52.4	63.1	c	24.3
control	15	79.9	3.41	Inf	73.5	86.8	d	0

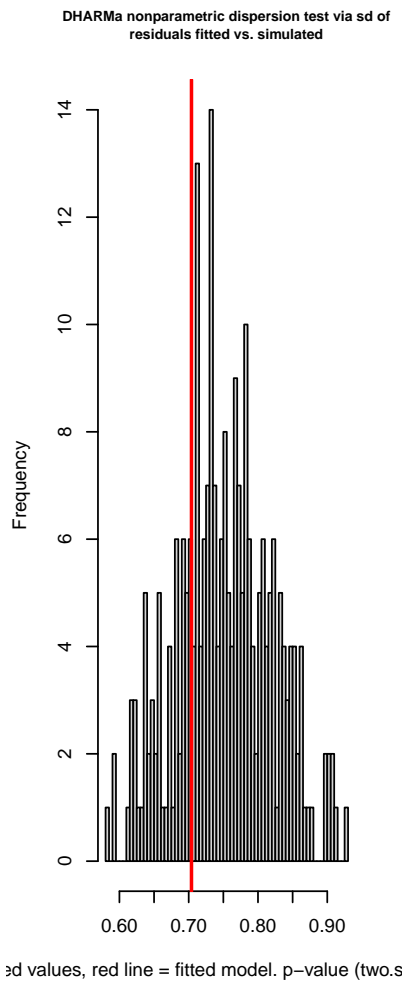
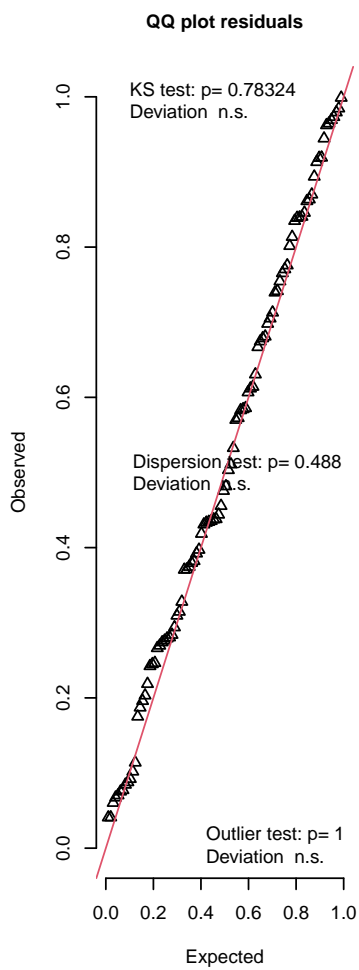


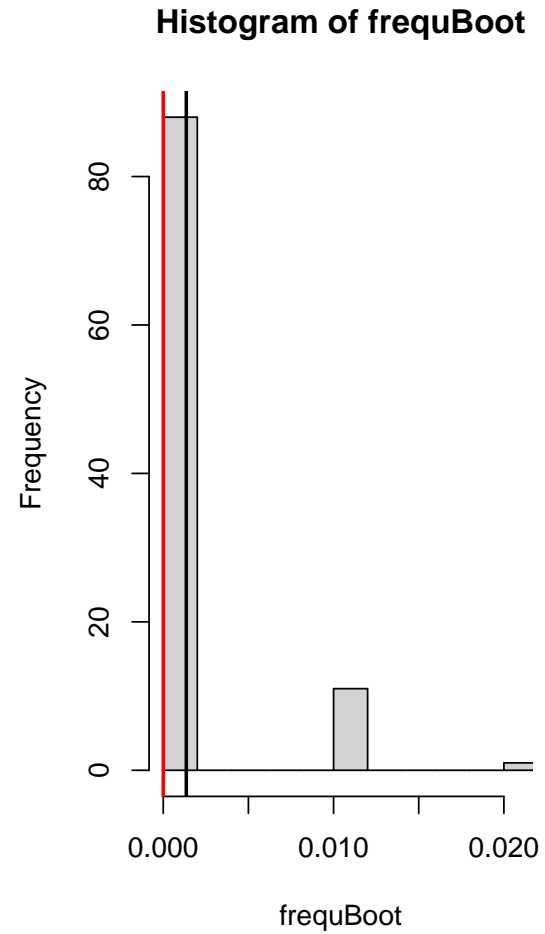
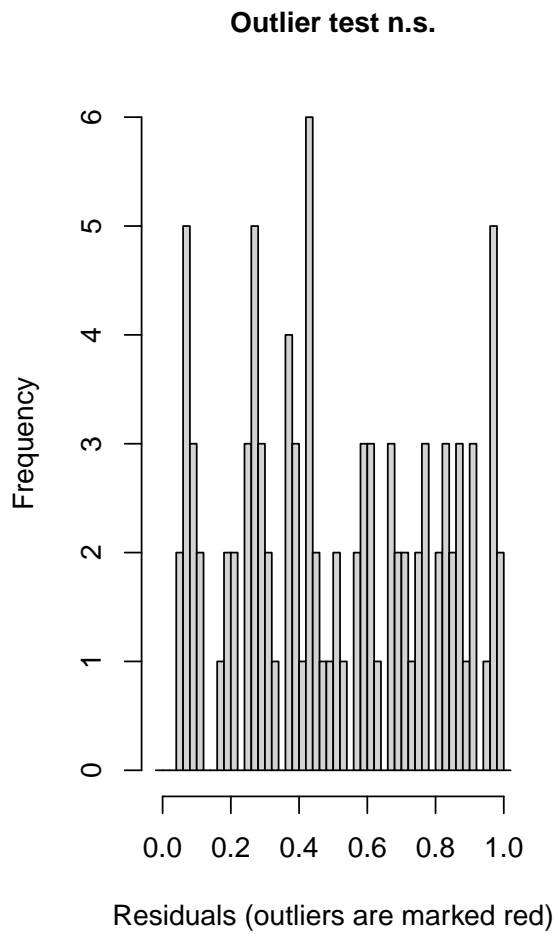
Disease incidence

```
dat_inc <- dat %>%  
  filter(!fungicide=="control") %>%  
  group_by(fungicide, repetition, day, replicate, fruit) %>%  
  summarise(diseased = spots_per_fruit > 0) %>%  
  group_by(fungicide, repetition, day, replicate) %>%  
  summarise(n= sum(!is.na(diseased)),  
            inc_prop=mean(diseased)) %>%  
  data.frame
```

