

Article

# A Meta-analysis of Fungicide Spray Schedules to Minimise Yield Loss from Powdery Mildew in Australian Mungbean

Paul Melloy<sup>1,\*</sup> , Emerson Del Ponte<sup>2</sup> , Adam H. Sparks<sup>1</sup> 

<sup>1</sup> University of Southern Queensland Centre for Crop Health Toowoomba, Queensland, 4350 Australia;

<sup>2</sup> Universidade Federal de Viçosa Departamento de Fitopatologia Viçosa, 36570-000 Minas Gerais, Brazil;

\* Correspondence: [Paul.Melloy@usq.edu.au](mailto:Paul.Melloy@usq.edu.au); Tel.: +61 7 4631 5527

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**Simple Summary:** A simple summary goes here.

**Abstract:** A single paragraph of about 200 words maximum. For research articles, abstracts should give a pertinent overview of the work. We strongly encourage authors to use the following style of structured abstracts, but without headings: 1) Background: Place the question addressed in a broad context and highlight the purpose of the study; 2) Methods: Describe briefly the main methods or treatments applied; 3) Results: Summarize the article's main findings; and 4) Conclusion: Indicate the main conclusions or interpretations. The abstract should be an objective representation of the article, it must not contain results which are not presented and substantiated in the main text and should not exaggerate the main conclusions.

**Keywords:** keyword 1; keyword 2; keyword 3 (list three to ten pertinent keywords specific to the article, yet reasonably common within the subject discipline.).

Mungbean [*Vigna radiata* (L.) Wilczek] is a pulse crop primarily grown in south-east Asia for human consumption as an affordable source of protein [1]. The bean pod or grain can be consumed raw or added to meals after sprouting. The dried grain can also be ground into a protein enriched flour for uses in noodles, biscuits and cakes [2].

Mungbean was first brought to Australia in the 1930s for use as a forage or green manure crop. However, it was only somewhat recently, during the 1960s that mungbean was grown commercially [3,4]. Prior to the 1970s the total area grown in Australia rarely exceeded 1000 hectares [3]. At the end of the 1980s, between 3,000 to 10,000 hectares were being harvested for dried beans in Australia, of which mungbean is categorised by the Food and Agriculture Organization of the United Nations' statistics data repository [5]. In the decade leading up to 2018, between 35 - 86.4 thousand hectares of dried beans were planted annually in Australia. This increase is attributed to high value export markets and to the improved yields in the new cultivars [6]. Currently mungbean is predominantly grown in southern Queensland and northern New South Wales as a short season summer legume crop. In 2019, approximately 90% of Australian mungbean was grown for export with lucrative returns of up to \$1300 AU per tonne [7]. Mungbean potentially yields up to 3 tonnes per hectare [8], but due to high variability in yields between seasons and locations, the average Australian farm still yields less than 1 tonne per hectare [4].

The high variability in yields can in part be attributed to a range of diseases that affect the crop that include fungi, bacteria and phytoplasmas [9–15]. Among the fungal diseases is powdery mildew, which can reduce yields by up to 40% [2] in susceptible cultivars. The disease in Australia currently is thought to be caused by *Podosphaera xanthii*, syn. *Podosphara fusca*. However, this description has

changed over time and a second species has also been cited as causing the disease but not yet officially classified [16]. While research outside of Australia refers to the causal agent of mungbean powdery mildew as *Erysiphe polygoni*.

The disease cycle of the pathogen can be as short as five days for a germinating conidia to infect the host and produce new reproductive structures, conidiophores, which produce more conidia, which disseminate to new infection sites [17,18]. Weather conditions that favour the rapid development of the disease are cool temperatures between 22 ° and 26 °C, and a leaf surface that is not overly wet for infection [19]. To date, the sexual cycle that produces chasmothecia has not been observed in Australian mungbean. And while the powdery mildew pathogens are obligate parasites, secondary hosts that allow the pathogens to over-season between mungbean crops have not yet been identified in Australia.

Due to the requirement for cool temperatures for infection, planting date is an effective strategy for preventing the development of powdery mildew in mungbean [20]. In southern Queensland and northern New South Wales, sowing dates between late spring (November) and mid-summer (January) are advised to avoid the conducive cooler autumn temperatures, which normally commence in March. Therefore, delayed sowing dates of late January into February increase the possibility powdery mildew will infect earlier in the crop's life cycle and cause greater yield losses than in earlier sown mungbean crops.

Cultivar selection is an additional strategy to mitigate yield damage from powdery mildew. While loci conferring resistance in mungbean have been identified [21,22]; to date, the focus in Australia has remained on breeding for disease resistance to bacterial diseases rather than powdery mildew due to the wide availability of fungicides for use in controlling powdery mildew. Quantitative disease resistance has been observed in some breeding lines overseas [2,23], however no evidence for how they compare to Australian varietal resistance could be found. Currently, Jade-AU has the the best disease resistance to powdery mildew (moderately susceptible), with cv. Green Diamond also having some resistance [18]. However, commercially available mungbean cultivars in Australia lack sufficient resistance to be used as the primary powdery mildew management strategy. Evidence of this is shown by field trials conducted in 2016 with the moderately susceptible cultivar, Jade-AU, where yield losses of up to 32.7% were recorded [24]. Additional strategies to cultivar choice, such as fungicide treatments, are necessary to limit yield loss when conditions for the disease are conducive.

