The definition of “no interaction”:

for detecting synergistic and antagonistic interactions

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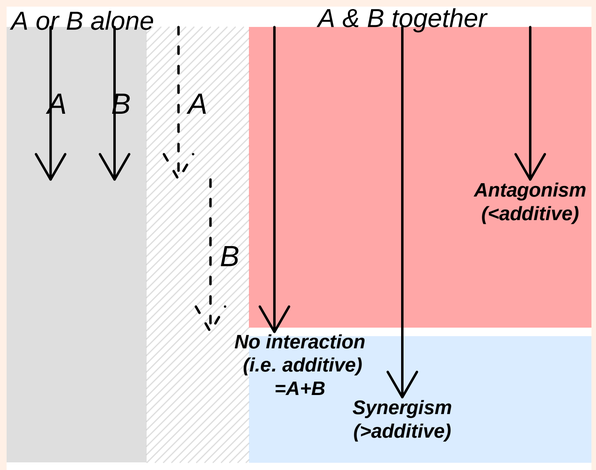
1. Motivation: Synergistic or antagonistic interactions between viroids on citrus growth

- viroids are single stranded RNA molecules infecting plant hosts
- viroids are often detrimental, with stunting, yield loss, death in citrus
  - but can also be beneficial (viz. dwarfing with no reduction in fruit size)

Right: Viroid effect on Citron plants, non-infected (left) vs infected (right).

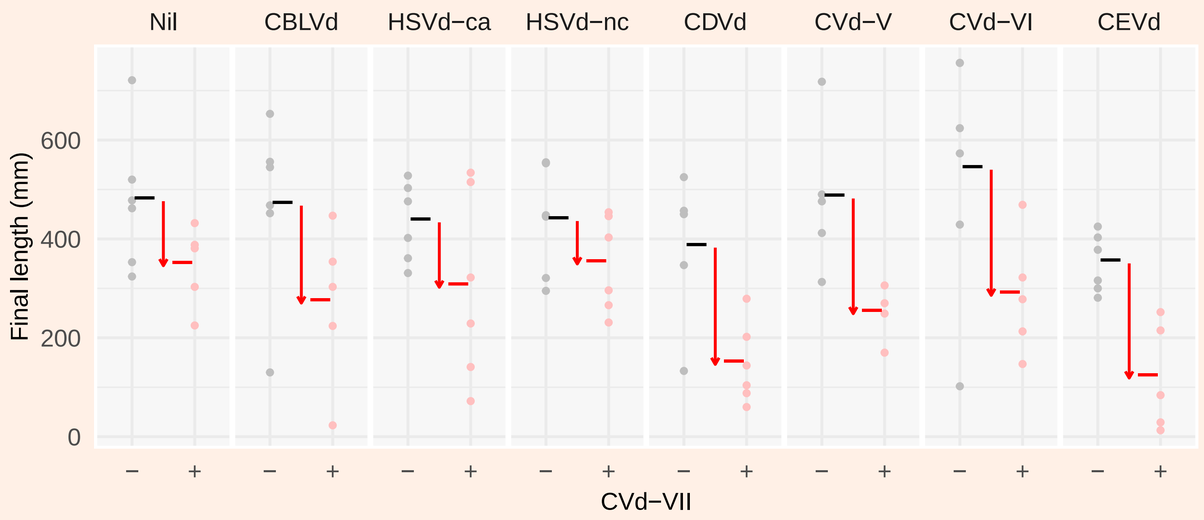
- what happens when there is co-infection of different viroids in citrus?
- we looked at co-infection of a recently discovered viroid (CVd-VII) (*Chambers et al, 2018*) and a range of known viroids in Citron (*C. medica L.*) indicator plants
- are there *synergistic* or *antagonistic* interactions on growth?
  - synergistic are *greater* than the sum of the parts
  - whereas antagonistic are *less* than the sum of the parts
  - see diagram on the RHS
  - I will call this the “null additive” model
    - i.e. the null hypothesis (of no interaction) is that the effects are *additive*

