1	Timing of Triazole-Based Spray Schedules for Managing Mungbean Powdery Mildew in
2	Australia: a Meta-Analysis
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4	Paul Melloy <sup>1*</sup> , Emerson Medeiros Del Ponte <sup>2</sup> and Adam H. Sparks <sup>1,3</sup>
5	
6	<sup>1</sup> University of Southern Queensland, Center for Crop Health, Toowoomba, Queensland,
7	4350 Australia
8	<sup>2</sup> Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa MG 36570-
9	900 Brazil
10	<sup>3</sup> Department of Primary Industries and Regional Development, Western Australia,
11	Locked Bag 4 Bentley, WA, 6983, Australia
12	*Corresponding author: P. Melloy; Email: paul.melloy@usq.edu.au
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#### 24 Abstract

25 Powdery mildew (PM), caused by two fungal species, Podosphaera xanthii and Erysiphe 26 *vignae*, is a yield limiting foliar disease commonly found in mungbean (*Vigna radiata*) 27 cropping areas of eastern region of Australia. Effective control of the disease relies largely 28 on fungicide applications, mainly of the triazole group. Uncertainty in the current fungicide 29 spray schedule recommendations, which advise commencing with a spray at the first 30 signs of PM, prompted this study to evaluate PM severity and crop yield data obtained 31 from fungicide trials which also tested spray schedules starting before (*early*) or after (*late*) 32 first signs, applied singly or combined with a follow-up spray. A meta-analytic approach 33 was employed to obtain mean differences of the PM severity and crop yield between plots 34 sprayed with specific triazole-based spray schedules and nontreated plots. From 26 trials, 35 14 and 15 met the criteria for inclusion in the respective PM severity and yield analyses. 36 The schedule with the first spray starting at first sign, with a follow-up spray 14 days later, 37 resulted in significantly lower disease severity compared to all other schedules. However, 38 the yield protected was only numerically higher and not statistically different compared to: 39 single-spray at first sign, single-spray late or two-spray starting late. PM severity and yield 40 in the *early* sprayed plots did not differ from the nontreated plots. These findings support 41 the current recommendations and provide additional evidence that yields are still 42 protected when delaying the first spray up to a week after disease onset. They also suggest that additional sprays may not always be necessary, thus reducing direct 43 44 fungicide costs, indirect costs due to fungicide insensitivity and potential adverse effects 45 to the environment.

47 Keywords: Podosphaera xanthii, Erysiphe polygoni, Erysiphe vignae, Vigna radiata,

48 demethylase inhibitors, network meta-analysis

### 49 Introduction

50 Mungbean [Vigna radiata (L.) Wilczek] is a pulse crop primarily grown in Southeast 51 Asia for human consumption either as raw pods, grain or added to meals after sprouting 52 as an affordable source of protein (Lambrides and Godwin 2007). The dried grain can 53 also be ground into a protein-enriched flour for uses in noodles, biscuits and cakes 54 (Chankaew et al. 2013). Even though mungbean was first brought to Australia in the 55 1930s for use as a forage or green manure crop, it is still considered a relatively new 56 commercial crop. In 2011, a cost-benefit analysis valued the return on investment for 57 mungbean research at 18:1 (Australian Mungbean Association n.d.). Since then, high 58 value export markets and new varieties with improved yields have increased profitability 59 and the crop has been more widely adopted. Between 35 and 86.4 thousand hectares of 60 dried beans are now planted annually in Australia (Clarry 2016). Nowadays, mungbean 61 is grown as a short season summer legume crop predominantly within southern 62 Queensland and northern New South Wales, this is the same region for which trials in this 63 meta-analysis were undertaken. In 2019, approximately 90% of Australian mungbean was 64 grown for export which received attractive returns of up to \$1300 AUD per tonne 65 (Queensland Government 2019).