There is a near complete lack of peer-reviewed literature summarising fungicide efficacy on powdery mildew in mungbean in Australia. Much of the experimental work has been published by funding agencies and state government extension departments as extension bulletins or other related type materials. Field trials conducted in eastern Australia over the past ten years have tested a range of fungicide active ingredients while also attempting to evaluate the best application time for the highest efficacy [19,24–29]. The trials showed that for fungicide applications to protect yield, a single fungicide application at first sign of the disease with another follow-up application two weeks later, if necessary, was the most effective [18,24]. However, due to the variability in the results between seasons and experiments, few of these studies produced a clear result based on a statistical analysis. This variability presents uncertainty for the best practice of fungicide spray schedules to mitigate yield losses from powdery mildew.

The collection of experiments mentioned above and other unpublished trials conducted in Australia presented an ideal opportunity for a meta-analysis to study fungicide timing to control mungbean powdery mildew. Meta-analyses are statistical tools which can analyse a collection of experiments that have a similar aim and produce a more accurate estimate of the true effect being measured. Using a meta-analysis can be useful in situations like this where several studies exist that have the same objective but may not provide a clear answer to the question due to variation in the results within the individual studies. The outcome of a meta-analysis provides a more accurate estimation of the true treatments effect because it considers the amount of variance in each study and weights the influence of each studies treatment effects according to its statistical accuracy. Typically

meta-analyses consider the effect of a single treatment against a control group across multiple independent studies [30]. However meta-analyses can also consider effect differences between multiple treatments and a control group. These are called multi-variate or network meta-analyses [31]. Multi-variate meta-analyses are particularly useful when there are no direct comparisons between two treatments in any of the included studies. Indirect comparisons can be made between these two treatments if the both had direct comparisons with one or more treatments in common, assuming no significant bias in the studies that investigated each of the treatments and which are subject to an indirect comparison [32].

Considering the November 2019 launch of the mobile app decision support system (DSS) PowderyMildewMBM [33], which provides a cost benefit analysis to assist growers in their decision “if” and “when” apply fungicide; a meta-analysis to evaluate and verify the best fungicide spray schedule seemed prudent. The aim of this meta-analysis was to determine, from a collection of unpublished studies, what spray management scenario provides the greatest yield protection from powdery mildew in Australian mungbean.

## 1. Materials and Methods

### 1.1. Trial Criteria for Inclusion and Description

The data for this study were obtained through studies that were conducted by the authors, FAIR (Findable, Accessible, Interoperable, Reusable) data available from GRDC Online Farm Trials <https://www.farmtrials.com.au/>, personal correspondence with colleagues and collaborating institutions. Trial data were obtained in different formats with varying levels of information. Raw data was obtained for some trials, others reported only the means of each treatment, with all but one trial reporting some form of variance along with the mean. In total, 26 trials were collated.

To ensure the correct question was asked of the analysis, the data needed to conform to strict criteria for inclusion. The criteria for inclusion of data in the meta-analysis first required a field trial testing fungicide efficacy for powdery mildew control on mungbean in the Grains Research and Development (GRDC) northern grains region of eastern Australia, which grows the majority of Australia’s summer crops. Data from trials conducted in this region had to include: the date when powdery mildew was first observed; disease incidence at the end of the growing season; fungicide application dates; the fungicide active ingredients; fungicide dose; and crop yield. The data were subset to only include fungicide treatments with the same mode of action; demethylase inhibitor (DMI) fungicides. Thus only tebuconazole and propiconazole were retained in the dataset and the 26 trials were reduced to 17 trials. Two more trials were removed because grain yield variance, which was required for the meta-analysis, was not reported. This reduced the final number of trials included in the meta-analysis to 15 (Table 1).

From the trials which were included in the meta-analysis, 2 were planted in late December, 5 in January and 8 were planted in February (Table 1).

In the meta-analysis, to ensure sufficient replication, we made no distinction between the tebuconazole and propiconazole fungicide treatments within the trials that met the selection criteria. The design of all trials included in this analysis were randomised complete block designs and were not previously published in peer-reviewed literature. Details of trial data are presented within a research compendium as a supplement to this publication [ ].