Synergistic vs Antagonistic



2. The data

- 2x8 factorial design: RCBD in greenhouse, n=6 replicate pots/plants
- Stem length measured for 6 weeks (7 measurements, week 0 to week 6), starting 10 weeks after inoculation
  - final length (week 6) shown
- effect of CVd-VII shown for each viroid (red arrow)
  - possibly some synergistic interactions with viroid?
    - i.e. effect of CVd-VII appears larger for many viroids than the control



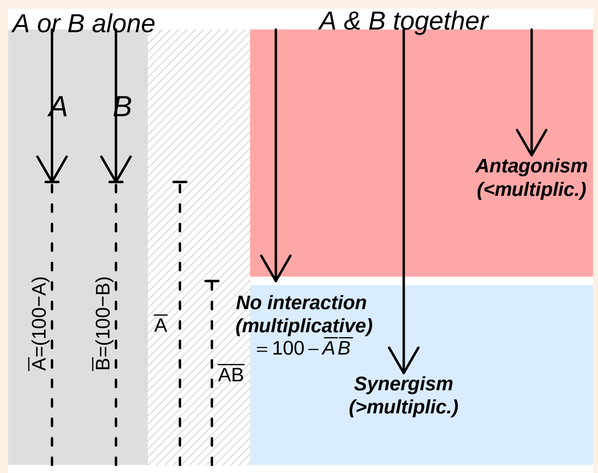
Diff between CVd−VII + and − shown by arrow (for each viroid)

3. A better definition of “no interaction”?

- we *do* want to be *quantitative* (a lot of these studies are *qualitative*)
- I argue it is more appropriate that “no interaction” means the viroid effects are *multiplicative* – the “null multiplicative” model (right)
  - if each viroid acts independently to kerb growth, the net effect should be multiplicative
  - viz. imagine that the 2nd viroid acts on what is “left” from the 1st viroid
  - compare to the “null additive” model in section 1

Synergistic vs Antagonistic

- the null multiplicative model



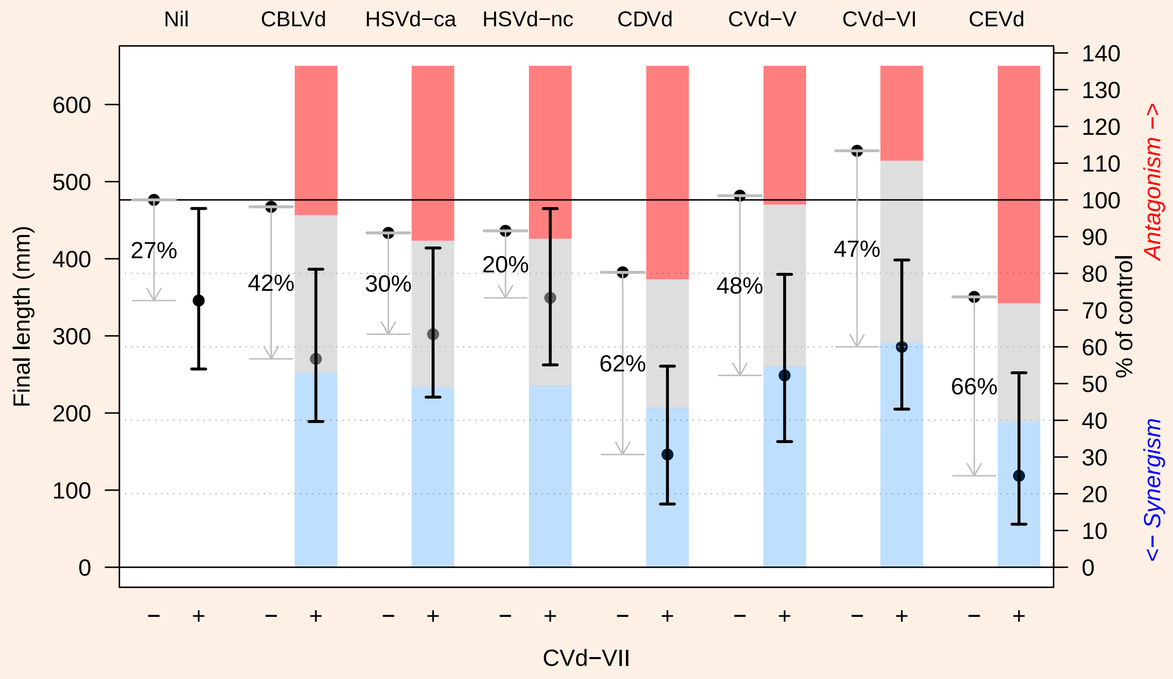
- e.g. if each viroid reduces growth by 40%, then resultant growth is 60% of 60% = 36% -- a net reduction of 64% (compared to 40+40=80% for the null additive)