Mungbean potentially yields up to 3 t/ha (Thomas et al. 2004) but the average Australian farm yields less than 1 t/ha (Chauhan and Williams 2018) with significant temporal and spatial variability, suggesting the urgent need for research efforts targeting production constraints. Abiotic constraints, insects and competition from weeds contribute

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70 to mungbean yield losses, in addition to the occurrence of diseases caused by fungi, 71 bacteria and phytoplasmas (Wood and Easdown 1990; Conde and Diatloff 1991; Thakur 72 and Agrawal 1995; Fuhlbohm et al. 1996, 2013; Wilson et al. 2001; Noble et al. 2019) 73 Among the fungal mungbean diseases in Australia, powdery mildew (PM), caused by 74 obligate biotroph fungi Podosphaera xanthii (syn. Podosphaera fusca and Erysiphe 75 polygoni) and Erysiphe vignae (Kelly et al. 2021), can reduce yield by 40% in susceptible 76 cultivars grown under disease-conducive conditions (Lambrides and Godwin 2007). To 77 date, ascospores produced in chasmothecia (sexual stage) have not been reported in 78 Australia; hence the asexual airborne conidial spores that are produced abundantly on 79 live hosts and dispersed by wind currents, are considered the primary inoculum for 80 epidemics, while the alternate host or hosts remain unknown. Conditions for infection by 81 PM pathogens are optimal when temperatures range between 22 °C and 26 °C 82 (Pérez-García et al. 2009; Kelly et al. 2017). Powdery mildew is a polycyclic disease that 83 develops guickly; the time from conidial germination to newly producing conidiophores 84 disseminating conidia (a monocycle) can be as short as five days (Sparks and Kelly 2017). 85 The characteristic white powdery fungal growth on the infected leaves negatively affects 86 photosynthesis, leading to reduced yield quantity and quality (Pérez-García et al. 2009). 87 In severe cases leaves become chlorotic, which can lead to premature leaf senescence. 88 Powdery mildew management involves the integration of cultural, genetic and 89 chemical practices (Pandey et al. 2018; Sparks and Kelly 2017). In southern Queensland 90 and northern New South Wales, sowing of mungbean crops is recommended between 91 late spring (November) and mid-summer (January) to avoid the cooler autumn 92 temperatures, which favor the disease (Australian Mungbean Association n.d.). While loci 93 conferring quantitative resistance to PM in mungbean have been incorporated in

94 mungbean breeding lines (Humphry et al. 2003; Zhang et al. 2008; Pandey et al. 2018; 95 Douglas 2008); ongoing breeding efforts in Australia have focused on resistance to 96 bacterial diseases given the feasibility to use fungicides for PM control. The recently 97 released Opal-AU cultivar is rated as moderately susceptible (MS) to moderately resistant 98 (MR) and is considered to contain the best resistance to PM (Douglas and McIntosh 2020) 99 followed by Jade-AU (MS) and Green Diamond (Australian Mungbean Association n.d.). 100 Losses up to 32.7% have been recorded in Jade-AU (Thompson 2016). However, cultivar 101 resistance alone is not sufficient to control PM, hence fungicide applications are 102 necessary when conditions for the disease are conducive.

103 Field trials conducted in eastern Australia over the past ten years have evaluated 104 fungicide efficacy with a range of active ingredients and spray timings. The trial results 105 are available only as gray literature, such as online trial reports (Kelly et al. 2017; 106 Thompson 2016; Northern Growers Alliance 2013a, 2013b, 2013c, 2013d). In general, 107 the best control of PM for yield protection has been achieved with a first application at the 108 first signs, with a follow-up application two weeks later if conditions are favorable for 109 disease development (Sparks and Kelly 2017; Thompson 2016). However, these results 110 have been inconsistent, possibly due to environmental or trial-specific conditions as well 111 as lack of statistical power in individual trials. Additionally, a fair comparison among 112 several treatment options was not possible due to different combinations of treatment 113 settings between the trials, such as fungicide active ingredient, number of sprays and 114 spray timing. These issues can be dealt with by combining results from the primary studies 115 to obtain estimates of both the size and uncertainty of the treatment effects via a 116 systematic review with meta-analysis (MA). The method has become standard in plant 117 pathology for summarizing data from uniform fungicide trials. Typically, MA models are

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fitted separately to data for each treatment against a control group (Madden and Paul 2011). Alternatively, a single model can be fitted in the form of a network of treatments where both direct and indirect evidence is used in the calculation of treatment effect sizes. The latter approach is called a network meta-analysis, one of the several meta-analytic methods that has found use for multiple treatment comparisons in plant disease management due to its estimates being considered more precise in some contexts (Madden et al. 2016).

125 The recently launched mobile app '*PowderyMildewMBM*' (Department of Primary 126 Industries and Regional Development 2019) is a decision support tool that provides a 127 cost-benefit analysis to assist growers evaluate whether applying fungicide to treat PM 128 would be profitable given their weather conditions and the estimated value of the 129 mungbean crop. More robust and reliable estimates of yield responses to different 130 fungicide spray schedules obtained using meta-analytic modelling could be important for 131 validating the assumptions the app makes about the timing of fungicide applications, such 132 as avoiding prophylactic sprays.