### 1.2. Response Variable - Grain Yield

Grain yield (tonnes per hectare), was used as the response variable for the meta-analysis. Mean grain yields for each treatment were either obtained directly from trial reports or calculated from the raw data when available. The sample variance was calculated from the raw data when available or the least squares statistic in the trial reports. Sample variance for the 2012 Kingaroy study was calculated from the reported least squares statistic using a T-critical value of 2.042 and the approach reported in

**Table 1.** Description of fungicide application trials included in meta-analysis to determine the most effective fungicide spray schedule to minimise yield loss from powdery mildew in Australian mungbean. Trials were all conducted in the Grains Research and Development (GRDC) Northern Grains Region of eastern Australia to test application timing for the best control of powdery mildew of mungbean.

| Unique Trial Reference | Year | Location     | Planting Date | Date First Sign | Research Organisation |
|------------------------|------|--------------|---------------|-----------------|-----------------------|
| mung1011/01            | 2011 | Hermitage    | 2011-01-24    | 2011-03-28      | DAF <sup>a</sup>      |
| mung1011/02            | 2011 | Kingaroy     | 2011-02-02    | 2011-03-22      | DAF <sup>a</sup>      |
| mung1112/01*           | 2012 | Gatton       | 2012-02-20    | 2012-04-02      | DAF <sup>a</sup>      |
| mung1112/02            | 2012 | Kingaroy     | 2012-02-03    | 2012-03-12      | DAF <sup>a</sup>      |
| AM1303                 | 2013 | Premer       | 2012-12-28    | 2013-02-28      | NGA <sup>b</sup>      |
| AM1304                 | 2013 | Marys Mount  | 2012-12-24    | 2013-03-16      | NGA <sup>b</sup>      |
| AM1305                 | 2013 | Goolhi       | 2013-01-23    | 2013-03-25      | NGA <sup>b</sup>      |
| BB1305                 | 2013 | Millmerran   | 2013-01-12    | 2013-03-13      | NGA <sup>b</sup>      |
| mung1415/01            | 2015 | Hermitage    | 2015-01-19    | 2015-03-16      | DAF <sup>a</sup>      |
| mung1516/01            | 2016 | Hermitage    | 2016-02-03    | 2016-03-08      | DAF <sup>a</sup>      |
| mung1516/02            | 2016 | Kingaroy     | 2016-02-11    | 2016-03-09      | DAF <sup>a</sup>      |
| mung1516/03*           | 2016 | Emerald      | 2016-02-12    | 2016-03-17      | DAF <sup>a</sup>      |
| mung1617/01            | 2017 | Hermitage    | 2017-02-13    | 2017-03-24      | USQ <sup>c</sup>      |
| mung1617/02            | 2017 | Missen Flats | 2017-01-27    | 2017-03-07      | USQ <sup>c</sup>      |
| mung1718/01            | 2018 | Wellcamp     | 2018-02-13    | 2018-03-21      | USQ <sup>c</sup>      |
| mung1819/01            | 2019 | Hermitage    | 2018-02-04    | 2018-04-12      | USQ <sup>c</sup>      |
| mung1819/02            | 2019 | Hermitage    | 2018-02-18    | 2018-04-12      | USQ <sup>c</sup>      |

<sup>a</sup> Queensland Department of Agriculture, Fisheries and Forestry

<sup>b</sup> Northern Growers Alliance

<sup>c</sup> University of Southern Queensland

Ngugi *et al.* [2011]. The T-critical value was obtained from a t-distribution table using, 30 degrees of freedom and probability of  $\alpha = 0.05$ .

Similar to Paul *et al.* [2007], we fitted a multivariate random effects model  $Y_i \sim N(\mu, s_i^2 + \sigma^2)$  where  $Y_i$  is the response, a vector of the grain yield measurements (tonnes per hectare) for each treatment in the  $i$ th trial over total number ( $K$ ) of trials ( $i = 1, \dots, K$ ).  $Y_i$  is estimated from a normal distribution with the mean  $\mu$ , sample variance of  $S_i^2$  and a between trial variance of  $\sigma^2$ .

### 1.3. Trial Variables and Moderators

While the main aim of all the identified trials was to assess the efficacy of fungicide for control of powdery mildew, some trials tested additional potential covariates in the experimental design. These additional variables included: row spacing, fungicide active ingredient, fungicide dose, planting date, number of fungicide applications and cultivar. To account for the variation attributed to some of these variables between trials, and within trials, a single *trial* factor was created. The *trial* random variable made distinctions between the following variables: unique trial reference code, trial location, trial season, host genotype, row spacing, and fungicide dose.

The random effects meta-analysis was expanded to evaluate if the residual heterogeneity could be explained by spray schedule. Spray schedule was assigned as an inside factor to the outside random factor *trial* to provide correlated random effects between the different spray schedule treatments within *trials*.

The timing of fungicide application treatments was defined in relation to the recorded date when powdery mildew first appeared in the trial. An *Early* fungicide application treatment referred to a fungicide spray schedule that commenced prior to the disease being observed in the trial. *Recommended* referred to a fungicide spray schedule which commenced 1 – 3 days following the first sign of disease. *Late* treatments commenced between 7 and 13 days after the first sign of disease. There were no

