Relationship between *Leptosphaeria maculans* (blackleg) infection and grain yield of *Brassica napus* (canola): experiment and meta-analysis

Norman Daurelle, Matthieu Babiar, Susan Sprague, Luke Barrett

Abstract

Canola yield, blackleg disease severity and environmental data from field trials including fungicide tests conducted between 2013 and 2016 with 22 cultivars sown in 16 locations throughout 4 Australian states (New South Wales, Victoria, Southern Australia, and Western Australia) were used to create a mixed-effect linear model relating yield to disease severity and environmental variables. This led to an estimate of the canola-blackleg disease-yield relationship of around -3.7 kg/ha/severity point (SP thereafter, scale 0-100) that accounts for Australian cultivation practices and, given sample size, number of locations and cultivars, should reflect the reality of the disease in Australia. The results obtained in this study confirmed the importance of environmental parameters and genetic resistance on disease severity and the disease-yield relationship, suggesting a higher tolerance to the disease symptoms from resistant cultivars. The work was expanded with a meta-analysis based on the results of 10 studies from the UK, Canada and Australia, synthesizing the results of the available literature, which led to a higher average relationship slope of -15.47 $kg.ha^{-1}.SP^{-1}$ within a confidence interval ranging from −7.78 to −23.17 $kg.ha^{-1}.SP^{-1}$. This value, with disease severity rated on a 0-100 scale (based on the percentage of stem base cross section suffering canker), is easily understandable and could be used to estimate the economic impact of blackleg. It could therefore also provide insight to advise farmers regarding the cost-effectiveness of their practices regarding blackleg on canola, based on their context (area sown to canola, cultivar grown, level of fertilizer and fungicide used).

Introduction

Making informed decisions regarding disease control requires an accurate understanding of the impact of pathogens on crop productivity. However, there is often limited data quantifying the precise effects that pathogens have on yield and profitability (citation). Accurate models describing the impact of plant diseases on crop yield are necessary in order to understand the economic consequences of epidemics, including the cost-benefit ratio of prospective research investments and crop management practices aiming to redress the problem (James 1974). For example, the ability to predict the likely costs associated with an outbreak of disease is important to help farmers make cost-effective decisions regarding the application of fungicide, or researchers to understand the value of R&D for novel tools for pathogen control.

Broken down into its component parts, yield loss to disease is the outcome of a complicated process that depends on multiple interacting factors, including both host resistance and pathogen aggressiveness traits. These, together with environmental conditions, interact to determine disease severity (cf. "the disease triangle"). The relationship between disease severity and yield (hereafter referred to as the yield-disease relationship) can further depend on host tolerance (Israel Pagán and Fernando García-Arenal 2018) and environmental variation. For example, consider a vascular pathogen. Following infection, changes in water availability could be predicted to alter the relationship between yield and disease severity. Under dry conditions, a given level of infection may result in a relatively high impact on yield because water becomes even more limiting. In an environment in which water is more readily available, the impact on yield will be lower, unless water is proportionally more absorbed by the pathogen. Therefore, the slope of yield-disease relationships may be expected to vary depending environmental conditions (Barrett et al. 2009).

The simplest model of the relationship between yield and disease is one derived from observations taken from single time points and locations, linking yield (or yield loss) to a given level of disease

severity or disease incidence. Predictions for future seasons or other locations can be extrapolated from these point estimates. However, such models, while relatively easy to derive, fail to account for variability of disease induced crop losses in different cultivars over space and time. Rather, as described above, relationships between yield and disease severity will likely vary considerably depending on environmental conditions and the genetics of the host-pathogen interaction. Accounting for such variability and establishing robust models of relationships between disease severity and yield therefore requires data from a broad range of environments, years, and cultivars. One approach to addressing this issue is to carry out large experiments encompassing trials in multiple years and locations and using many cultivars (Madden and Paul 2009). While such an approach can be powerful, broad spatio-temporal coverage of yield-disease relationships is beyond the scope of many studies, particularly for crops grown around the world. From an empirical perspective, accounting for such variability requires data from as many locations, genotypes, and years as possible in different farming systems to establish robust models of relationships between disease severity and yield.

In this respect, meta-analysis can be a powerful tool to synthesize the results of multiple independent studies of relationships between disease and yield. It produces an estimate and range for the metric of interest that is collected from all studies included in the sample, based on each study's value(s) of the metric and the variance associated with it (these). Though theoretical roots of meta-analysis are historically numerous and seem to partly predate it (Group 1904), the term "meta-analysis" seems to have been coined by Gene V. Glass (Gene 1976). Meta-analysis is now much used in the medical field, and plant pathologists have started using it to get overall results from several studies (Madden and Paul 2009; Dalla Lana et al. 2015; Lehner et al. 2017).

Here we focus on developing an understanding of the yield-disease relationship for blackleg disease (syn. Phoma lingam) of canola, or oil-seed rape, or rapeseed (*Brassica napus*) caused by the fungal pathogen *Leptosphaeria maculans*. In this interaction, disease is initiated via foliar infection, typically early in the growing season. The fungus then grows through the petiole to the stem, where

it can cause canker, resulting in reduced water and nutrient transport through the plant (Sprague et al. 2009a). Genetic resistance to *L. maculans* conferred by major Resistance genes (R-genes) has repeatedly broken down due to evolutionary change in pathogen populations: e.g. Rlm1, Rlm6 (Sprague et al. 2006a; Van De Wouw et al. 2016), and fungicides are currently important for disease management. Previous studies indicate strong links between environmental conditions and disease severity, with temperature (Barbetti 1975), rainfall (Guo and Fernando 2005), inoculum load (Bousset et al. 2018) and pathogen genotype (Bousset et al. 2018) all demonstrated to influence Blackleg severity. Under some conditions, Blackleg can be a devastating disease (McGee and Emmett 1977; Steed et al. 2007), with reports of up to 90 % yield loss in Australian canola crops (Sprague et al. 2006a). However, relatively few studies have drawn explicit links between disease severity and yield, particularly with respect to how such a relationship might vary in time and space. The expression *major resistance genes* is sometimes used in literature to describe host loci implicated in avirulence of a pathogen strain against a host plant genotype, in gene-for-gene context (Flor 1971), where virulence is considered a categorical variable. On the other hand, *Quantitative Trait Loci (QTL)* are used to describe quantitative traits, and pathogen virulence on host plants (or host resistance) can also be considered a quantitative variable (Kushalappa et al. 2016). Quantitative resistance is also sometimes referred to as *tolerance* (Riedel et al. 2011). While QTL can lead to detection of resistance (R) genes (alleles), presence / absence of multiple resistance-associated QTL with potential additive effects is the basis of the quantitative resistance concept (Leonards-Schippers et al. 1994; Parlevliet 2002; Soufflet-Freslon et al. 2008; Kushalappa et al. 2016).

In this study we adopt a yield-targeted approach to address the following questions: what is the relationship between blackleg disease severity and canola yield, and does it vary across years, locations and cultivars? We define disease severity as the percentage of stem base cross section suffering canker averaged over the sampling unit (e.g. plot, or individuals chosen to account for the plot) (Fitt 2006; Hwang et al. 2016). To address these questions, we first assess the relationship between blackleg disease severity and canola yield using data collected from a series of field

experiments conducted over multiple years and across a large range of environments in Australia. In addition, we gathered estimates of the relationship between blackleg disease severity and yield from published independent studies focusing on the same questions and analysed them along with the estimate obtained from the field experiments using a meta-analysis. This is useful to compare farming systems and develop more general estimates of yield loss that may be useful for larger scale modelling studies. Answering these questions for blackleg disease of Canola could serve as an example for other crop-pathogen interactions and should allow better evaluation of the cost-effectiveness of agricultural practices aimed at mitigating blackleg-induced yield losses.