Two questions were addressed in our study: i) when in time, relative to PM first signs (prior, during or after), should the first fungicide spray be applied on mungbean to maximize yield protection?; ii) given those timings at which the first spray was made is a second spray worthwhile yield protection? To answer these questions, we systematically reviewed all sources of fungicide trial data for PM control in the eastern Australian mungbean production scenarios that we were able to obtain and conducted a metaanalysis of the effect of different sprays schedules on mungbean yields.

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#### 141 Materials and Methods

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142 Data source and inclusion criteria. As of August 2020, no peer-reviewed study on the 143 efficacy of fungicide on powdery mildew in mungbean was found to be conducted in 144 Australia after searching article databases, Web of Science, Scopus and Google Scholar 145 with the search string: "mungbean AND powdery mildew AND fungicide" and filtering the 146 results by country of publication. On the other hand, we were able to find and gather data 147 from 26 trials reporting the fungicide effect on PM intensity and mungbean yields in 148 databases at the University of Southern Queensland and collaborating institutions, 149 including Northern Growers Alliance and the Queensland Department of Agriculture and 150 Fisheries. Trial locations from these databases represented the main mungbean growing 151 areas in Australia, from Central Queensland to Northern New South Wales. Raw data 152 were available for thirteen trials, the remaining 13 trials reported only the means of each 153 treatment.

Field trials not meeting the following criteria were excluded: i) the field trial was conducted in Australia ii) the date when powdery mildew was first observed; iii) disease severity at the end of the growing season; iv) fungicide application dates; v) the fungicide active ingredient(s); vi) fungicide application dose(s); vii) and crop yield.

158 Treatments with fungicide active ingredients evaluated in fewer than eight trials were 159 discarded from the analysis. This resulted in the retention of two DMI fungicides 160 (tebuconazole and propiconazole) in the dataset, reducing the initial set of 26 trials to 17 161 trials. Variance accompanying the treatment means was required for the trials to be 162 included in the meta-analysis. Three trials, 1516/01, 1516/02, 1516/03, were removed for 163 not reporting PM severity variance, resulting in a PM severity meta-analysis on 14 trials 164 (Table 1). Separately, two trials, 1112/01 and 1516/03, lacking grain yield variance were 165 removed from the 17 trials, resulting in a grain yield meta-analysis of 15 trials. All trials

included in this analysis were conducted in a randomized complete block design. Further
 trial details can be found in the supplementary materials and accompanying research
 compendium.

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170 **Treatments of interest.** In this study, we were interested in testing whether variations in spray schedules which used triazole fungicide (tebuconazole and/or propiconazole) are 171 172 more beneficial than the recommended practice. The 'Recommended' practice refers to 173 a single fungicide spray schedule starting one to three days following the first sign of PM. 174 After inspecting data from the trials which met our inclusion criteria, four other spray 175 schedules were defined based on a combination of spray timing, and if one or more follow-176 up applications were made after the first spray (coded with the suffix +). A total of five triazole-based spray schedules were then defined: Recommended ( $n_{vield}$  = 27,  $n_{severity}$  = 177 28); Recommended+ ( $n_{yield}$ = 40,  $n_{severity}$  = 41); Early, single spray prior to disease 178 detection ( $n_{vield}$  = 13,  $n_{severity}$  = 13); *Late*, a single spray between seven and 13 days after 179 the first sign of PM ( $n_{vield}$ = 17,  $n_{severity}$  = 15); and Late+ ( $n_{vield}$ = 19,  $n_{severity}$  = 13), a Late 180 spray which included one or more follow-up sprays.  $n_{severity}$  represents the total number 181 of respective treatments used in the PM severity meta-analysis and  $n_{yield}$  the total number 182 183 of respective treatments in the mungbean yield meta-analysis. There were no spray 184 schedule treatments that began between four and six days after the first sign of PM. Given the small number of trials with *Early+* treatments ( $n_{vield}$  = 5,  $n_{severity}$  = 5) this moderator 185 186 level was excluded from the meta-analysis.