**Table 2.** Powdery mildew severity scoring scale.

| Scale | Scale.Description  |
|-------|--|
| 1     | No sign of powdery mildew  |
| 2     | Small colonies in lower 1/3 of the canopy, up to 75% of the plants affected                                      |
| 3     | Colonies in lower 1/2 of the canopy, > 75% of the plants affected  |
| 4     | Colonies in lower 2/3 of the canopy, up to 75% of the plants affected  |
| 5     | Colonies in lower 2/3 of the canopy, > 75% of the plants affected  |
| 6     | Colonies in lower 2/3 of the canopy, 100% of the plants affected   |
| 7     | Colonies in lower 2/3 of the canopy, 100% of plants, with some plants with colonies in the top 1/3 of the canopy |
| 8     | Colonies to top of the plant with > 75% of the plants affected   |
| 9     | Colonies to top of the plant with > 100% of the plants affected and heavy leaf drop                              |

spray schedule treatments which began between 4 and 6 days after the first sign of powdery mildew. The number of fungicide applications was binned into two categories. A spray schedule with a *single* spray, and a spray schedule with two or three spray applications *plus*. The division of the treatments produced: 13 *Early<sub>single</sub>* treatments; 5 *Early<sub>plus</sub>*; 32 *Recommended<sub>single</sub>*; 46 *Recommended<sub>plus</sub>*; 17 *Late<sub>single</sub>*; and 20 *Late<sub>plus</sub>*. *Early<sub>plus</sub>* treatments were removed from the subsequent meta-analysis, due to an insufficient sample size.

What remained was the mean yield for a total of 173 treatments in the following five spray schedule categories: no spray control, *Early<sub>single</sub>*, *Recommended<sub>single</sub>*, *Recommended<sub>plus</sub>*, *Late<sub>single</sub>*, and *Late<sub>plus</sub>*.

An ordinal 1 – 9 scale was used to describe the mean powdery mildew plot severity in most trials (Table 2). However, the 2013 NGA trials (Table 1) reported mean plot severity as the percentage of leaves covered by powdery mildew and the location of the infected leaves in the lower, middle or upper canopy. Given the ordinal scale ranked plots on the percent of foliage showing the disease from the lower canopy to the upper canopy, a conversion from percentage of the diseased canopy, to the 1 – 9 scale was straight-forward. The end of season mean plot severity, and date of first sign of disease were used to calculate the area under the disease progress curve (AUDPC) for each treatment. Additional powdery mildew mean plot severity observations made in-between these two observations were also included in the AUDPC calculation if they were available. AUDPC was calculated using the *agricolae* package in R [36]. A categorical disease pressure factor was created by binning trials into two levels based on the mean AUDPC of the no spray control plot. The two levels, “low disease pressure” and “high disease pressure”, were separated by the median AUDPC (153.625) of the no spray control plots.

The mean difference in grain yield was estimated from the difference in model estimates of the non-treated control and each of the spray schedule treatments using fungicide.

Disease pressure was tested against the basic model to determine if, when introduced as a moderator variable, it could significantly explain additional heterogeneity between the spray schedule treatments. A Wald-type test was used to test the impact of the disease pressure factor.

Linear contrasts were used to produce the mean difference in effect sizes, and their respective standard errors and 95% confidence intervals, between each spray schedule treatment.

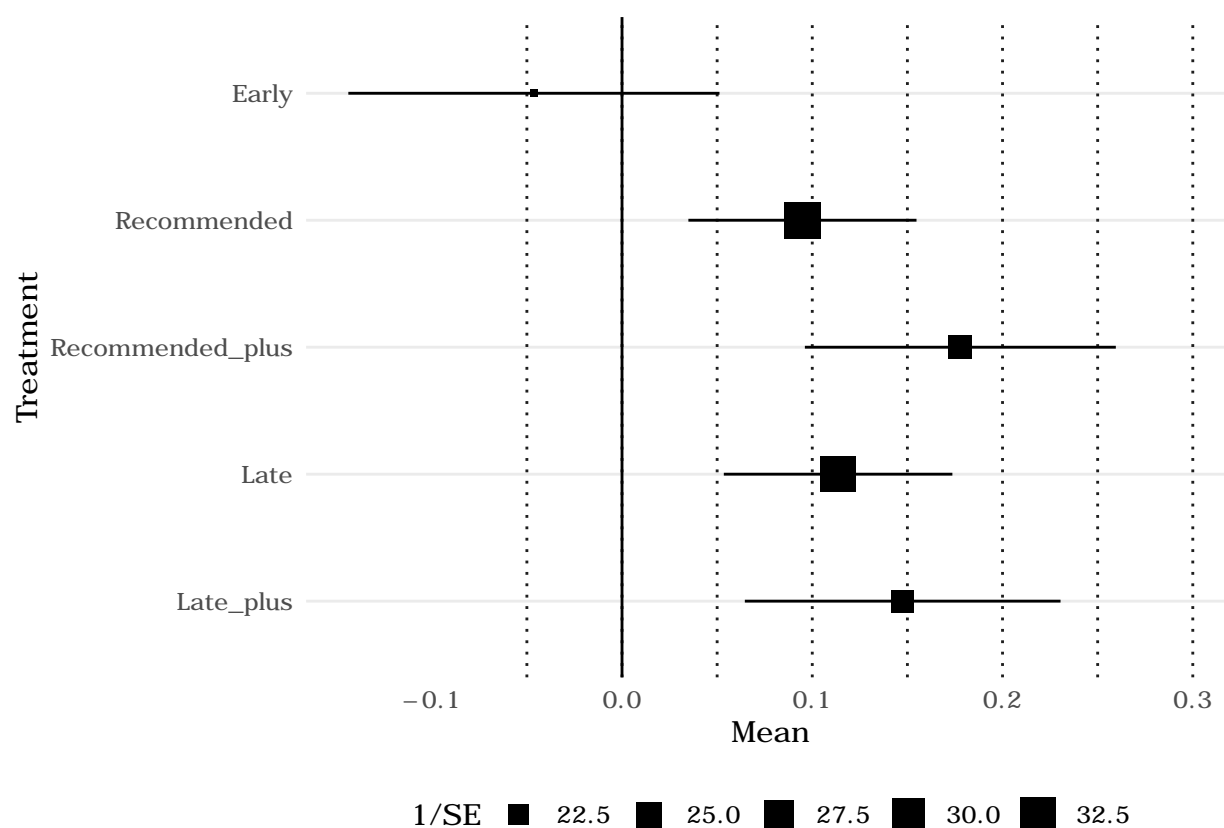
Additional meta-analysis models tested variables ... for their influence on the SpraySchedule variables ability to mitigate yield loss. For the variable disease pressure a Wald-type test was used to test the impact of this variable on the model as a whole.