Model

```
mod_inc <- glmer(inc_prop ~ fungicide*day +  
                 (1|repetition/replicate),  
                 weights=n,family=binomial,  
                 data=dat_inc)  
  
simulateResiduals(mod_inc) %>% testResiduals()
```





`$uniformity`

Exact one-sample Kolmogorov-Smirnov test

data: `simulationOutput$scaledResiduals`

D = 0.065276, p-value = 0.7832

alternative hypothesis: two-sided

`$dispersion`

DHARMA nonparametric dispersion test via sd of residuals fitted vs. simulated

```
data: simulationOutput
dispersion = 0.93691, p-value = 0.488
alternative hypothesis: two.sided
```

```
$outliers
```

```
    DHARMa bootstrapped outlier test
```

```
data: simulationOutput
outliers at both margin(s) = 0, observations = 96, p-value = 1
alternative hypothesis: two.sided
percent confidence interval:
 0.00000000 0.01041667
sample estimates:
outlier frequency (expected: 0.00135416666666667 )
0
```

```
$uniformity
```

```
    Exact one-sample Kolmogorov-Smirnov test
```

```
data: simulationOutput$scaledResiduals
D = 0.065276, p-value = 0.7832
alternative hypothesis: two-sided
```

```
$dispersion
```

```
    DHARMa nonparametric dispersion test via sd of residuals fitted vs.
    simulated
```

```
data: simulationOutput
dispersion = 0.93691, p-value = 0.488
alternative hypothesis: two.sided
```

```
$outliers
```

```
    DHARMa bootstrapped outlier test
```

```

data: simulationOutput
outliers at both margin(s) = 0, observations = 96, p-value = 1
alternative hypothesis: two.sided
percent confidence interval:
 0.00000000 0.01041667
sample estimates:
outlier frequency (expected: 0.00135416666666667 )
0

```

Means comparison test

```

emm_inc <- emmeans(mod_inc, ~ day|fungicide, type="response")
res_inc <- cld(emm_inc, alpha=0.05, Letters=letters, type="response")
res_inc