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188 **Response variable and meta-analytic procedures.** Following methods in published 189 meta-analyses evaluating the effect of fungicides on crop yield (Paul et al. 2008), we 190 focused this meta-analysis on obtaining estimates of crop yield and proportion disease 191 severity between spray schedules and the nontreated control. Grain yield means of each 192 fungicide treatment, expressed as metric tons per hectare, were either obtained directly 193 from the summaries of trial reports, or summarized from the raw data when available. PM 194 severity was recorded on an ordinal scale from one (no signs of the pathogen) to nine 195 (100 % of plant infested and dropping leaves) (Table 2). The sampling variance was also 196 calculated from the raw data when available or the least squares statistic, using the 197 method described in Ngugi et. al. (2011), when trial data was obtained from summarized 198 trial results.

A preliminary inspection of mean grain yields at the trial level suggested that a normal distribution could be assumed without need for transformation. The distribution of PM severity ratings on the other hand were skewed and needed adjustment. The ordinal one to nine scale was converted to a proportion based on the description of each scale level (Table 2). Converting to a proportion retained the upper (1) and lower (0) scale boundaries and permitted a logit transformation which improved normality of the PM severity response.

206 The severity scale conversion to a severity proportion was aided by fitting proportion 207 severity to the respective ordinal PM severity scale with a generalized additive model 208 (GAM) using the 'gam' R package (Hastie 2020). The GAM can be defined by  $S_{\rho} \sim s(S_{\rho})$ 209 where  $S_{\rho}$  is a vector of nine severity proportion values equivalent to each level of the 210 ordinal 1 - 9 scale, and  $S_{\alpha}$  the ordinal one to nine scale ( $S_{\alpha}$ ). The default 'gam()' 'family' 211 value, "Gaussian", was used. The smoothing parameter, '*spar*', was set to 0.1 for  $S_o$  which allowed a better fit and an R<sup>2</sup> value close to 1. The GAM was used to predict the 212 213 corresponding percent severity and sampling variance.

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Environmental effects between trials would likely lead to spray schedule treatments effects which were correlated within trials. A network meta-analysis, also referred to "twoway unconditional linear mixed model", was chosen because it assumes correlations between treatments within the same trials and makes weighted comparisons between trials even if all treatments are not present within each included trial (Paul 2008; Machado 2017). The model is given by

$$Y_i \sim Norm(\mu + \delta, \Sigma + S_i^2)$$

221 Where  $Y_{i}$  the response, is either a vector of the grain yield measurements (tonnes 222 per hectare) or a vector of PM severity proportions, for the *i*th trial over the total number 223 (K) of trials (i = 1,...K).  $Y_i$  is assumed to be a normal distribution with  $\mu$  the estimated 224 weighted mean of treatments across all trials;  $\delta$  is the estimate for each moderator level. 225 in this case '*spray schedule*,' corresponding to each fungicide treatment level;  $\Sigma$ , a 6 x 6 unstructured matrix of treatment variances between trial; and  $S_i^2$  a variance-covariance 226 matrix. Weighted estimates of this random effects model were calculated using the 227 228 inverse of the model-implied variances (Viechtbauer 2010). The model was fit so 'spray 229 schedule' treatments were added as correlated random effects within each trial and the 230 variance co-variance matrix of the random effects specified as 'unstructured'. This allows 231 the random effects for each spray schedule treatment to have different variances, which 232 are also allowed to be correlated. Each treatment mean/variance entry was given an 233 individual 'id variable, which was incorporated as a random effect to the model.

The model was fitted in the R statistical software environment, version 4.0.1, (R Core Team 2020) using the *maximum likelihood* parameter and '*optim*' optimizer with the '*metafor*', version 2-4.0, R package (Viechtbauer 2010). Linear contrasts of the spray schedule moderator estimates and variances for grain yield were used to obtain the mean difference and associated 95% confidence intervals between each spray schedule. These
mean differences were used to infer the optimum fungicide spray strategy for maximizing
mungbean grain yields. The percentage of variation attributed to within trial variation and
between trial variation was approximated for both meta-analyses using the l<sup>2</sup> statistic
(Higgins and Thompson 2002).

A stability test was used for the PM severity and yield meta-analyses by excluding one of the trials and running the meta-analysis. This was done 14 or 15 times, for each trial in the respective PM severity or yield meta-analysis. Each of the models with a 'dropped' trial were examined to detect if any of the estimates varied significantly indicating an unstable network meta-analysis.

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#### 249 **Results**

250 Trial characteristics and overall summary. Characteristics of the trials which met the 251 study inclusion criteria were summarized in Table 1. Planting dates for the trials ranged 252 from late December to the middle of February. The first signs of PM were observed as 253 early as February 28 and as late as April 12. As expected, PM severity was higher and 254 the mean grain yields were lower in the unsprayed control treatments as compared with 255 those treatments which fungicide was applied (Fig. 1). The median yields at the trial level 256 ranged from 289.4 kg/ha to 2045 kg/ha in the control, while in the spray schedules they 257 ranged from 275.9 kg/ha to 2526.3 kg/ha (Table 1).