The data were analysed in the R statistical software environment, version 4.0.1, [37] using two contributed meta-analysis packages, *metafor*, version 2-4.0, [38] and *netmeta*, version 1.2-1, [39].

## 2. Results

Overall the meta-analysis indicated that, with the exception of the *Early<sub>single</sub>* treatment, all the spray schedules were effective at protecting mungbean yields relative to the no spray controls (Figure





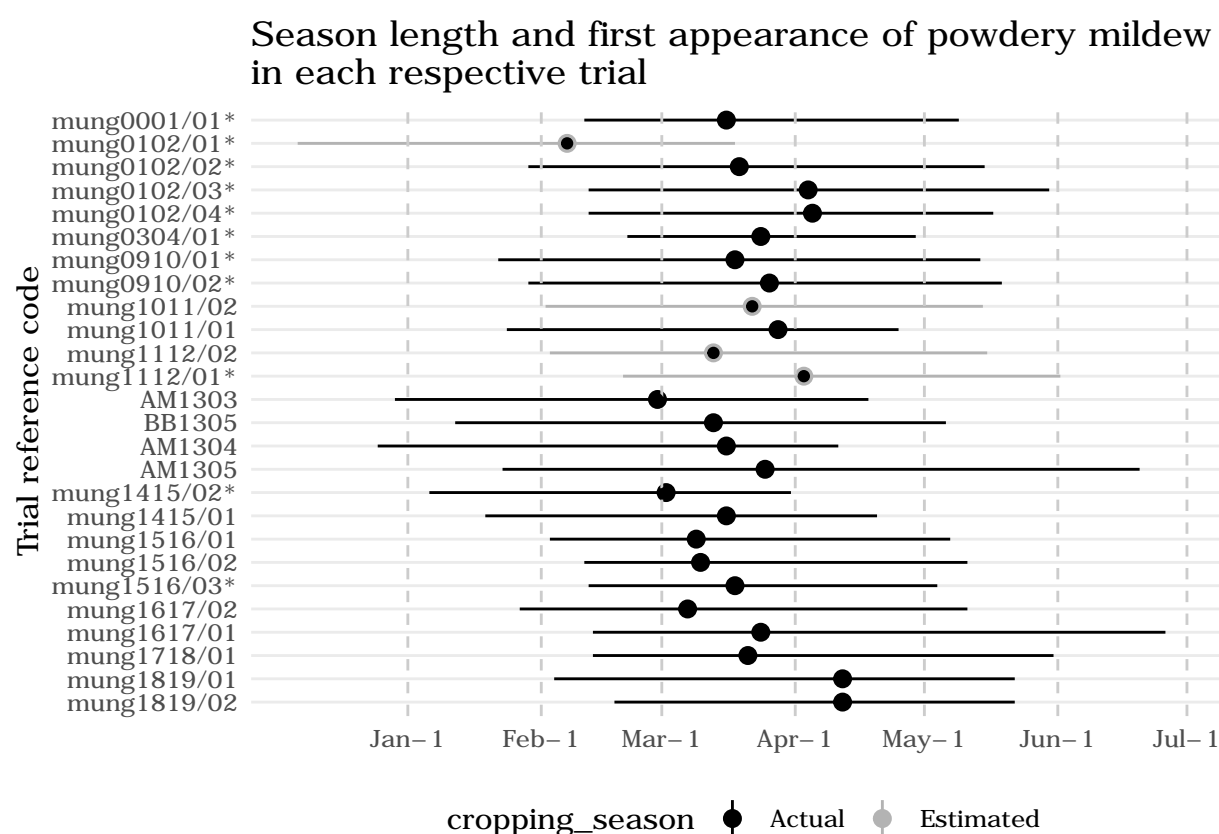
**Figure 1.** Yield contrasts

1). Treatments which incorporated multiple fungicide applications, *Recommended<sub>plus</sub>* and *Late<sub>plus</sub>*, produced the highest mean yield protection estimates at 177.83 (SE = 41.69 kg / ha) and 147.51 kg / ha (SE = 42.33 kg / ha) respectively (Table 3). The treatment that showed the most yield saved compared to the control, *Recommended<sub>plus</sub>*, was also significantly higher than all other single applications, (*Early<sub>single</sub>*,  $P < 0.0001$ , *Recommended<sub>single</sub>*,  $P = 0.0051$  and *Late<sub>single</sub>*,  $P = 0.0153$ ). However the *Late<sub>plus</sub>* only showed significantly greater yield saved when compared to the *Early<sub>single</sub>* treatment ( $P = 0.0004$ ). Consideration should be given when comparing *Late<sub>plus</sub>* and *Early<sub>single</sub>* treatments given they did not occur within the same trial for any of the 15 trials included in the meta-analysis. Within the single fungicide application treatments *Late<sub>single</sub>* provided the highest yield protection, and was estimated at saving an average of 113.57 kg / ha (SE = 30.65 kg / ha) relative to the no spray control ( $P = 0.0002$ ). *Early<sub>single</sub>* treatments were significantly lower than all other treatments (Table 3) and reported a mean estimate 46.3 kg / ha (SE = 49.77 kg / ha) lower than the no spray control ( $P = 0.3521$ ).

The  $Q_M$  omnibus test of moderators, shows the moderators significantly influence the model ( $Q_M = 31.1217$ ,  $df = 5$ ,  $P < 0.0001$ ) and we can reject the null hypothesis ( $H_0 : \beta_1 = \beta_2 = \beta_3 = \beta_4 = 0$ ) that there is no difference between the moderators [38]. The analysis shows there is still a significant amount of residual heterogeneity ( $Q_E = 37805.5158$ ,  $df = 149$ ,  $P < 0.0001$ ) not captured by the spray management moderator indicating other possible moderators, which might influence grain yield.