Material and methods

1. Analysis of experimental data

Small plot (2x10m) field experiments were conducted between 2013 and 2016, comprising a total of 22 cultivars (Table S1) sown in 16 different locations (Table S2), throughout all canola-growing regions of Australia. Variation in disease severity within year/location/cultivar combinations was engineered by applying fungicides with different levels of efficacy, allowing comparisons under otherwise identical experimental conditions. Indeed, the experiments were initially designed to test the effects of different fungicide treatments on blackleg severity and canola yield. 13 different fungicide treatments were tested including untreated control, seed treatment (Jockey), fungicide-amended fertilizer (Impact) or in-crop application of foliar fungicide at the 4-6 leaf growth stage (Prosaro) both individually and in combination. Observed cultivars ranged in genetic resistance from resistant to susceptible (R, R-MR, MR, MR-MS, MS, and MS-S) with the exception of S-category cultivars (not grown in Australia). While not originally designed to assess relationships between disease severity and yield, the genetic resistance and fungicide treatments interact to generate a large

dataset which provides an opportunity to explore and analyze large amounts of data (Supplementary 1), to address the question at hand.

Disease assessment

The canker severity internal infection (CSII) was used as an estimate of disease severity. CSII of mature plants was assessed on 20-40 individuals at 1-2 weeks prior to desiccation. For each replicate plot (1-4 reps), this involved rating the cankered surface from 0 (0% of the stem cross section cankered) to 100 (100% of the stem cross section cankered) (Fitt 2006; Hwang et al. 2016).

Grain yield

According to standard cultivation practices, plots were harvested after 50% of seeds changed color. Plots were either harvested with a mechanical harvester, or with a border quadrat (1 or 2 m^2) in Goulburn (2014, NSW), Monteagle (2015, NSW), Canowindra (2016, NSW) and Kaniva (2016, VIC). Seeds were dried in an oven (48h, 70°C) and weighted, resulting in a yield measure per plot, expressed as a yield in kg/ha.

Additional data

Rainfall measurements during the growing season (April-October) were collected for each experimental site from the Australian Bureau of Meteorology website ([CSL STYLE ERROR: reference with no printed form.]). Rainfall was separated in two periods: rainfall from April to June (vegetative growth when crops are exposed to blackleg infection), and from July to October (flowering/pod filling period).

Genetic quantitative resistance

Statistical analysis

The precise complement of fungicide treatments and canola cultivars varied among individual trials. Hence, the dataset was incomplete and unbalanced. Linear mixed-effects model analysis has proven robust under such conditions (Smith et al. 2005), and is appropriate especially when all cultivars are not present in all trials. Need to explain how we ignored fungicide treatment and instead focused on disease severity

In order to broadly analyse variation in the yield-disease relationship, we used a mixed-effects linear model relating yield to quantitative variables disease severity, rainfall from April to June (the first, vegetative, part of the growing season), and rainfall from April to October (throughout most of the whole growing season), while accounting for yield potential differences due to factor variables location, cultivar and year. Disease severity and rainfall variables were used as fixed effects whereas location, cultivar and year were used as intercept random effects. Two measures of rainfall were used because it was hypothesized that timing of rainfall may influence the yield-disease relationship. The final model took the form:

$$Y_{i,j,k,l} = \beta_0 + \beta_1 X_{1,l} + \beta_2 X_{2,l} + \beta_3 X_{3,l} + S_{0,i} + S_{1,j} + S_{2,k} + e_{i,j,k,l} \quad (1)$$

For all resistance groups

Where $Y_{i,j,k,l}$ represents the yield of the l^{th} observation of the i-th year, j-th location, k-th cultivar combination ($l \in [1{:}\, n_{i,j,k}]$ with $n_{i,j,k}$ the number observations for the i-j-k combination); β_0, the intercept of the model, represents the average yield without disease at minimal rainfall values; β_1 the effect of X_1, disease severity; β_2 the effect of X_2, rainfall during the first growth period; β_3 the effect of X_3, rainfall during the whole growing season; $X_{1,l}$, $X_{2,l}$, $X_{3,l}$ the respective l^{th} observation of each variable for the i-j-k combination; $S_{0,i \in [2013:2016]}$ the effect of year, as random effect on the intercept; $S_{1,j \in locations}$ the effect of location, as random effect on the intercept; $S_{2,k \in cultivars}$ the effect of cultivar, as random effect on the intercept; $e \sim \mathcal{N}(0, \sigma^2)$ the residuals (verified to be

normally distributed with σ^2 = 331.2918), or errors of the model-predicted values compared to actual values, with $e_{i,j,k,l}$ the l^{th} observation for the i-j-k combination.

We subsequently used the slope β_1 (representing yield-loss per mean disease severity point) to describe a general yield-disease relationship independent of the effects of year, location and cultivar, which were accounted for in the model.

In the overall model, the effect of blackleg resistance group was not considered (due to confounding effect of cultivar). To assess the effect of blackleg resistance rating on β_1, the same model was run separately for each resistance group. As per the overall model, the effects of year, location, and cultivar were accounted for in models run for each of the six resistance groups (noting that each cultivar belongs to a single resistance group), therefore no explanatory terms were removed from the overall model.

$$Y_{i,j,k,l} = \beta_0 + \beta_1 X_{1,l} + \beta_2 X_{2,l} + \beta_3 X_{3,l} + S_{0,i} + S_{1,j} + S_{2,k} + e_{i,j,k,l} \quad (2)$$

Within each resistance group

To get a clearer understanding of how location influences the parameter β_1, the dataset was divided into location subsets. This allowed models to be run for each location separately and therefore examine the effect of location on the yield-disease relationship, while still accounting for yield potential differences between cultivars. Apart from removing location (the $S_{1,j}$ term), year was changed from random to fixed effect in this model, and rainfall variables were also removed from the model because rank deficiency of the fixed effects matrix rendered them unused even when included:

$$Y_{i,k,l} = \beta_0 + \beta_1 X_{1,l} + \beta_{4,i} + S_{0,k} + e_{i,k,l} \quad (3)$$

For each location

Where $\beta_{4,i}$ represents the fixed effect of year i on the intercept. This change was operated because some locations were observed only on certain years. $S_{0,k}$ is the random effect of cultivar on the intercept included in this model.

Effect significance and coefficients for each of the mixed-effects linear models were calculated using standard approaches (Bates et al. 2014).

In addition to the mixed-effects model analysis, we also generated estimates of the coefficient β_1 by running a series of simple linear models for each Year x Location x Cultivar (YLC) cluster:

$$Y_l = \beta_0 + \beta_1 X_{1,l} + e_l \qquad (4)$$

With Y_l as the yield of the l^{th} observation; β_0 the intercept of the model; β_1 the effect of X_1, disease severity; e_l the l^{th} residual, $e \sim \mathcal{N}(0, \sigma^2)$. Significant ($p < 0.05$) regression coefficients were then used to generate a distribution of yield-disease relationships.

To translate our results into percentage of yield loss, the ratio (observed yield / cluster intercept) was computed and multiplied by 100 for the YLC clusters that showed a significant effect of disease on yield. This % of potential yield without disease was then related to disease severity in a simple linear regression model. There is a 1:1 ratio of yield:severity -coupled- values used. In other words, no yield is related to a disease severity value that did not happen in the plot where it was recorded. This model is described by the same statistical formula (4).