In general trials that yielded below 700 kg/ha showed no yield benefit from any fungicide
application and two-thirds of the trials produced yields which were on average higher in
the fungicide treated plots compared with the no spray control (Table 1).

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261 Meta-analytic estimates. The end of season PM severity was significantly lower in all 262 fungicide spray schedules compared to the no spray controls, with exception to *Early* 263 sprays (*P* = 0.313). The analysis estimated the *Recommended*+ spray schedule provided 264 the highest efficacy for controlling PM (Table 3), significantly lowering PM severity 265 compared to all other spray schedules. The other spray treatments, *Recommended* (P < 266 0.001), Late (P = 0.0315) and Late+ (P = 0.0073), still provided effective control of PM 267 and did not differ significantly from each other with exception to the *Recommended* spray 268 schedule which was significantly higher than a single *Early* application (P = 0.0116). 269 Between trial variance accounted for approximately 99.94% of the total PM severity 270 variance as indicated by the P statistic. More than half the PM severity sampling variance 271 which accompanied the means were recorded as zero, which could explain why between 272 trial variance accounted for almost all heterogeneity.

A sensitivity analysis of the PM severity and yield models indicated the consistency for all estimates, with insignificant variation for each moderator when trials were sequentially excluded from the analysis with exception to the exclusion of trial 1617/02 at Missen Flats. Excluding 1617/02 presented the greatest change in estimates with the largest changes occurring in the yield meta-analyses.

The spray schedule effect on mungbean yields were somewhat similar to the PM severity meta-analysis. All spray schedules, except for *Early* (P = 0.946), resulted in a significant yield increase relative to the unsprayed controls (Table 4). For those spray schedules, the difference in yield relative to the control ranged from 130.1 to 189.1 kg/ha, with the highest estimated yield increase produced by *Recommended+* followed by *Recommended*, *Late* and *Late+* (Table 4). Between trial heterogeneity was also high in the yield meta-analysis, consisting of 99.66% of the model variance according to the l<sup>2</sup> statistic test. A Wald-test of moderators indicated a disease pressure in the no spray control was significant (P = 0.047) when improving yield protection estimates for each spray schedule. However, due to low replication between some disease pressure categories and no *Late*+ spray schedules observed in low disease pressure trials, further analysis was not pursued.

An inspection of log-likelihood profile plots showed the models for each random interactive term provided a reasonable estimate of  $\tau^2$ , with no indication the variables were over-fit.

293

#### 294 **Discussion**

295 The current advice provided to eastern Australian mungbean growers for when to 296 commence fungicide applications for controlling powdery mildew is to spray at the first 297 appearance of the disease (Thompson 2016; Sparks and Kelly 2017). Our meta-analysis 298 corroborates the current advice but also suggests that if the first spray is delayed by up to 299 one to two weeks mungbean yields may still be protected given the statistically similar 300 results of the *Late* categorized spray schedules to the *Recommended* practice. Reducing 301 PM severity however was most effective in the *Recommended+* spray schedule despite 302 the results of the yield analysis. Regardless, if the goal is to save yield and maximize 303 return these results are reassuring for mungbean growers who lack time and resources 304 to scout crops regularly for disease or for situations when logistic issues prevent growers 305 from getting machinery in the paddock immediately after PM is spotted. A flexible window 306 for commencing a spray program permits growers to consider better planning for their 307 integrated pest management by timing sprays in combination with other chemicals and 308 thus reduce costs with spray application. However, if the grower decides to apply a second

309 spray, consideration should be given to the fungicide withholding periods, which in 310 Australia require the second application to be applied at no earlier than 10 to 14 days after 311 the first application, and not within 21 days of harvest (Australian Pesticides and 312 Veterinary Medicines Authority 2014). Time permitting, a second spray after a "*Late*" 313 application may not be necessary, as suggested by our results (Table 4). More research 314 is needed to evaluate whether crop age at the first sign of PM may influence potential crop 315 yield reductions given the timing of spray schedule interventions.