A log-ratio test, based on a chi-squared distribution, indicated that retaining the *SpraySchedule* variable as a random interactive term provided a significantly better model ( $P = 0.0179$ ). An inspection of log-likelihood profile plots showed the model, for each random interactive term, provided a reasonable estimate of  $\tau^2$ , with no indication the variables were over-fit.

While the addition of disease pressure, as an interactive moderator term to *SpraySchedule*, did explain slightly more residual heterogeneity, shown by a lower  $Q_E = 409.91$ . The model including disease pressure did not significantly improve the model ( $P = 0.4859$ ) and did not significantly explain



**Figure 2.** Season plot

differences in the `SpraySchedule` term, and therefore disease pressure was not included in the final model.

First sign of powdery mildew was observed as early as the 28th of February in the 2013 trial at ... ; and as late as the 12th of April in the 2018 trial at ...

### 3. Discussion

The current advice provided to growers for when to commence fungicide applications for control of powdery mildew, is to spray at the first sign of the disease then if necessary a follow-up spray two weeks later [18,24]. This meta-analysis confirms that advice, by showing that applying the first fungicide application, within three days of first sign will significantly mitigate yield loss from powdery mildew by between 34.89 to 154.78 kg / ha (*Recommended*,  $P = 0.0019$ ). Additionally, spray schedules which commenced “late”, up to 13 days after first sign of the disease were still effective at mitigating yield loss by 53.5 to 173.64 kg / ha (*Late*,  $P = 0.0002$ ). Spray schedules which included follow-up fungicide applications were on average more effective at reducing yield loss due to powdery mildew by; 96.11 to 259.54 kg / ha in the *RecommendedPlus* treatments ( $P < 0.0001$ ) and 64.55 to 230.47 kg / ha in the *LatePlus* treatments ( $P = 0.0005$ ) (Figure 2) which again follows the advice given by Thompson et al. [24]. This meta-analysis adds certainty to the advice, which is currently given to growers, especially around any uncertainty that Early sprays might be effective at suppressing the disease. We show here Early fungicide applications, which were made prior to any sign of the disease, were not effective at mitigating yield loss.

This meta-analysis also provides a effective spray window for fungicide applications, given that spray schedules, which commenced “late” were still significantly effective at mitigating yield loss (*Late*  $P = 0.0002$ ) 7 - 13 days after the first sign of disease. Growers typically do not have the time to inspect their crop regularly for the first sign of powdery mildew and therefore the disease may not be spotted