This dataset was analyzed with R, under the RStudio environment, using basic R and various packages car (Fox et al. 2021) dplyr (Wickham et al. 2021) ggpubr (Kassambara 2020) lme4 (Bates et al. 2021) rmarkdown (Allaire et al. 2021) and tidyverse (Wickham and RStudio 2021).

2. Meta-analysis

To build a broader understanding of the relationship between yield and disease in space and time, studies of canola and blackleg reporting disease-yield regression slopes or data that could be

used to create such slopes were gathered from the literature. With the exception of the present study, data from unpublished works was not considered. For studies that did not report disease-yield relationship slopes, coupled yield and disease severity values were extracted and slopes were then computed. In total, 10 publications and data from this study were used in the meta-analysis (table 1). From these studies, we identified 24 experiments from which yield-disease slopes could be extracted. In all cases, experiments were conducted under field conditions, and yield was estimated on a per-plot basis. In 6 out of 11 studies (including the present study), disease levels were manipulated via use of fungicides. In all studies, disease severity was based on the relative proportion of internal stem infection, although different metrics were used: In (Ballinger et al. 1988), (GC Upadhaya et al. 2019), (Khangura and MacLeod 2011) and (Sprague et al. 2009b) stem canker was already recorded as %, fitting a [0-100] scale. In (Hwang et al. 2016) and (Kutcher), median disease severity over different site-years was recorded on a [0-5] scale of stem base canker. The data was converted to [0-100] scale using simple cross product ($severity_{\%} = 20 * severity_{0\text{-}5}$), as yield was available in $kg.ha^{-1}$. Similarly, for (Steed et al. 2007) data, the following was applied: ($severity_{\%} = 25 * severity_{0\text{-}4}$). In (Zhou et al. 1999), raw severity or yield data was not available, but 4 slopes of the yield-disease relationship were reported, with the information needed to transform these to the desired metric. Severity had been measured on a [0-4] scale and yield was expressed in $t.ha^{-1}$ therefore slopes could be converted: $\frac{25 * \text{slope}\{t.ha-1/severity0-4\}}{1000}$ = slope{$kg.ha^{-1}/severity_{0\text{-}100}$} . In (Khangura and Barbetti 2005), no transformation was required to use the 8 slopes reported.

The slopes used as effect-sizes all came from simple linear regression models of the kind

$$Y_i = \beta_0 + \beta_1 X_{1,i} + e_i \quad (5)$$

with Y_i the i-th observation of yield, in kg/ha, for the study; β_0 the intercept of the model, representing the average yield without disease; $X_{1,i}$ the i-th observation of disease severity rated on a 0-100 scale; β_1 the effect of X_1 on Y, or the slope; $e \sim \mathcal{N}(0, \sigma^2)$ the residuals (e_i the *i*-th residual), or errors of the model-predicted values compared to actual values. We assumed and, as far as

possible, verified residuals to be independent and normally distributed inside each identified experiment. Other explanatory variables were not included because none were common to all the data taken from all studies included in the meta-analysis.

Meta-analysis was conducted to estimate the overall weighted average effect size (here, the slope) and to determine the variability in effect size across studies, using a random effect model. The amount of variability (i.e., τ^2) was estimated using the restricted maximum-likelihood estimator (Viechtbauer 2005). In addition to the estimate of τ^2, the Q-test for heterogeneity (Cochran 1954) is reported. Studentized residuals and Cook's distances are used to examine whether studies may be outliers and/or influential in the context of the model (Viechtbauer and Cheung 2010). Effect-sizes with a studentized residual larger than a threshold given by literature (Viechtbauer and Cheung 2010) are considered potential outliers. Studies with a Cook's distance larger than the median plus six times the interquartile range of the Cook's distances are considered to be influential. The rank correlation test (Begg and Mazumdar 1994), using the standard error of the observed effect-sizes as predictor, is used to check for funnel plot asymmetry. The analysis was carried out using R (version 4.1.1) (R Core Team, 2021) and the metafor package version 3.0-2 (Viechtbauer 2021). The R function rma.mv() was used to account for the fact that some studies contributed multiple effect-sizes.

Results

1. Analysis of experimental data

Disease severity and grain yield were highly variable (Figure 1). Grain yield (mean = 1743 kg.ha^{-1} ; SD = 660 kg.ha^{-1}) ranged between 1.2 and 2.3 t/ha for 50% of the data, with up to 3 - 4t/ha yields achieved in some cases (less than 3%). In the overall model (formula 1), location was the main source of yield variation (Table 2). Disease severity values followed a right-skewed distribution, with a preponderance of values at or close to zero (Figure 1). Nevertheless, the experiments captured a wide range of disease severity levels. Results from the overall analysis of the Australian experimental data

263 (model 1) show a significant relationship between canola yield and disease severity ($p < 0.0001$), with
264 an estimated slope of -3.73 kg/ha per disease progression point.

265 *Context dependency in the relationship between disease severity and yield*

266 There was a significant impact of resistance rating on the relationship between disease
267 severity and yield (Figure 3). In general, the relationship was negative, such that on average, cultivars
268 with higher levels of genetic resistance (rated R and R-MR) had lower levels of disease severity,
269 whereas more susceptible groups showed significantly (p-value ≤ 0.05) higher disease levels (Fig. 2b,
270 Table 3). Estimated slopes ranged from -1.32 kg/ha per disease progression point (group R) to -6.05
271 kg/ha per disease progression point (group MS-S).

272 Disease severity also varied according to environmental conditions (Tables S2, S3), with
273 locations showing highly different disease severity levels. (Figure 2a, Table S2). For the separate
274 models run for each location (Figure 4), there was a wide range of relationship slopes. 6 out of 16
275 models yielded significant (p-value ≤ 0.05) yield-disease relationships, with estimated slopes ranging
276 from -2.81 to -28.48 kg/ha per disease progression (mean -9.11 kg/ha). Non-significant slopes likely
277 reflect small sample sizes for many localities. Environment and location were also major sources of
278 variation for disease severity in our experimental data and was also responsible for almost 69% of
279 yield variability not explained by fixed-effect explanatory variables in the main mixed-effects model.

280 Yield observations were clustered together for each Year x Location x Cultivar combination
281 and a univariate linear regression model was used to compute yield-disease slopes. Outputs from the
282 model derived to estimate yield loss in percentage terms (Fig. 5), indicate that a fully diseased crop
283 (i.e. CSII = 100) would result in a 34 % reduction in yield, a CSII score of 50 results in a 17% yield
284 reduction, and at a 25 CSII severity score (which was observed more frequently), yield is reduced by
285 8.5% of its potential without disease.

286

2. Meta-analysis

A total of 11 studies comprising 24 yield-disease slopes were included in the final analysis. Effect sizes were highly variable among experiments (Q(df = 23)= 249.3558, p<0.0001). Disease-yield relationship slopes obtained from studies ranged from −51.22 to −1.9610 kg.ha^{-1}.SP^{-1}. The estimated average outcome based on the random-effect model was μ^ = $slope\ hat$ = -16.69 kg.ha^{-1}.SP^{-1} (95% CI: -24.08 to -9.31), with a standard error of 3.77 kg.ha^{-1}.SP^{-1}. For example, using this estimate, the predicted mean yield penalty for an intermediate level of blackleg crown canker severity (i.e. 50%) would be 834.5 kg per hectare. A forest plot showing all slopes and the overall estimate based on the random-effects model, along with a summary estimate for each country-based group of values is shown in figure 6. An examination of the studentized residuals revealed that the effect-size from (Hwang et al. 2016) may be a potential outlier in the context of this model. According to the Cook's distances, this effect-size could be influential. The estimate taken from this study was based on data reporting the median disease severity and mean yield values for grouped observations. This led to a slope based on less observations of yield and disease severity than would have been originally available in the experiment, therefore this slope value had a bigger standard error than what the original data would have led to, and the association between yield and disease severity was weaker.