```

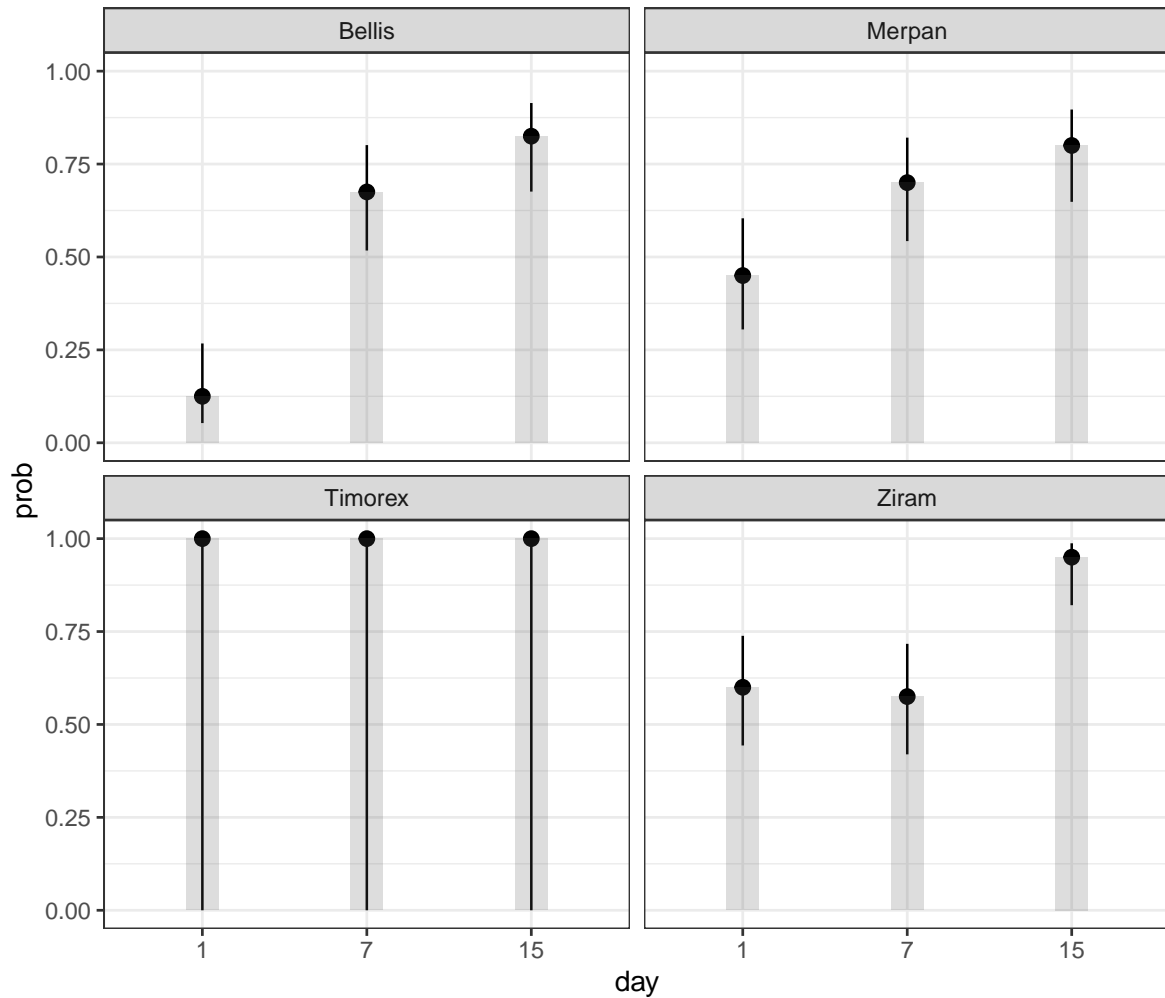
day	fungicide	prob	SE	df	asympt.LCL	asympt.UCL	.group
1	Bellis	0.125	0.0523	Inf	0.053	0.267	a
7	Bellis	0.675	0.0741	Inf	0.517	0.801	b
15	Bellis	0.825	0.0601	Inf	0.676	0.914	b
1	Merpan	0.45	0.0787	Inf	0.305	0.604	a
7	Merpan	0.7	0.0725	Inf	0.543	0.821	ab
15	Merpan	0.8	0.0632	Inf	0.648	0.897	b
1	Timorex	1	1.37e-09	Inf	2.22e-16	1	a
7	Timorex	1	4.92e-10	Inf	2.22e-16	1	a
15	Timorex	1	3.13e-10	Inf	2.22e-16	1	a
7	Ziram	0.575	0.0782	Inf	0.42	0.717	a
1	Ziram	0.6	0.0775	Inf	0.443	0.738	a
15	Ziram	0.95	0.0345	Inf	0.821	0.987	b

```

res_inc %>%
  ggplot()+
  aes(x=day, y =prob)+
  geom_pointrange(aes(ymin=asympt.LCL , ymax=asympt.UCL))+