4. Analysis

- analysis of final length (week 6) (also week 0 as a covariate or growth (not shown))
- Generalised linear model (GLM) with Gaussian distribution and logarithm link
  - $\eta = \log(\mu) \sim$ CVdVII + viroid + CVdVII × viroid
  - in other words, $y \sim N\left(e^{\eta}, \sigma^2\right)$ instead of the usual $y \sim N\left(\eta, \sigma^2\right)$
  - interest here is the interaction term, CVdVII × viroid
    - are there (non-multiplicative) interactions?

5. Results

- no significant interactions
  - but still want to show indications of interactions (and uncertainties)
- following graphic inspired by *Darling et al (2009)*
  - showing the CVd-VII effect of each viroid (and its uncertainty) as *black error bars*
    - e.g. 27% decrease for control (Nil)
    - compared to regions of no interaction (grey), synergy (blue) and antagonism (red)
  - note: everything back-transformed from the logarithmic scale (details below)
  - using 83.4% CIs for pairwise comparisons (e.g. *Cave, 2022*)



- note: all error bars straddle the gray region (viz. non-significant at the 5% level)

Gory details on the calculations!

error bars= $\exp\left(\hat{\eta}_{+ve,i} \pm 1.344\text{SED}_{+vs-,i}\right)$ $\hat{\eta}_{+ve,i}$ =lin. pred. for VII +ve, viroid i

gray region= $\exp\left(\hat{\eta}_{-ve,i} - D_{none} \pm 1.344\text{SED}_{+vs-,none}\right)$ $\hat{\eta}_{-ve,i}$ =lin. pred. for VII -ve, viroid i

$D_{none} = \hat{\eta}_{+ve,none} - \hat{\eta}_{-ve,none}$ $\text{SED}_{+vs-,i}$ =SED for $\hat{\eta}_{+ve,i}$ vs $\hat{\eta}_{-ve,i}$

6. Discussion

- though a negative result (viz. no significant interaction)...
  - if there are any interactions, almost certainly *synergistic* rather than *antagonistic*
  - *Wang et al (2024)* suggests either synergistic and antagonistic are possible
- univariate analysis of final length (week 6) only
  - with a Gaussian distribution and logarithmic link, extension to repeated measures not easy with “off the shelf” software
    - viz. GLMM requires covariance on underlying scale, GEE uses working correlation only (not covariance) and NLMM is not designed for this...
  - i.e. we want the model for multivariate $\boldsymbol{y}$ with covariance $\boldsymbol{\Sigma}$ to be
    - $\boldsymbol{y} \sim N\left(e^{\boldsymbol{\eta}}, \boldsymbol{\Sigma}\right)$ rather than $\boldsymbol{y} \sim N\left(\boldsymbol{\eta}, \boldsymbol{\Sigma}\right)$
- for non-Gaussian distributions (e.g. Gamma), the logarithm link is often the default link function (leading to a null multiplicative model)

References

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[3] Cave, V (2022), *Confidence tricks: the 83.4% confidence interval for comparing means,* VSN International Ltd, https://vsni.co.uk/blogs/confidence-tricks-the-83-4-confidence-interval-for-comparing-means/

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