A funnel plot was used to assess potential bias in the reporting of results in published studies. (Fig 7). The rank correlation test indicated funnel plot asymmetry was significant (Kendall's tau = -0.4420, p-value = 0.0021). More specifically, the results show that the standard error is not normally distributed around the predicted mean effect size. Rather, the observed distribution of standard error is more consistent with an inverse of the predicted, in that the standard error peaks around the mean. (Fig 7). While this result could be interpreted as showing potential for publication bias, the increased variance at intermediate values likely reflects the fact that yield-disease outcomes are likely more stochastic at intermediate values. Furthermore, the studies included in this work were not selected out of a pool but rather represent the entirety of the available literature containing yield-disease

312 relationship slope estimates for canola and blackleg or data allowing to create such metric. Therefore,
313 results generated from this sample of studies (as a sample of all studies, including studies that will or
314 might have been conducted) is considered valid for further interpretation.

315

Discussion

The potential for *Leptosphaeria maculans* to cause severe yield losses in canola when environmental conditions are favourable and the disease is left unchecked are well documented (Li et al. 2003; Sprague et al. 2006a). Here, we extend previous work on the impacts of blackleg on canola by examining relationships between canola yield and blackleg disease severity across a wide range of environmental, genetic and agronomic conditions. Using data collected from a series of field experiments conducted over multiple years and across a large range of environments in Australia, we show that blackleg severity has a statistically significant impact on canola yield, despite high levels of variability in yield due to spatial and environmental factors. Results from these experiments further show that the relationship between severity and yield is context dependent. Regression slopes describing the yield-disease relationship varied depending on the resistance rating of canola cultivars and environmental conditions. We further placed these results within a broader geographic and agronomic context by gathering estimates of the relationship between blackleg disease severity and yield from all identified, published independent studies using a meta-analysis.

A 'universal' linear model fitted to the full dataset demonstrated that crown canker caused by *L. maculans* significantly impacted canola yield under modern Australian farming conditions, generating an estimate of yield loss to disease of approximately -3.7 $kg.ha^{-1}.SP^{-1}$ across all trials and cultivars. This value was derived from experiments performed over 4 years across a wide range of localities, using a diverse array of modern canola varieties. It can therefore be argued that this value represents a sound estimate of average, absolute crop losses relating to disease severity across current Australian farming systems. While using this single point estimate is unlikely to be accurate for specific cultivars and environments, this value is currently the best available general estimate for extrapolating average crop losses due to varying amounts of disease severity at the

national scale in Australia (e.g. economic forecasting of losses to disease). Specifically, generating estimates of yield loss using the parameter generated in the "universal" model requires only basic data on area planted to canola and an estimate of mean disease severity. For example, data from long-term monitoring trials indicate that in the absence of fungicide, blackleg disease severity levels among commonly commercially available cultivars of Canola in Australia average around 30% internal stem infection (Van de Wouw et al. 2021), while in 2021, approximately 3 million ha was planted to Canola in Australia (https://www.agriculture.gov.au/abares/research-topics/agricultural-outlook/australian-crop-report/overview). Assuming a severity of 30% internal stem infection across all Australian grain growing regions, a simple estimate of potential yield loss (in the absence of management using fungicides) attributable to blackleg of 333,000 tonnes can be forecast for Canola in the 2021/22 harvest without requiring any other information.

In addition to deriving an absolute estimate of crop losses across all trials, we extended the analysis to generate an estimate of the relationship between yield and disease severity in relative terms. Specifically, for the Year x Location x Cultivar (YLC) combinations that showed a significant effect of disease on yield, we used the intercept of each model as an estimate of yield potential without disease, and calculated proportional yield (i.e. observed yield / model intercept) as it related to disease severity. We then used this data to perform a simple univariate linear regression between disease severity and relative yield for each YLC combination. While this is a more complex and derived approach than that based on observed yield data as described above, estimates of relative yield loss are valuable for estimating yield gaps because yield loss per severity point is likely to vary with yield potential (ref). While relative yield is most simply assessed as yield relative to a disease-free control treatment, in this dataset, the absence of standard controls across trials prohibited the calculation of relative yield using such an approach.

Regression analysis of relative yield and disease severity generated an estimate of 0.34% yield loss per disease point across all trials and cultivars (i.e. CSII scores of 100, 50 and 25 are predicted to result in a 34%, 17% and 8.5% reductions in yield respectively). Revisiting the above scenario of a mean 30% CSII across all growing regions, estimates of yield loss of canola to blackleg, using this relative yield loss parameter, are predicted to be 10.2% overall. Canola production for the 2021/22 harvest is forecast to approximately 5 million tonnes (Abares link), leading to an estimate of potential yield loss attributable to blackleg of 510,000 tonnes with an approximate market value of $450 million AUD at $880 per tonne. An estimate of 34% yield reduction for complete stem cankering seems very low given reports of nearly total crop loss to disease in past epidemics (Sprague et al. 2006b). This disparity potentially reflects model inaccuracy model at high severity values due to the small number of experimental observations above 75%. We note the trend for relative yield to decline sharply at CSII values greater than 75% and it is possible there is a threshold at which the slope of the yield-disease relationship becomes non-linear. Structural damage to the stem may be more critical to yield than host plant metabolic costs (redirection of water and nutrients along with the fungus's feeding off plant cells) beyond a threshold, especially if paired with heavy rainfall. Hence, for further analyses we suggest that use of this relative yield loss parameter be restricted to values of CSII at 60% or less.

This study clearly demonstrated that the impact of blackleg disease on canola yield was variable depending on environmental parameters and the genetics of the interaction. While previous work has repeatedly demonstrated that disease severity can be influenced by environment and host-pathogen genetics, there are relatively few studies showing that the relationship between severity and yield can likewise vary. Our analysis of resistance groups indicated a general decrease in the yield-disease severity slope as cultivar resistance rating increased. More resistant cultivars not only experienced lower levels of severity, but also suffered less yield loss for a given level of disease (with an aberration for the Medium-Susceptible group, potentially reflecting small sample size for this class). This result is consistent with an increase in disease tolerance corresponding with increasing resistance, where tolerance can be defined as the rate of change in crop yield as disease severity

increases, such that a more tolerant cultivar will suffer a smaller loss in yield per unit increase of disease severity (ref). While more work is obviously needed to demonstrate variation in tolerance in this system, a positive relationship between resistance and tolerance in canola cultivars can be explained quite simply. Breeding for resistance to *L. maculans* does not occur in isolation. Rather, selection for resistance likely occurs concurrently (at least to some extent) with selection for yield and other agronomic traits. As a result, it is quite feasible that breeders simultaneously select for cultivars with lower levels of stem infection and increased tolerance to infection. A similar scenario has been postulated for selection of wheat varieties with tolerance to *Zymoseptoria tritici* (Mikaberidze and McDonald 2020).