```

```
geom_col(alpha=.2, width=.2)+
facet_wrap("fungicide")
```



Curative treatments

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

Data scheme


```
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 ...
```

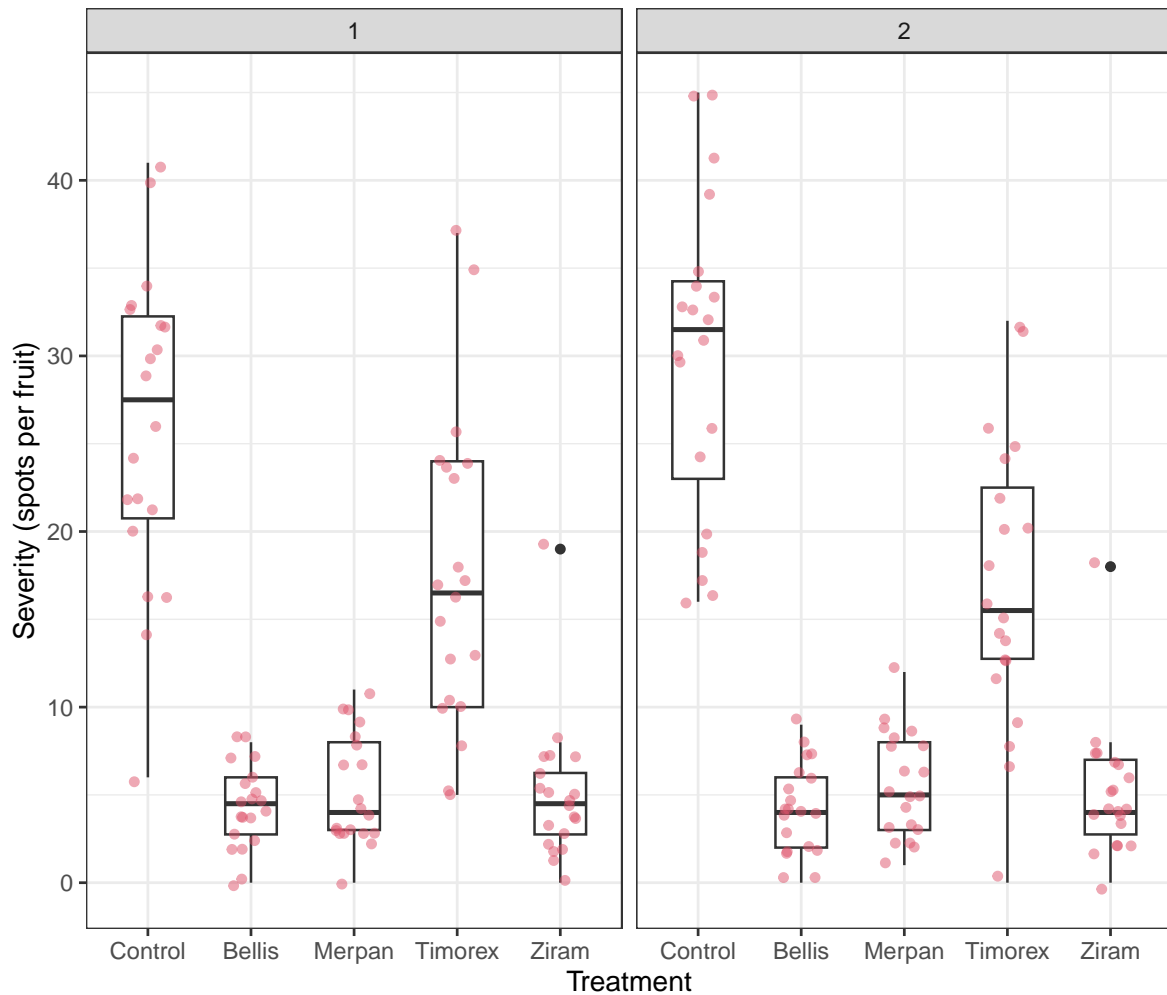
```
ftable(xtabs(~ fungicide + repetition + replicate, cura))
```

		replicate 1	2	3	4
fungicide	repetition				
Control	1		5	5	5
	2		5	5	5
Bellis	1		5	5	5
	2		5	5	5
Merpan	1		5	5	5
	2		5	5	5
Timorex	1		5	5	5
	2		5	5	5
Ziram	1		5	5	5
	2		5	5	5

Disease severity (spots per fruit)

Box plot: severity per treatment and repetition (1 and 2)

```
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)
# performance::check_overdispersion(fit_zipoisson)
# simulateResiduals(fit_zipoisson) %>% testResiduals()
```

```

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
8	1.26e+03
9	1.18e+03
9	1.18e+03
10	1.18e+03