**Table 3.** Estimated grain yield contrasts between each spray schedule.

| Treatment contrasts            | Estimate | Standard Error | Z-score | P-value |    |
|--------------------------------|----------|----------------|---------|---------|----|
| Early - Recommended            | 0.1221   | 0.0421         | 2.8990  | 0.0037  | ** |
| Early - Recommended_plus       | 0.1587   | 0.0596         | 2.6617  | 0.0078  | ** |
| Early - Late                   | 0.0843   | 0.0541         | 1.5595  | 0.1189  |    |
| Early - Late_plus              | 0.1821   | 0.0603         | 3.0203  | 0.0025  | ** |
| Recommended - Recommended_plus | 0.0365   | 0.0371         | 0.9858  | 0.3242  |    |
| Recommended - Late             | -0.0378  | 0.0349         | -1.0850 | 0.2779  |    |
| Recommended - Late_plus        | 0.0600   | 0.0446         | 1.3445  | 0.1788  |    |
| Recommended_plus - Late        | -0.0744  | 0.0375         | -1.9812 | 0.0476  | *  |
| Recommended_plus - Late_plus   | 0.0235   | 0.0453         | 0.5174  | 0.6049  |    |
| Late - Late_plus               | 0.0978   | 0.0446         | 2.1932  | 0.0283  | *  |

Note:

\*\* - significant at 0.005 level

\* - significant at 0.05 level

within the first couple of days of it establishing. Also, weather conditions and logistical issues might prevent growers from getting machinery in the paddock to treat the disease immediately after it is spotted. These issues, and perhaps other on farm duties, might delay a timely application, permitting the disease more time to establish. Knowing a delay of up to two weeks may not lead to yield losses, can allow growers to incorporate other integrated pest management options, such as insecticide, with the fungicide spray saving on spray application costs. However attention should be made to the label requirements when first planning a spray schedule. Second applications, if they are needed, should be applied at least 10 - 14 days after the first application and not within 21 days of harvest [40]. If time permitted a second spray after the first “late” application, on average the meta-analysis shows this would be optimal, as the *LatePlus* treatment significantly limited yield loss by 97.8 kg / ha, when compared to the *Late*, single spray treatment  $P = 0.0283$ .

It is important to note, the studies included in this meta-analysis may not reflect a “normal” mungbean season. All the included experiments were intentionally sown late in the season, from January through to as late as early March, to ensure powdery mildew infection (Figure 2). While certain circumstances may force growers to sow mungbeans late, many may decide to follow their fields instead of risking a powdery mildew epidemics and an early frost damaging the crop and yields. Regardless, according to the Australian Mungbean Association sowing guide [20], mungbeans should not be sown later than January in the Darling Downs, the region where the majority of trials were undertaken. If the planting guide is adhered to the mungbean would be at a lower risk of powdery mildew, reducing the requirement for fungicide applications. An example of this is if we inspect the six trials in which powdery mildew manifested 60 days or greater after planting (Trial references: AM1304, mung1819/01, mung1011/01, AM1303, AM1305 and BB1305). Of these trials, four directly compared single *Recommended* fungicide applications and *RecommendedPlus* (AM1303, AM1305, BB1305 and mung1819/01), two of which (AM1305 and mung1819/01) produced at least one treatment *RecommendedPlus* treatment with a higher grain yield the *Recommended* treatments. However, neither AM1305 or mung1819/01 neither contained *Recommended* or *RecommendedPlus* treatments with higher mean yields than the no-spray control. This indicating there is likely no benefit to applying two fungicide applications when the disease manifests later than 60 days into the growing season. Further the trials undertaken in 2013, such as Premier (AM1303), Goolhi (AM1305) Marys Mount (AM1304) and Millmerran (BB1305) reported a significant difference in powdery mildew severity between the fungicide treatments, however neither trial showed a significant difference in grain yield between treatments [27, Northern Growers Alliance [26], Northern Growers Alliance [28], Northern Growers Alliance [25]]. Therefore, discretion is still required for if fungicide applications are necessary given the crop growth stage and forecast weather conditions. The PowderyMildewMBM



considers crop growth stage and weather, to assist decision making whether to spray or not to spray [33].

PowderyMildewMBM also provides a cost-benefit analysis to growers for fungicide spray schedule scenarios. The user inputs weather, crop, and economic inputs into the DSS and the application returns the probability that one or two fungicide sprays would protect yields sufficiently to increase the net profit. The scope of the meta-analysis is limited to the timing of fungicide application and therefore we can not validate all the variables available in PowderyMildewMBM. However our results validate the decision of the app developers not to recommend prophylactic fungicide applications. If the powdery mildew is not observed in the crop the app advises the user to keep monitoring for disease.

Scouting the crop for powdery mildew might not be necessary during the warmer months of the season. Data from the trials included in this experiment show regardless of crop sowing date, powdery mildew establishes on average in the month of March, which marks the beginning of autumn (Figure 2). Considering the results of the meta-analysis, weekly inspections would be ideal to ensure timely fungicide applications if necessary. Scouting at intervals greater than 12 - 13 days, might risk crop losses if a spray schedule does not commence within aforementioned the spray window. More work is needed to understand when “late” fungicide applications are too late.