In addition to plant genotype, environment and location were major sources of variation in the relationship between canola yield and blackleg disease severity. This variance likely reflects complicated interactions between plant genotype, pathogen genotype and environment. In the interaction between *B. napus* and *L. maculans*, yield reductions are largely the consequence of disease-related damage to the stem vasculature, which results in reduced water and nutrient transport through the plant (Sprague et al. 2009a). Hence, any environmental variation in water or nutrient availability has potential to influence the relationship between crop yield and disease severity. For example, assuming blackleg-related damage to the vascular system reduces water transfer from roots to seed pods, impacts on yield could be expected to be stronger when water is limiting (i.e. under dry conditions). However, in our analyses we were unable to identify any clear links with seasonal rainfall or other environmental patterns. This may in part reflect that we were unable to control for both location and rainfall in the analysis due to limitations in the experimental design and collinearity between these two variables. As a consequence, we were unable to account for variation in other factors linked to spatial location (e.g. soil nutrients, temperature), which may also influence the relationship between disease severity and yield. We expect that soil characteristics, and weather conditions (and various interactions with plant and pathogen genotypes) are likely nested within the effect of location.

Controlling for such variation would require specifically accounting for such interactions during the experimental design phase. Such a deliberate approach was not possible here given that this study was enabled by repurposing a diverse mix of data collected during multi-year fungicide trials. Indeed, the dataset used in our analyses was unbalanced and incomplete. This limited the power and range of analyses we were able to perform. Nevertheless, the data provide a unique snapshot of canola yield in response to varying levels of disease over multiple years and locations, using a range of different canola cultivars. The results derived from it reflect the a realistic assessment of blackleg disease on canola in the context of modern Australian cultivation practices The consistency of yield distribution with Australian statistics on yield (D.E. Seberry *et al*, 2018) [3] supports this idea and although low severity values were much more frequent than high ones, this is to be expected when working with resistant cultivars that are ranked using a quantitative scale.

Transition to Meta-analysis results

Overall, it seemed the yield losses and yield-disease relationships estimated from this data (-3.7 $kg.ha^{-1}.SP^{-1}$ for the overall relationship and ranging from -1.32 $kg.ha^{-1}.SP^{-1}$ to -6.05 $kg.ha^{-1}.SP^{-1}$ for different resistance groups) were weaker than the estimations usually found in the literature. This was confirmed in the meta-analysis. The meta-analysis performed in this study showed that future observations of the canola-blackleg yield-disease relationship should fall between -9.31 and -24.08 $kg.ha^{-1}.SP^{-1}$, centered on -16.69 $kg.ha^{-1}.SP^{-1}$. Although analysis of the funnel plot obtained from the studies included in this work showed asymmetry, and therefore possible bias of the study sample, all the data that could be found in the literature to create yield-disease relationships was gathered. Therefore, any bias revealed here may affect the whole field of research on blackleg of canola.

Overall, relatively few studies have focused on quantifying disease-yield relationships (Khangura and Barbetti 2005), or made available data that can be used to create such a metric (Ballinger et al. 1988; Khangura and Barbetti 2002; Sprague et al. 2009a; Khangura and MacLeod 2011; Hwang et al. 2016; GC Upadhaya et al. 2019; Kutcher) and most instead focus on the effects of chemical treatments (Madden and Paul 2009).

Remarks on Meta-analysis

It is important to keep in mind that the disease-yield regression models used in our meta-analysis do not include environmental parameters. They only relate yield to disease severity because the data gathered from the studies used did not always include environmental variables, and only models that use the same variables can be compared. However, the variability in the slope of the relationship based on environmental conditions and level of genetic resistance exists in the meta-analysis data because studies were performed in different locations, over several years, and with different cultivars. Therefore, we can be confident that the results of this work are not just representative of blackleg on canola under specific conditions, but account for the overall disease-yield relationship, with data coming from Australia, the UK, and Canada.

Remark on the need for harmonisation of methods and metrics.

Harmonisation of metrics used to describe the disease would be valuable. That argument had already been made by Teng and Gaunt in 1980 and can also be found in Cooke et al. (2006), citing Nutter et al, 1991 and Watson and Morton, 1990. Defining a set of metrics that should be reported in all studies dealing with this disease would also make results from the overall literature, such as the meta-analysis conducted here, more robust. Databases could be established to gather such metrics from all studies conducted worldwide. If companies allocated budget to assessing mean disease severity in farmers' fields, such databases would quickly become large, allowing more accurate results and maybe finer analysis.

In the future

Remaining to be developed is a method of predicting severity at the end of the growing season based on early infection symptoms. Along with an accurate estimate of the yield-disease relationship, this would allow predicting yield loss and therefore help decide whether the use of a fungicide is economically justified. The predicted range of yield loss may also be made more accurate by using yield-disease relationships calculated for the resistance group to which the observed cultivar belongs. This would require gathering enough coupled yield and severity observations in each resistance group to make such slopes reliable.

Genetic resistance durability: look back and look forward

Genetic resistance durability has become an ongoing highly studied scientific topic (Parlevliet 2002; Palloix et al. 2009; Rimbaud et al. 2018a; Rimbaud et al. 2018b; Rousseau et al. 2019) but land use (e.g. intensive crop rotations) (Kutcher et al. 2013) and cultivar breeding programs (Palloix et al. 2009) along with associated deployment strategies (Rimbaud et al. 2018a) still present challenges to ensuring ecologically sound yield maintenance / improvement.

Conclusion

The dataset collected led to an estimate of the canola-blackleg yield-disease relationship of around -3.7 $kg.ha^{-1}.SP^{-1}$ accounting for Australian cultivation practices. The meta-analysis showed there is heterogeneity in the yield-disease relationship across scientific studies. It also led to an estimate of -16.69 $kg.ha^{-1}.SP^{-1}$ within a range going from -9.31 to -24.08 $kg.ha^{-1}.SP^{-1}$ point. This suggests that the average slope of the yield-disease relationship may be stronger than the estimate computed from this dataset. On the other hand, 6 out of 10 studies included in the meta-analysis were designed to assess the effects of fungicide treatments, and these studies usually require high pathogen

pressure. Therefore, we may think that the "real" average slope of the yield-disease relationship in the fields of growers may thus more likely lie between the estimated slope value −16.69 $kg.ha^{-1}.SP^{-1}$ and the upper bound of the confidence interval, -9.31 $kg.ha^{-1}.SP^{-1}$ rather than in the lower part of the confidence interval. This work highlights the necessity for independent replications of experiments: through meta-analysis, more robust results will come from including more independent studies and effect-sizes. In this case, as was said in the introduction, having an accurate value of this parameter, and developing a way of diagnosing the potential severity level at maturity early might, through eased decision-making, contribute to securing farmers' income while limiting fungicide use. This makes for an interesting goal.

In addition, the values derived from the literature search and meta-analysis provide potential to build a sampling distribution of the global severity-yield relationship for additional parametrization of such models.

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512 **Table 1. Studies included in the meta-analysis**. Experimental details are summarized to give an idea
513 of each dataset.