```

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

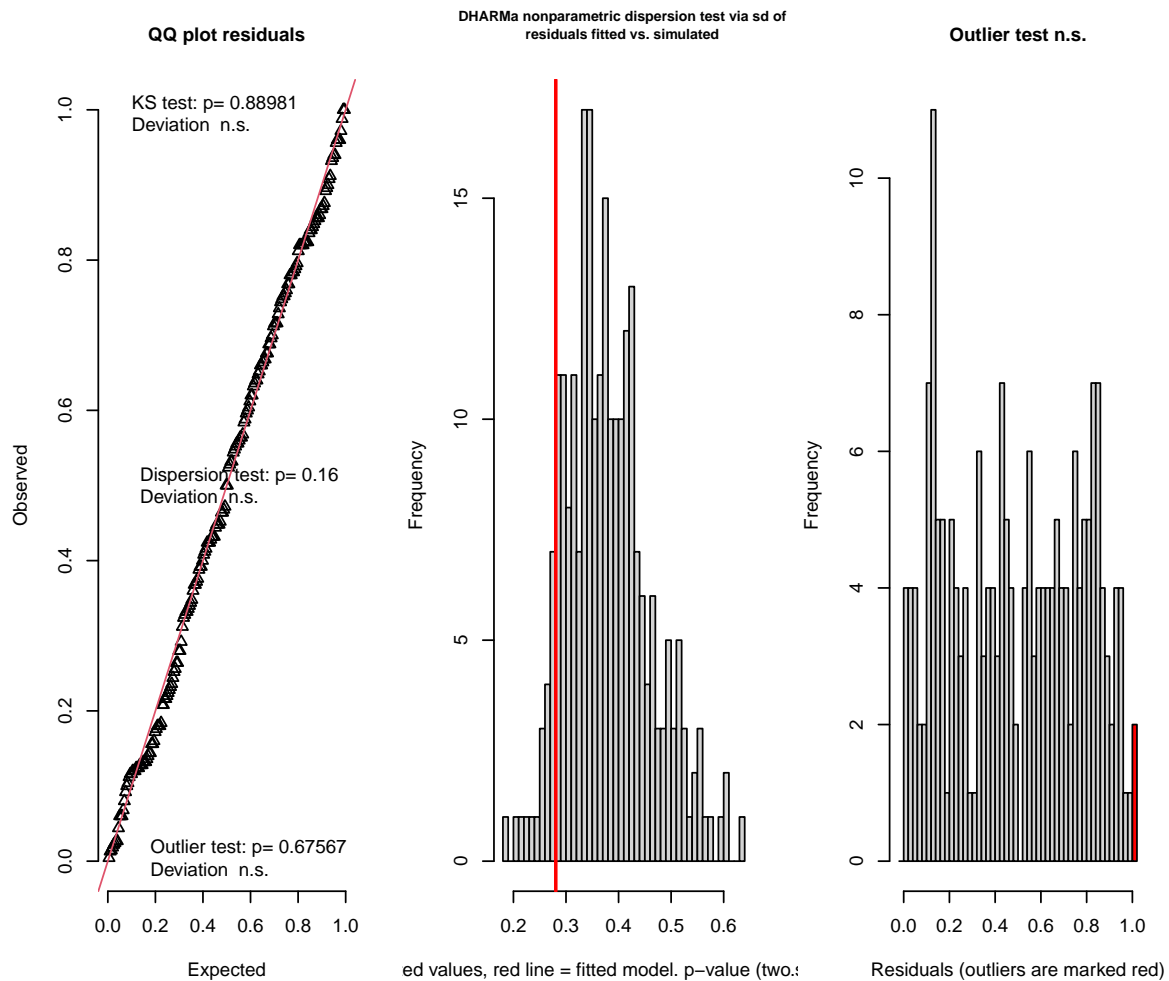
```

Goodness of fit

```

simulateResiduals(fit_twediee_cur) %>% testResiduals()

```



`$uniformity`

Asymptotic one-sample Kolmogorov-Smirnov test

```
data: simulationOutput$scaledResiduals
D = 0.041, p-value = 0.8898
alternative hypothesis: two-sided
```

`$dispersion`

DHARMA nonparametric dispersion test via sd of residuals fitted vs. simulated

```
data: simulationOutput
dispersion = 0.73874, p-value = 0.16
alternative hypothesis: two.sided
```

```
$outliers
```

```
    DHARMa outlier test based on exact binomial test with approximate
    expectations
```

```
data: simulationOutput
outliers at both margin(s) = 2, observations = 200, p-value = 0.6757
alternative hypothesis: true probability of success is not equal to 0.007968127
95 percent confidence interval:
  0.001213349 0.035654668
sample estimates:
frequency of outliers (expected: 0.00796812749003984 )
                                0.01
```