316 Results from a stability analysis on the yield meta-analysis shows the removal of trial 317 1617/02 at Missen Flats caused the greatest change in yield estimates and lowered 318 confidence in the differences between treatments. This shows that results from this trial 319 had greater weight in the meta-analysis due to lower sample variances. In addition, the 320 Missen Flats trial produced higher yields (Table 1) and was subject to high disease 321 pressure resulting in greater losses due to the disease, which may also have contributed 322 to this trial having the most influence on the meta-analysis estimates. The number of trials 323 collated in this meta-analysis could be considered small, however results from future trials 324 could easily be added to the results published here to improve confidence for the best 325 fungicide spray schedule timing.

In this study, between trial variance accounted for almost all variance for both yield and disease severity meta-analyses. Climatic variation and management differences between the trials, such as planting date and supplemented irrigation, are likely to be major contributing factors to the between trial variance.

According to the Australian Mungbean Association sowing guide (Australian Mungbean Association n.d.), mungbeans should not be sown later than January in the Darling Downs region of southern Queensland, the region where most of the trials were Page 15 of 31

333 undertaken. If the planting guide is adhered to, powdery mildew may present later in the 334 crop cycle and yields may be at a lower risk from powdery mildew, reducing the 335 requirement for fungicide applications. An example of this is the 2013 trials, Premer 336 (AM1303), Goolhi (AM1305) Marys Mount (AM1304) and Millmerran (BB1305) where PM 337 manifested 60 days after sowing or later (Table 1), approximately when the plant would 338 be filling pods. The trial reports indicated significant differences in powdery mildew 339 severity between the fungicide treatments, however none of the trials showed a significant 340 difference in grain yield between the same treatments (Northern Growers Alliance 2013a, 341 2013b, 2013c, 2013d). Crop age at first infection may also explain the large amount of 342 between-trial heterogeneity. Therefore, in lieu of further research, discretion is still 343 required to determine if fungicide applications are necessary, and consideration given to 344 the crop growth stage and forecast weather conditions at the first sign of PM.

345 Early detection of PM remains vital for an effective fungicidal control response. Data 346 from the trials included in this experiment show regardless of crop sowing date, PM 347 establishes on average in the month of March, which marks the beginning of autumn in 348 Australia. Such information, together with additional observations, may permit models to 349 predict the likely onset of the disease given weather parameters, providing an early 350 warning system for growers to scout their crop for signs of powdery mildew. The results 351 herein suggest, ideally, that inspections for the first signs of PM occur weekly in March to 352 ensure timely fungicide applications. Scouting at intervals greater than 12 to 13 days 353 might risk crop losses if a spray schedule does not commence within the aforementioned 354 spray window. More work is needed to understand when "Late" fungicide applications are 355 too late. However regardless of the number of sprays, fungicide applications commencing at the *Recommended* time on average were more effective at limiting PM severity and
 protecting mungbean yields.

358 The approximate costs of applying tebuconazole fungicide at \$ 4.18 AUD/ha, if 359 fungicide is purchased at \$ 15 AUD/L (Simpfendorfer and Taylor 2011) and application 360 cost are \$ 2 AUD/ha (Queensland Government 2019). This meta-analysis estimates 361 average yield savings of between 137.8 kg/ha (SE = 33.9) (Recommended) and 189.1 362 kg/ha (SE = 53.0) (*Recommended+*). Assuming mungbean is sold at \$ 1200 AUD/t, these 363 yield savings would save between \$71 to 234 AUD/ha for a single application at first sign 364 and \$ 83 to 337 AUD/ha if a follow-up application is made. Such profit levels and efficacy 365 for controlling visual signs of disease may tempt growers into applying fungicide to control 366 diseases such as powdery mildew when they are unnecessary or unlikely to result in a 367 financially beneficial yield saving. Excessive use of fungicides is likely to lead to ongoing 368 problems such as fungicide resistance. The evolution of fungicide resistance can occur 369 quickly, especially when spray schedules are not designed with potential fungicide 370 resistance in mind. Powdery mildew is one such group of fungal pathogens with an 371 extensive track record of rapidly developing fungicide resistance. P. xanthii is categorized 372 by the Australian Fungicide Resistance Action Committee (FRAC) as a "High Risk" 373 candidate to evolve fungicide resistance (Fungicide resistance action committee 2019: 374 Peterson 1973; McGrath 2001; Chin et al. 2001).