Authors	Year	Study	Experimental details
Ballinger et al	1988	Evaluation of flutriafol for control of blackleg of rapeseed.	1 year, 2 sites, 2 cultivars (cv. Tatyoon and Wesbrook) with one not harvested in one site, 7 fungicide treatments in one site, 3 in the other, 3 replicates for each treatment.
Kutcher	1990	Studies on blackleg disease of oilseed rapes: germplasm evaluation, variation for virulence and yield loss/disease relationships.	2 years, 2 sites one year and 4 sites the next with one site in common, 6 cultivars.
Zhou et al	1999	Effects of severity and timing of stem canker (Leptosphaeria maculans) symptoms on yield of winter oilseed rape (Brassica napus) in the UK	4 sites, 1 year in each site (but different actual years). 1 cultivar in 3 site-years, 2 cultivars in 1 site-year. Different fungicide treatments in different site-years.
Khangura & Barbetti	2005	Time of sowing and fungicides affect blackleg (Leptosphaeria maculans) severity and yield in canola.	2 years, 2 sites one year and 5 sites the second year. Total of 6 different sites, 4 replicates of 5 treatments (including fungicides) with 6 cultivars.
Steed et al	2007	Relating plant and pathogen development to optimise fungicide control of phoma stem canker (Leptosphaeria maculans) on winter oilseed rape (Brassica napus).	3 years, 1 sites, 1 cultivar (cv. Apex). 9 treatments (combination of different fungicide application timings), 3 replicates.
Sprague et al	2010	Effect of root rot and stem canker cause by Leptosphaeria maculans on yield of Brassica napus and measures for control in the field.	2 cultivars (cv. Rainbow & Grace), 3 years (1 with one cultivar and 2 with the other), 1 site, fungicide present/absent.
Khangura & MacLeod	2011	Yield losses from blackleg (Leptosphaeria maculans) in canola varieties under moderate disease pressure.	1 year, 2 sites, 10 cultivars, 4 replicates of different fungicide treatments.
Kutcher et al	2013	Blackleg disease of canola mitigated by resistant cultivars and four-year crop rotations in western Canada	2 sites, 10 years in one site, 8 years in the other, 2 cultivars, 4-replicate split-plot of 7 rotations.
Hwang et al	2016	Blackleg (Leptosphaeria maculans) severity and yield loss in canola in Alberta, Canada.	3 years, 2 sites, 4 cultivars.
Upadhaya et al	2019	Efficacy of fungicide seed treatments in controlling blackleg of Canola	2 years, 1 site, 1 cultivar (cv. Westar), 4 seed fungicide treatments + untreated, 4 replications, randomized complete blocks.
Daurelle, et al	2021	Relationship between Leptosphaeria maculans (blackleg) infection and grain yield of Brassica napus (canola): experiment and meta-analysis	4 years, 22 cultivars, 16 locations, 4 replications of 13 different fungicide tratments.

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Table 2. percentage of yield variance explained by formula (1) random-effect model variables. Year, Location and Cultivar are used as random effects on the intercept of the multiple linear regression linking yield to disease severity, rainfall from April to June and total rainfall, making it a mixed-effects linear model. Each variable used as random effect accounts for a part of the deviation of observed yield values from fixed-effects predicted values.

Variable	Yield variance	% of total
Year	15724	2.11
Location	512674	68.81
Cultivar	106888	14.35
Residual	109754	14.73
Total	745040	100
Model	635286	85.27

Table 3. median, mean and difference from mean to group MR mean (estimate) disease severity across blackleg resistance groups. Standard error and p-value relate to the estimate computed through one-factor linear model: random effect of blackleg resistance group on disease severity. Model summary in R showed all means were significantly different from MR mean (p-values reported here), and Type II (Anova() command) showed significant effect of resistance group (Blackleg rating) on disease severity (p-value = 1.818627e-75). n = number of severity (and yield) observations in each group.

Blackleg rating	n	median severity	mean severity	estimate	estimate std error	p-value
R	288	2.013513514	7.581814	-12.21632913	1.543479828	5.13705e-15
R-MR	101	5.5	8.554455446	-11.24368769	2.189216231	3.21996e-07
MR	347	16.5	19.79814313	19.79814313	1.039466121	8.40192e-72
MR-MS	217	22.85714286	27.59539492	7.797251792	1.675791658	3.59589e-06
MS	217	35.5	35.2986081	15.50046497	1.675791658	8.56134e-20
MS-S	182	27.1	34.66642127	14.86827814	1.77215817	1.21422e-16

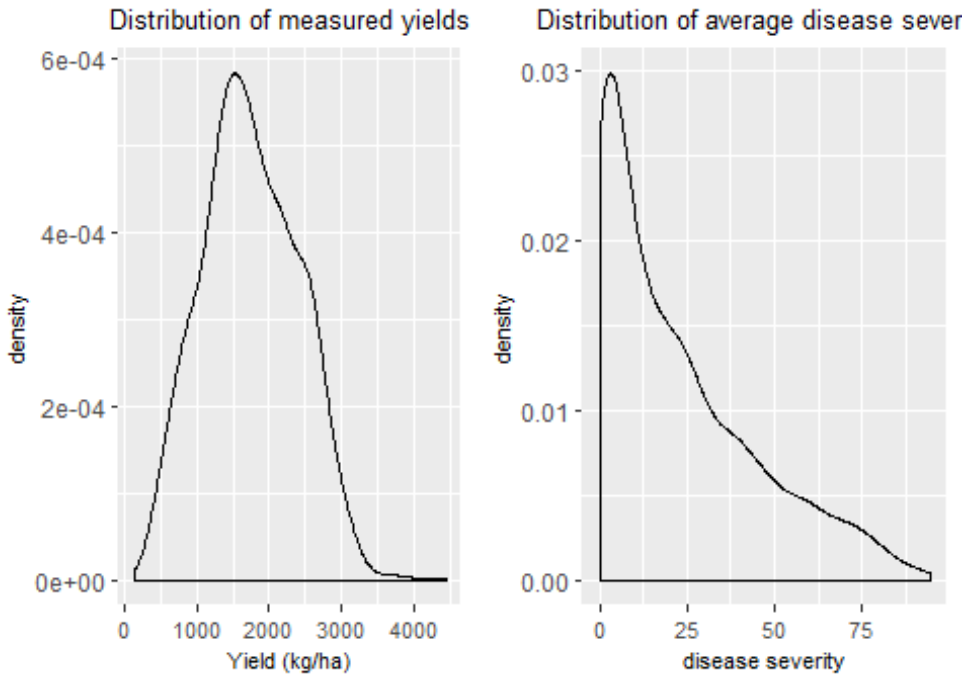

Figure 1. Distributions of yields and disease severities observed in the field experiments of this study. Ranges of disease severity and grain yield were both broad, and normal distribution can be assumed for yield, but not for severity. Values at or close to zero predominated in disease severity. Nevertheless, it is clear the experimental design managed to capture a wide range of severity levels.