```
$uniformity
```

```
    Asymptotic one-sample Kolmogorov-Smirnov test
```

```
data: simulationOutput$scaledResiduals
D = 0.041, p-value = 0.8898
alternative hypothesis: two-sided
```

```
$dispersion
```

```
    DHARMa nonparametric dispersion test via sd of residuals fitted vs.
    simulated
```

```
data: simulationOutput
dispersion = 0.73874, p-value = 0.16
alternative hypothesis: two.sided
```

```
$outliers
```

```
    DHARMa outlier test based on exact binomial test with approximate
```

expectations

```
data: simulationOutput
outliers at both margin(s) = 2, observations = 200, p-value = 0.6757
alternative hypothesis: true probability of success is not equal to 0.007968127
95 percent confidence interval:
 0.001213349 0.035654668
sample estimates:
frequency of outliers (expected: 0.00796812749003984 )
                                0.01
```

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

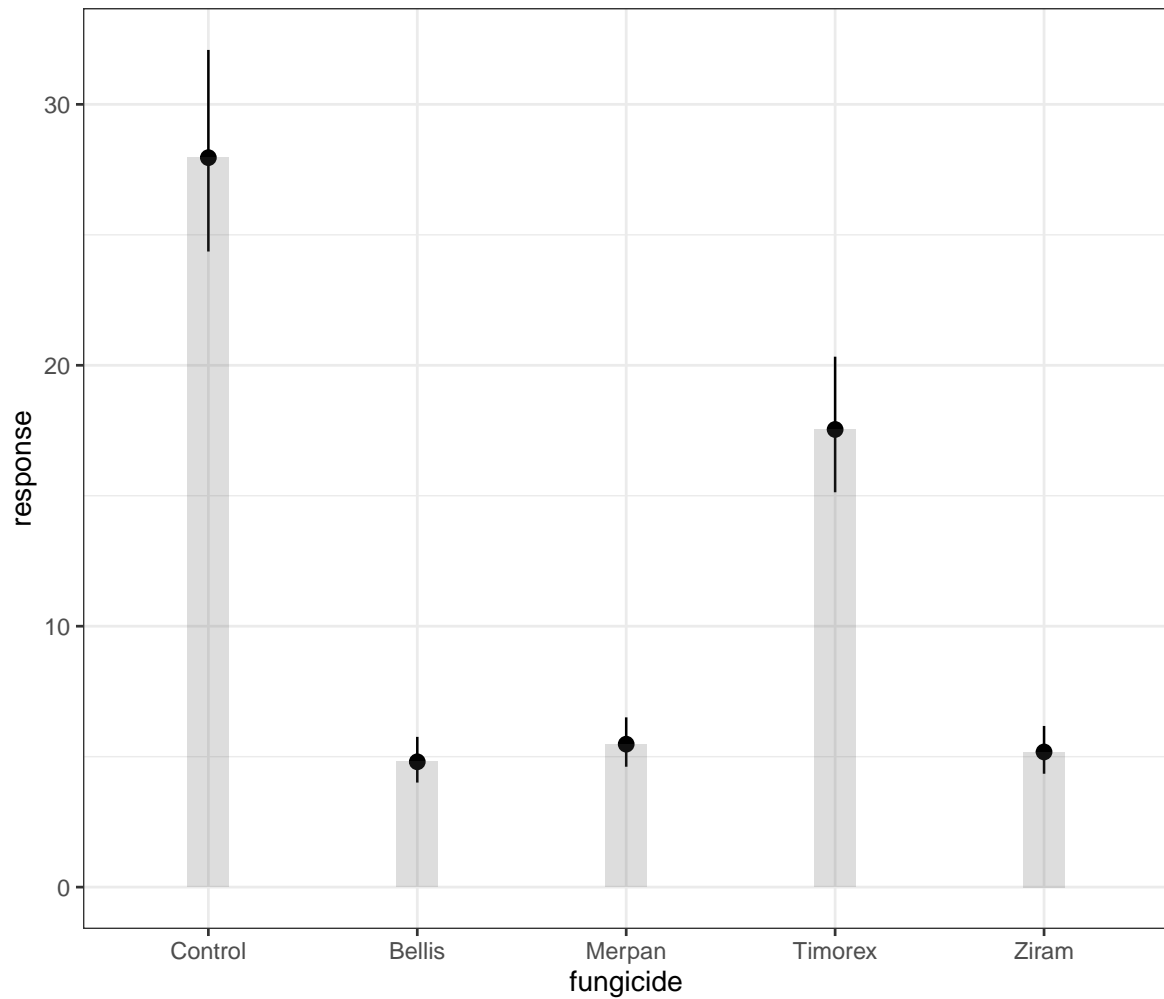
Chisq	Df	Pr(>Chisq)
533	4	3.98e-114

Means comparison test