We estimate the costs of applying tebuconazole fungicide at \$4.18 AU / ha, if fungicide is purchased at \$15 / L [41] and applications cost are \$2 per hectare [7]. The meta-analysis estimates average yield savings of between kg / ha (*Recommended*) and kg / ha (*RecommendedPlus*). These average yield savings would save \$ AU / ha for a single application at first sign and \$ AU / ha if a follow-up application is made. Lucrative returns such as these may tempt growers into applying fungicide to control diseases such as powdery mildew when they are unnecessary. Excessive use of fungicides are highly likely to lead ongoing problems such as fungicide resistance. *Podosphaera xanthii* is categorised by the Australian Fungicide resistance Action Committee (FRAC) as a “High risk” candidate to evolve fungicide resistance [42]. Evolution of fungicide resistance can occur quickly, especially when spray schedules are not designed with potential fungicide resistance in mind. Powdery mildew is one such group of fungal pathogens with an extensive track record of rapidly developing fungicide resistance. In cucurbits, *P. xanthii* evolved resistance to benzimidazole within three years of its registration [43] and has evolved resistance to numerous fungicide classes, such as benzimidazoles, demethylation inhibitors, organophosphates, hydroxypyrimidines, quinone inhibitors and quinoxalines since [44]. In cereal crops *Blumeria graminis* showed reduced sensitivity to strobilurins in 1998, two years after the fungicides began being used in Europe [45].

According to the Fungicide Resistance Action Committee (FRAC) it is important to alternate or mix fungicides with different modes of action to avoid fungicide resistance. At the time of writing, in Australia one fungicide is registered with the to treat powdery mildew in mungbean, Veritas/Custodia (200 g/L tebuconazole with 120 g/L azoxystrobin). This fungicide provides dual action: tebuconazole disrupts C-14 demethylase, required in sterol biosynthesis for cell membranes; and azoxystrobin, a Quinone outside inhibitor. Quinone is required in cell metabolism and active in the mitochondrial complex III. However FRAC recommends that applications of QoIs or strobilurins, singularly or as a mixture, should not be applied consecutively and alternated with a fungicide from a different group [46]. For mungbean, past experiments have shown some success with sulphur applications to control powdery mildew. Sulphur is a non-systemic protectant and thus provides an excellent option for rotation to limit the emergence of fungicide resistance.

To extend the time that a fungicide remains effective against the target pathogen, strategic use of the fungicide must be considered. Excessive fungicide sprays by a single active increase the selection pressure on the pathogen to evolve resistance [46]. If any strains of the pathogen with resistance to the fungicide are present in a population regular use of the fungicide will increase its proportion in the population. Therefore, managing the disease with fungicides for the highest economic return is likely

not to require eradication of the causal pathogen. Integrated pest management practices focus on using only the required number of chemical control to protect the economic return. These strategies permit the presence of yield limiting diseases and pests if intervening would cost more than the economic return of protection.

Advising use of fungicide for disease control to protect yields should also consider the harm it may do to symbiotic relationships with microbiota. While some nitrogen fixing symbiont show a level of tolerance to fungicides, all are detrimentally affected by increasing concentrations of fungicide leading to lower nodulation and biomass accumulation [47, Shahid and Khan [48]]. Therefore if a grower is relying on inoculating with rhizobia to help meet the crops nitrogen requirements, untimely and excessive applications of a systemic fungicide for powdery mildew control may actually reduce yield.

### 3.1. Future work

This meta-analysis provides a ballast for the direction of future powdery mildew research in mungbean. However it does not provide enough certainty to when late fungicide applications would be too late to protect yield losses due to the disease. Future experiments should take note of physiological maturity of the crop throughout the season so “crop age” might be considered as a covariate in future analyses. In cases where mungbean physiological maturity may not have been recorded the APSIM mungbean module [49].

Early detection of powdery mildew is vital for an effective fungicidal control response. Data from the collection of 26 studies in the Northern Grain region, indicate powdery mildew is likely to manifest on crops in the first month of autumn. This data together with additional observations may permit models to predict the likely onset of the disease given weather parameters, providing an early warning system for growers to scout their crop for signs of powdery mildew. However an improved understanding of the pathogen biology is also needed to ensure models are as accurate as they can be. The population genetics, epidemiology and alternate hosts are all poorly understood in this host-pathogen relationship. The recent discovery of a second powdery mildew species, which infects mungbean highlights the paucity of knowledge on this disease.

## 4. Conclusion

## 5. Supplemental Material

Supplementary material can be found in the following research compendium.

## 6. Data Availability

All data and code used in the preparation of this manuscript can be found in the associated research compendium.

## 7. Conflict of Interest Statement

The authors of this paper have no conflicting interest in the research presented in this manuscript

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**Author Contributions:** For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used “X.X. and Y.Y. conceive and designed the experiments; X.X. performed the experiments; X.X. and Y.Y. analyzed the data; W.W. contributed reagents/materials/analysis tools; Y.Y. wrote the paper.” Authorship must be limited to those who have contributed substantially to the work reported.

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## Abbreviations

The following abbreviations are used in this manuscript:

|       |   |
|-------|---|
| DSS   | Decision support system                     |
| AUDPC | Area under the disease progress curve       |
| NGA   | Northern Growers Alliance                   |
| GRDC  | Grains Research and Development Corporation |
| DMI   | Demethylase inhibitor                       |

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