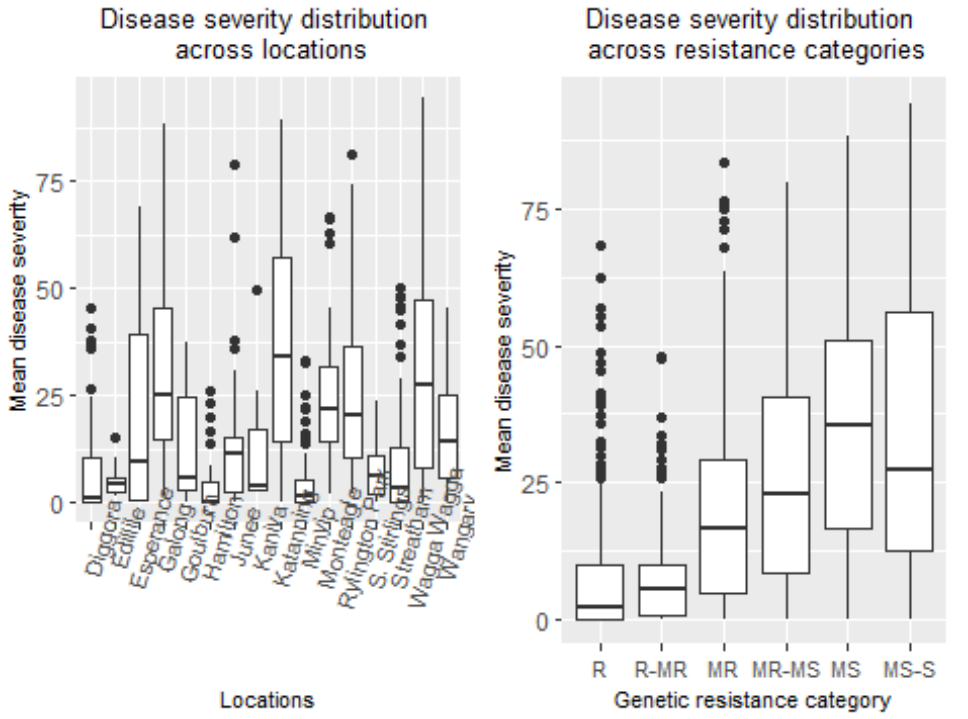

Figure 2. Distribution of severity across locations (a) and resistance categories (b). a) Heterogeneity of severity values across locations displays the impact of environmental conditions (rainfall, temperature, soil parameters) on disease severity. b) Cultivars with higher genetic resistance (rated R and R-MR) tend to show lower disease severity, whereas more susceptible groups (rated MS and MS-

S) show significantly higher disease levels. This displays the effect of genetic resistance level on disease severity.

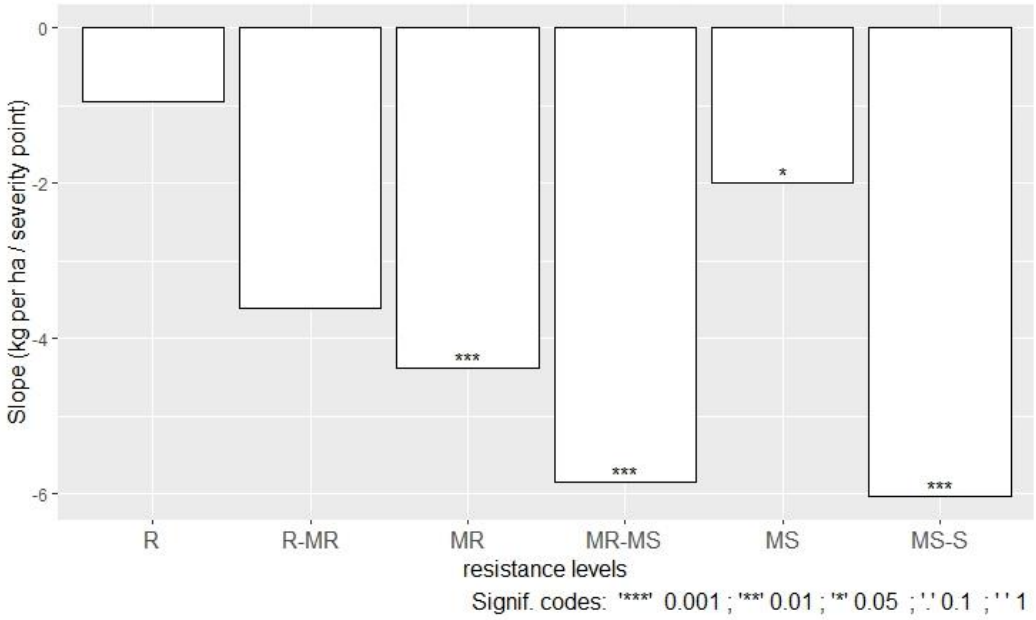

Figure 3. Yield-disease relationship slopes across resistance groups. Yield-disease relationships are weaker for cultivars of higher genetic resistance groups. Those lose less yield than cultivars belonging to more susceptible groups when confronted to the same disease severity level. This indicates tolerance mechanisms are associated with the resistance genes selected in resistant cultivars. The MS group relationship (-2.05 kg/ha per disease severity point) is expected to be due to sampling issues.

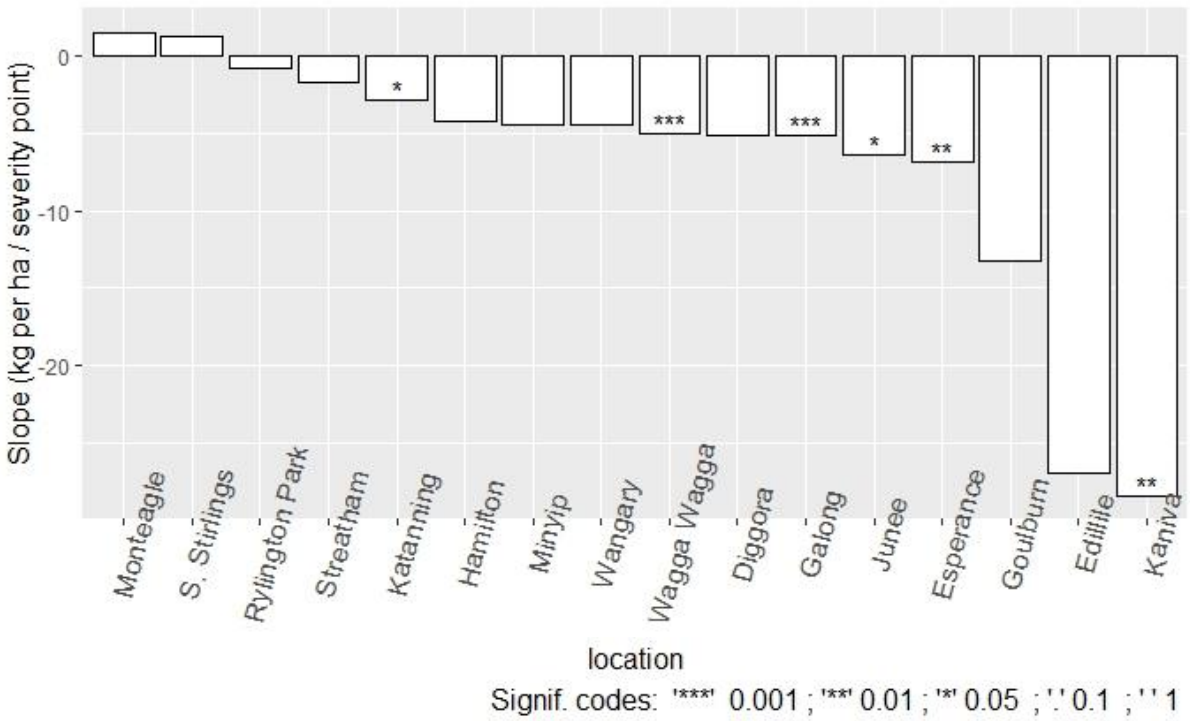

Figure 4. **Disease-yield relationship slopes across locations**. Different significant slopes for some locations suggest environmental parameters (rainfall, temperature, soil characteristics) – and to some extent the genetics of the host-pathogen encounter, given then unbalanced quality of the dataset – affect the strength of the yield-disease relationship.