```
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	asympt.LCL	asympt.UCL	.group	%Control
Bellis	4.8	0.445	Inf	4.01	5.76	a	82.8
Ziram	5.18	0.465	Inf	4.35	6.18	a	81.5
Merpan	5.48	0.48	Inf	4.61	6.51	a	80.4
Timorex	17.5	1.32	Inf	15.1	20.3	b	37.3
Control	28	1.96	Inf	24.4	32.1	c	0

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



Disease incidence

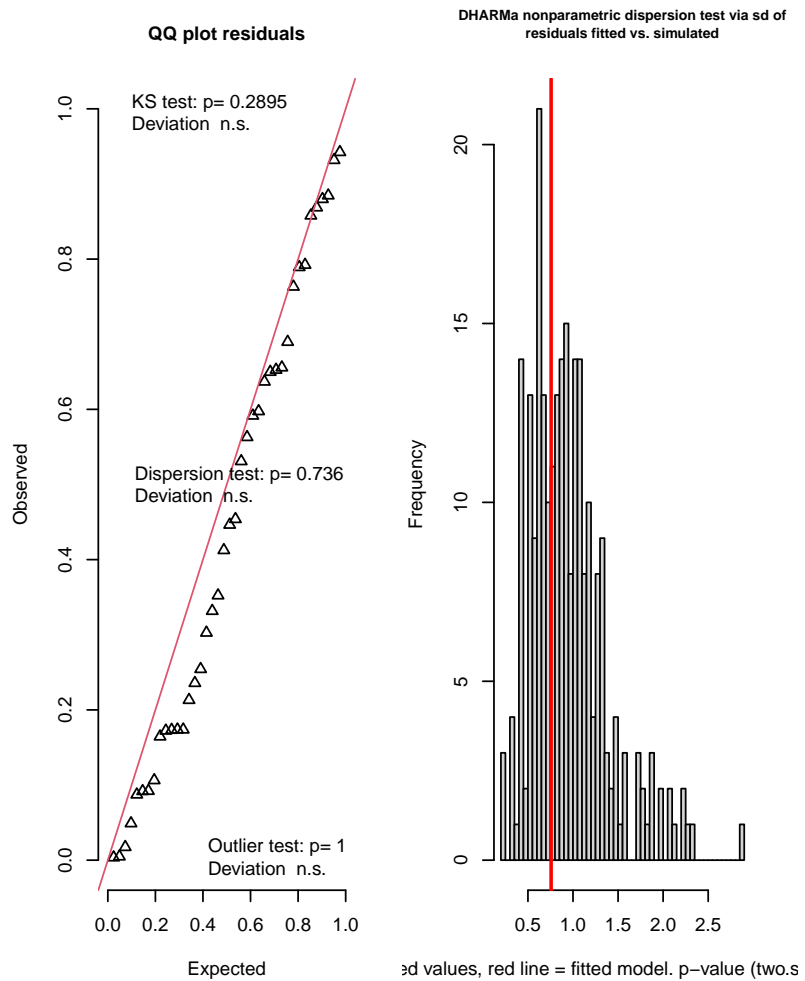
```
cura_inc <- cura %>%
  # filter(!fungicide=="control") %>%
  group_by(fungicide, repetition, replicate, fruit) %>%
  summarise(diseased = spots_per_fruit > 0) %>%
  group_by(fungicide, repetition, replicate) %>%
  summarise(n= sum(!is.na(diseased)),
            inc_prop=mean(diseased)) %>%
  data.frame
```

Model

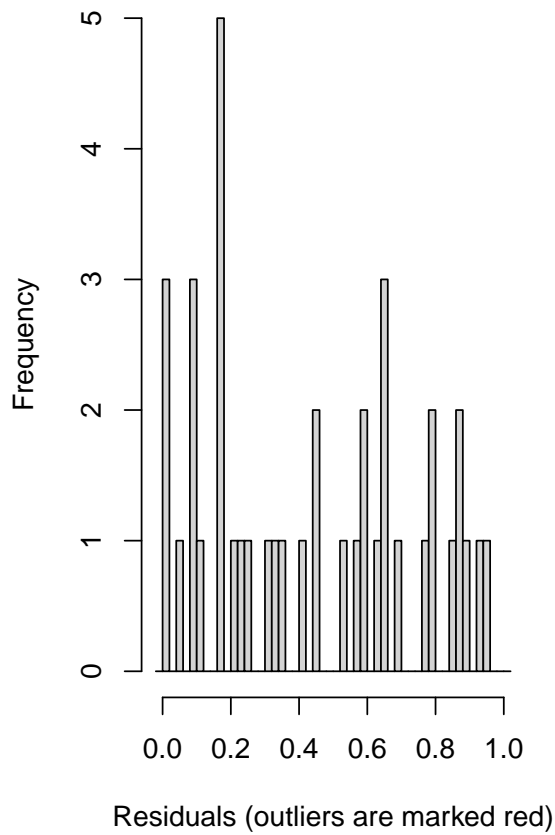
```
mod_inc_cura <- glmer(inc_prop ~ fungicide +
  (1|repetition/replicate),
  weights=n,family=binomial,
  data=cura_inc)
```