To extend the time that a fungicide remains effective against the target pathogen, the strategic use of the fungicide must be considered. To aid in making these decisions and devising a good strategy, DSS tools such as the *PowderyMildewMBM* app consider crop growth stage and weather, informing users when deciding whether to spray or not to spray (Department of Primary Industries and Regional Development 2019). Using these such tools can reduce unnecessary and unprofitable fungicide applications, which may help
lead to the development of fungicide resistance. To offer more benefit for future research,
experiments should record physiological maturity of the crop throughout the season so
"crop age" might be considered as a covariate in future analyses.

384 Currently, the recommendations that are advised to mungbean growers appear to be 385 the best practices for reducing losses due to powdery mildew. Care needs to be taken to 386 adhere to them, especially avoiding prophylactic sprays, to limit the development of 387 fungicide resistance in the powdery mildew pathogen populations. While the number of 388 included trials in these meta-analyses is low, this study provides direction for future 389 powdery mildew control research in Australian mungbean. Future trials might build on this 390 work to provide greater certainty as to when *Late* fungicide applications would be too late 391 to protect yield losses due to the disease. Given the high value of mungbean at the farm-392 gate, further work could be done in this area that would benefit the Australian mungbean 393 industry.

394

#### 395 Supplemental Material

396 Supplementary material can be found in the following research compendium.397 https://openplantpathology.github.io/Mungbean\_PM/

#### 398 Data Availability

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

401 **Conflict of Interest Statement** 

402 The authors of this paper have no conflicting interest in the research presented in this 403 manuscript.

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- 410

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### 541 Tables and Figures

542 **Table 1.** Trial summaries which met the inclusion criteria for yield and powdery mildew

543 (PM) severity meta-analyses.

Trial code	Location	Year	Sowing date	PM onset <sup>a</sup>	n <sup>b</sup>	Control median PM severity <sup>c</sup>	PM severity range	Control median yield	Yield range (t / ha)
1011/01	Hermitage	2011	2011-01-24	2011-03-28	4	4.83 (2)	2 - 2.33	1.525	1.544 - 1.769
1011/02	Kingaroy	2011	2011-02-02	2011-03-22	6	7.83 (2)	3.33 - 5.33	0.798	0.73 - 1.059
1112/01	Gatton	2012	2012-02-20	2012-04-02	7	7.5 (1)	2 - 6.3	0.738	0.81 - 0.948
1112/02 <sup>d</sup>	Kingaroy	2012	2012-02-03	2012-03-12	7	8 (1)	1.7 - 8	0.751	1.036 - 1.323
AM1303	Premer	2013	2012-12-28	2013-02-28	4	8 (1)	2 - 7.83	1.422	1.334 - 1.45
AM1304	Marys Mount	2013	2012-12-24	2013-03-16	2	3 (1)	3.17 - 3.17	1.094	1.265 - 1.265
AM1305	Goolhi	2013	2013-01-23	2013-03-25	4	9 (1)	1 - 3.75	0.694	0.604 - 0.722
BB1305	Millmerran	2013	2013-01-12	2013-03-13	4	8 (1)	1.62 - 8	0.802	0.744 - 0.803
1415/01	Hermitage	2015	2015-01-19	2015-03-16	5	7.8 (1)	5.4 - 6.6	2.045	2.018 - 2.176
1516/01	Hermitage	2016	2016-02-03	2016-03-08	7	8 (1)	3 - 7.5	1.803	2.141 - 2.37
1516/02 <sup>e</sup>	Kingaroy	2016	2016-02-11	2016-03-09	7	8.25 (1)	2.25 - 7.75	0.811	0.873 - 1.034
1617/01 <sup>e</sup>	Hermitage	2017	2017-02-13	2017-03-24	39	8 (9)	5.33 - 8	0.474	0.327 - 0.683
1617/02	Missen Flats	2017	2017-01-27	2017-03-07	39	9 (9)	7.67 - 9	1.505	1.13 - 2.526
1718/01	Wellcamp	2018	2018-02-13	2018-03-21	18	8.17 (6)	5.67 - 7.67	1.091	1.084 - 1.69
1819/01	Hermitage	2019	2018-02-04	2018-04-12	4	7.5 (1)	5 - 7.17	0.587	0.543 - 0.571
1819/02	Hermitage	2019	2018-02-18	2018-04-12	4	7.83 (1)	4.6 - 7.33	0.289	0.276 - 0.312

<sup>a</sup> Date of first powdery mildew (PM) observation.

<sup>b</sup> number of spray treatments per trial (n).

<sup>c</sup> Bracketed numbers following nontreated control PM severity refers to the number of pooled control treatments summarized.