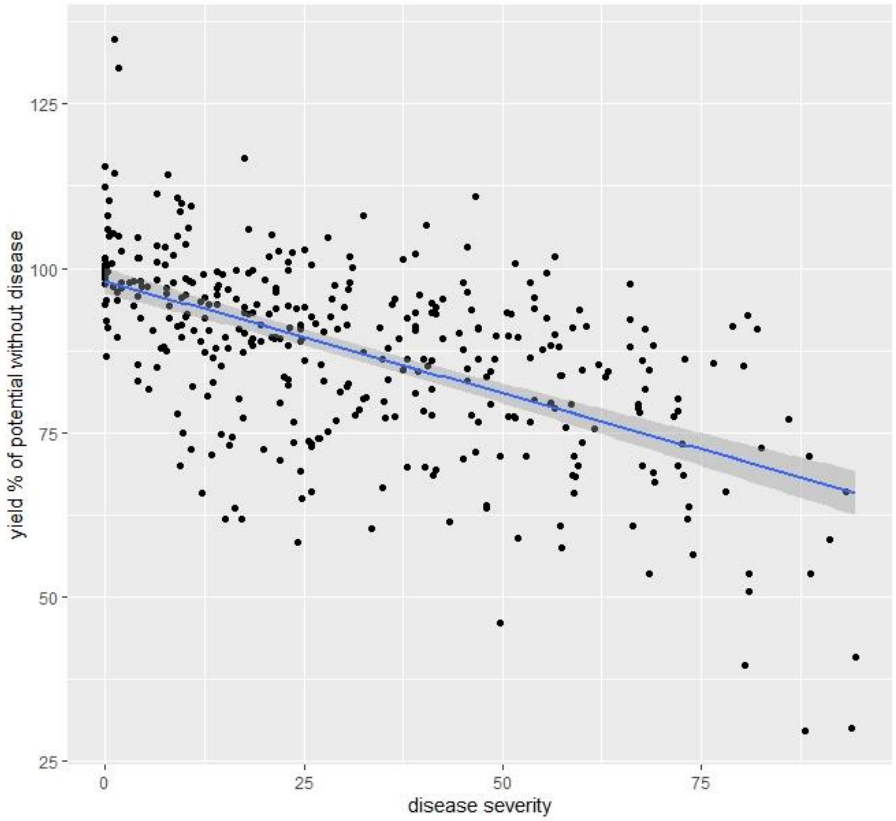

Figure 5. **Linear regression of yield percentage of potential without disease against disease severity**. Yield-disease relationship is established for yield in kg/ha among YLC (Year x Location x Cultivar) clusters, then clusters which exhibit a significant relationship between severity and yield are selected (= separated from those which don't). Yield is then ratioed to the cluster intercept of the yield(kg/ha)-disease linear model and multiplied by 100. Yield % of potential without disease values are then gathered back and a linear regression model is established between this transformed metric of yield and disease severity across selected clusters. Nota bene: there is a 1:1 ratio of yield:severity -coupled- values used. In other words, no yield is related to a disease severity value that did not happen in the plot where it was recorded.

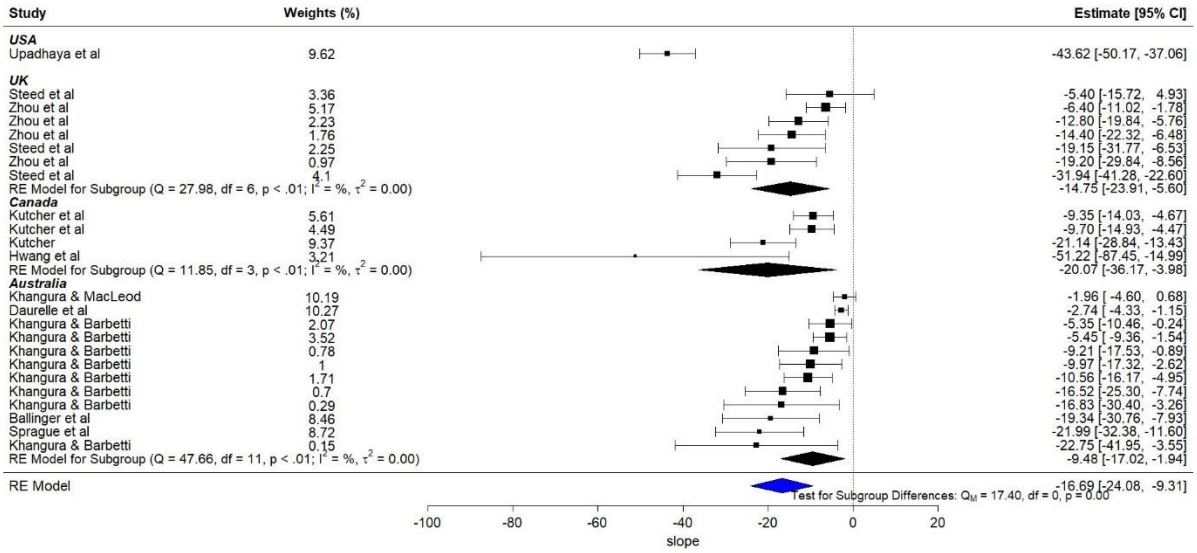

Figure 6. Forest plot of the slopes, summary estimates for each country, and overall summary estimate of the random-effect model. All slope values included are represented along with their confidence interval. The edges of the blue lozenge mark the confidence interval of the summary, average slope, which lies at its center. This graph includes the estimate calculated from Hwang et al (2016) data.

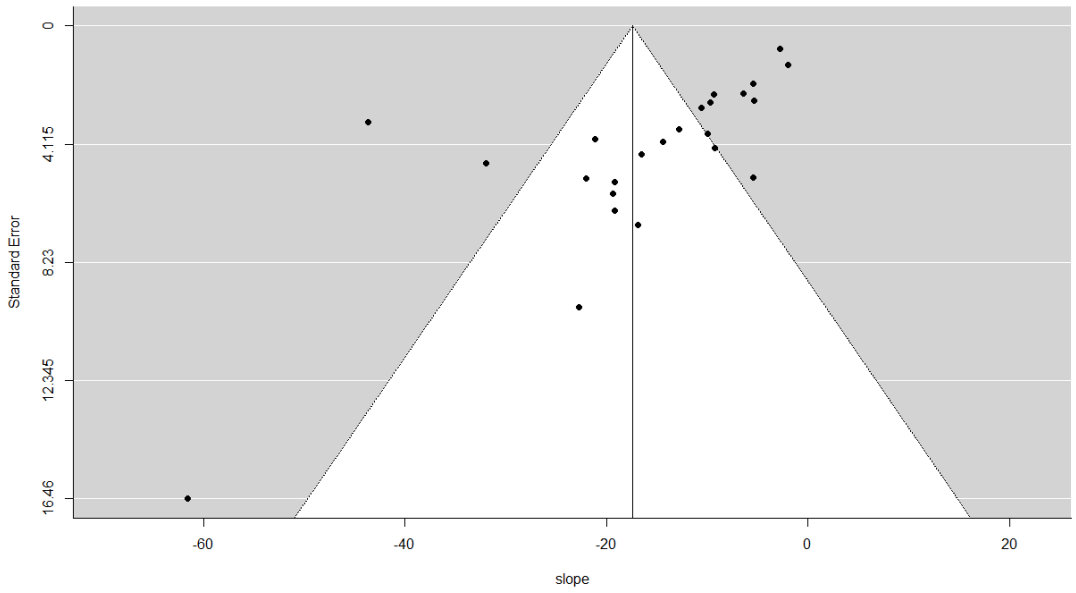

Figure 7. Funnel plot of the studies used in the meta-analysis. On the x axis is the value of the slope of each identified experiment. On the y axis is the standard error associated with it. Dots are supposed to be distributed symmetrically within the white triangle (or funnel), because estimates are supposed to be normally distributed around the true value of the slope. Values the furthest from the average are supposed to bear the greatest standard errors. Asymmetry indicates bias in the study sample, however all studies found in the literature were included. This graph also includes the estimate from Hwang et al (2016).

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