Goodness of fit

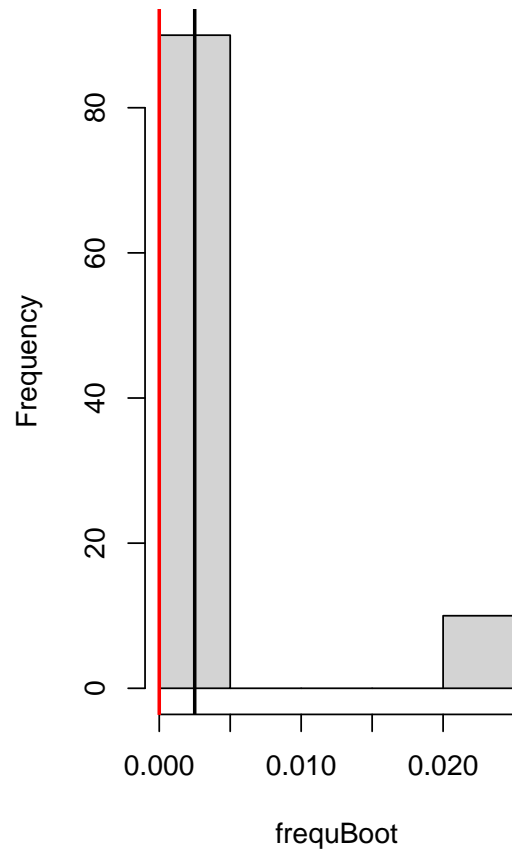
```
simulateResiduals(mod_inc_cura) %>% testResiduals()
```



Outlier test n.s.



Histogram of frequBoot



\$uniformity

Exact one-sample Kolmogorov-Smirnov test

data: simulationOutput\$scaledResiduals

D = 0.15123, p-value = 0.2895

alternative hypothesis: two-sided

\$dispersion

DHARMA nonparametric dispersion test via sd of residuals fitted vs. simulated

```
data: simulationOutput
dispersion = 0.79438, p-value = 0.736
alternative hypothesis: two.sided
```

```
$outliers
```

```
    DHARMa bootstrapped outlier test
```

```
data: simulationOutput
outliers at both margin(s) = 0, observations = 40, p-value = 1
alternative hypothesis: two.sided
percent confidence interval:
 0.000 0.025
sample estimates:
outlier frequency (expected: 0.0025 )
                                0
```

```
$uniformity
```

```
    Exact one-sample Kolmogorov-Smirnov test
```

```
data: simulationOutput$scaledResiduals
D = 0.15123, p-value = 0.2895
alternative hypothesis: two-sided
```

```
$dispersion
```

```
    DHARMa nonparametric dispersion test via sd of residuals fitted vs.
    simulated
```

```
data: simulationOutput
dispersion = 0.79438, p-value = 0.736
alternative hypothesis: two.sided
```

```
$outliers
```

```
    DHARMa bootstrapped outlier test
```

```

data:  simulationOutput
outliers at both margin(s) = 0, observations = 40, p-value = 1
alternative hypothesis: two.sided
percent confidence interval:
 0.000 0.025
sample estimates:
outlier frequency (expected: 0.0025 )
                                0

```

Means comparison test

```

emm_inc_cura <- emmeans(mod_inc_cura, ~ fungicide, type="response")
res_inc_cura <- cld(emm_inc_cura, alpha=0.05, Letters=letters, type="response")
res_inc_cura

```

fungicide	prob	SE	df	asympt.LCL	asympt.UCL	.group
Bellis	0.9	0.0474	Inf	0.762	0.962	a
Ziram	0.95	0.0345	Inf	0.821	0.987	a
Merpan	0.975	0.0247	Inf	0.843	0.996	a
Timorex	0.975	0.0247	Inf	0.843	0.996	a
Control	1	3.43e-06	Inf	2.22e-16	1	a