<sup>d</sup>Trials which reported PM severity variance and not yield variance and therefore excluded in the yield protection meta-analysis.

<sup>e</sup>Trials which did not report powdery mildew (PM) severity variance and therefore excluded in the PM severity meta-analysis.

# 545 **Table 2**. Powdery mildew severity rating scale and calculated percent severity for each

## 546 ordinal rating

Severity rating	Description	Equivalent percent severity
1	No sign of powdery mildew	0.00
2	Small colonies in the lower 1/3 of canopy with up to 75% of plants infested	16.50
3	Colonies in lower half of canopy with more than 75% of plants infested	43.50
4	Colonies in lower 2/3 of canopy with up to 75% of plants infested	49.50
5	Colonies in lower 2/3 of canopy with more than 75% of plants infested	57.42
6	Colonies in lower 2/3 of canopy with 100% of plants infested	66.00
7	Colonies in lower 2/3 of canopy with 100% of plants infested, some plants with colonies in the top 1/3 of the canopy	75.00
8	Colonies all the way to the top of the plant with more than 75% of plants affected	87.00
9	Colonies to the top of the plant with severe leaf drop	100.00

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- 550 **Table 3.** Estimated powdery mildew severity mean difference for each spray schedule
- 551 treatment to the unsprayed control treatments. Meta-analysis estimates were back
- transformed using an inverse logit. Data were obtained from the gray literature reports of
- 553 (k) field trials undertaken in Eastern Australia.

Moderator	Nª	<b>K</b> <sup>b</sup>	ти <sup>с</sup>	se	P <sup>d</sup>	<b>Cl</b> L <sup>e</sup>	CI <sub>U</sub> <sup>f</sup>
Early	13	3	-0.0433	0.0287	0.313	-1.1047	-0.1841
Recommended	28	12	-0.2725	0.0261	< 0.0001	-1.3974	-0.4768
Recommended+	41	11	-0.4408	0.0257	< 0.0001	-1.5522	-0.6317
Late	15	5	-0.1458	0.0327	0.0315	-1.3091	-0.3885
Late+	13	2	-0.2101	0.0333	0.0073	-1.4000	-0.4795

<sup>a</sup>Number of treatment means categorized to each spray schedule.

<sup>b</sup>Number of trials with the respective spray schedule.

°Estimated mean disease severity.

<sup>d</sup>Indicates the significance between each respective spray schedule and the no spray control (intercept).

eLower range of the 95% confidence interval.

<sup>f</sup>Upper range of the 95% confidence interval.

Table 4. Estimated mungbean yield mean difference to the no spray control (intercept) for
each spray schedule treatment. Yield estimates (μ) were calculated from a network metaanalysis of data obtained from gray literature reports of 'k' field trials undertaken in Eastern
Australia.

Moderator	Nª	<i>k</i> ⁵	μ <sup>c</sup>	se	CI_{L} <sup>d</sup>	CI_{U}e	$P^{f}$
Early	13	3	0.0029	0.0439	-0.0830	0.0889	0.9464
Recommended	27	13	0.1378	0.0339	0.0713	0.2043	< 0.0001
Recommended+	40	12	0.1891	0.0530	0.0853	0.2930	0.0004
Late	17	7	0.1374	0.0440	0.0512	0.2237	0.0018
Late+	19	4	0.1301	0.0578	0.0168	0.2433	0.0244

<sup>a</sup>Number of treatment means categorized to each spray schedule.

<sup>b</sup>Number of trials with the respective spray schedule.

°Estimated mean yield determined by the meta-analysis.

<sup>d</sup>Lower range of the 95% confidence interval.

<sup>e</sup>Upper range of the 95% confidence interval.

fIndicates the significance between each respective spray schedule and the no spray control (intercept)

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Figure 1: Box plots of the unsprayed control treatment means for both PM severity (top) and
crop yield (bottom), indicating within-season variation A, and trial treatment means for each
spray schedule B. The middle box plot line indicates the median, box boundaries indicate the
lower and upper quartiles; circles indicate trial treatment means for each respective crop
season and spray schedule.

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571 https://github.com/openplantpathology/Mungbean\_PM



Box plots of the unsprayed control treatment means for both PM severity (top) and crop yield (bottom), indicating within-season variation (**A**<sub>*i*</sub>), and trial treatment means for each spray schedule **B**. The middle box plot line indicates the median, box boundaries indicate the lower and upper quartiles; circles indicate trial treatment means for each respective crop season and spray schedule.

177x152mm (300 x 300 DPI)