A network meta-analysis provides new insight into fungicide scheduling for the control of *Botrytis cinerea* in vineyards

Elisa González-Domínguez[a], Giorgia Fedele[a], Caffi Tito[a], Laurent Delière[b], Pierre Sauris[b], David Gramaje[c], José Luis Ramos-Sáez de Ojer[d], Emilia Díaz-Losada[e], Ana M. Díez-Navajas[f], Paul Bengoa [f], and Vittorio Rossi[a*].

[*]Correspondence to: Vittorio Rossi, [a]*Department of Sustainable Crop Production, Facoltà di Scienze Agrarie, Alimentari e Ambientali, Università Cattolica del Sacro Cuore, Via Emilia Parmense, 84 29122 Piacenza, Italy. E-mail: vittorio.rossi@unicatt.it*

[b]SAVE, INRA, Villenave d'Ornon, France.

[c]*Instituto de Ciencias de la Vid y del Vino, Consejo Superior de Investigaciones Científicas – Universidad de La Rioja – Gobierno de La Rioja, Logroño, Spain.*

[d]*Consejería de Agricultura, Ganadería y Medio Ambiente, Logroño, Spain.*

[e] *Estación de Viticultura e Enoloxía de Galicia – INGACAL, Leiro, Spain.*

[f] NEIKER-Tecnalia, Centro de Arkaute-ko Zentroa, Vitoria-Gasteiz, Spain.

Abstract

BACKGROUND: Control of Botrytis bunch rot (BBR) is currently based on the application of fungicides at four timings corresponding to specific growth stages of vines: end of flowering (A), pre-bunch closure (B), veraison (C) and before harvest (D). The current research provides a network meta-analysis of 116 studies conducted between 1963 and 2016 in nine countries, in which 14 strategies (based on combinations of 1, 2, 3, or 4 sprays applied in A, B, C, and/or D) were compared.

RESULTS: When a 1-spray strategy was applied, BBR control was more effective with sprays applied in A, C, or D than B. With a 2-spray strategy, strategy AC provided similar control as strategy BC; strategy CD also

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provided good control. For a 3-spray strategy, the best disease control was consistently obtained with strategy ACD. Four sprays strategy ABCD provided the best control but often involved needless sprays so that the routine application of four sprays is not justified.

CONCLUSIONS: Spraying at timing A seems to be very important for achieving efficient and flexible disease control. Flexibility is reduced by spraying at timing B rather than A.

1 INTRODUCTION

The fungus *Botrytis cinerea* Pers. Fr. (teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel) infects more than 200 plant species and causes among the most important plant diseases worldwide[1]. On grapevines, *B. cinerea* causes the devastating disease called Botrytis bunch rot (BBR)[2], which can affect all of the herbaceous organs of the vines; damage to ripening berries is especially serious, leading to severe losses in yield and reductions in wine quality[3].

The biology of *B. cinerea* and its epidemiology on vine crops have been studied in detail[2,4-7], and multiple infection pathways have been identified that occur in two periods: from flowering to young cluster development, and after veraison. In the early season, *B. cinerea* infects inflorescences and young berries, resulting in (i) inflorescence and blossom blight, (ii) latent infections of berries, and (iii) saprophytic colonisation of grape bunch trash[7]. After veraison, latent infections may become visible as rotted berries, and the colonized bunch trash may serve as a source of inoculum inside the bunches. In addition to conidial infection, ripening berries can be infected through contact with the aerial mycelium produced on adjacent infected berries (berry-to-berry infection)[8]. The susceptibility of berries from veraison to ripening increases according to a sigmoid curve[6,9]. Modifications of the berry cuticle also make cracks more likely, and the wounded berries can be easily infected[10].

The complexity of the life cycle of *B. cinerea* has caused growers to rely heavily on routine applications of fungicides at four specific grape growth stages: A, end of flowering (growth stage 69 of Lorenz et al. [11]); B, pre-bunch closure (growth stage 77); C, veraison (growth stage 83); and D, before harvest (before growth

stage 89). This calendar schedule of applications, sometimes called the "phenological method", was conceived based on the experiments in the 1960s[12-14]. Baldacci et al.[12] proposed 3 sprays: at the end of flowering, to reduce flower infections and infestation of floral debris; at pre-closure of bunches, as the last chance to disinfest the trash inside the bunch; and at veraison because of the increase in susceptibility of bunches from this period until harvest. Agulhon[14] improved the method suggesting that a fourth treatment can be applied 3 to 4 weeks before harvest.

The phenological method is easy to follow and provides good protection against BBR[15-17]. However, the method has important limitations. First, because the treatments are preventive and do not take into account the real risk of infection, the treatments are sometimes unnecessary. Second, the phenological method can increase the probability that *B. cinerea* develops resistance to botryticides[18]. Finally, public concerns about the possible effects of chemicals on human health[19] and environmental pollution[20] require that fungicides not be applied when unnecessary.

In response to these limitations in the phenological method, researchers have studied the possibility of reducing the number of fungicide applications by identifying the key timings in which fungicides should be recommended. In Europe, these studies have been performed in France[14-16], Italy[21-23], Germany[24], Spain[25], and Switzerland[26]. The findings have resulted in varying and sometimes conflicting recommendations for BBR management.

There is therefore a need to assess the effectiveness and consistency of the different management strategies for BBR control. To our knowledge, a quantitative review of multiple studies on different control strategies has not been published. An excellent tool for integrating and interpreting multiple individual studies is meta-analysis[27].

In this work, a multi-treatment (or network) meta-analysis was used to integrate the results of different strategies for BBR control. Network meta-analysis allows direct comparisons of all the strategies to each other and takes into account all of the correlations[28]. This multi-treatment analysis can also use a large number of individual studies, because it does not require that all of the studies include all of the treatments

to be compared. The meta-analysis reported here was preceded by a systematic review of peer- and non-peer-reviewed studies; unpublished data from studies developed or collected by some of the co-authors were also included.

2 MATERIALS AND METHODS

2.1. Database of studies on Botrytis bunch rot control

A database concerning studies of BBR control was assembled from the following sources: (i) JCR (Journal Citation Reports)-indexed journals, (ii) non-JCR journals, and (iii) experimental reports. For (i) and (ii), a structured search on the Web Of Science was carried out using the following search string: ("Botrytis" OR "mould") AND "grapevine" AND "control". For (ii), additional searches were performed in the following journals: *Giornate Fitopatologiche*, *EPPO Bulletin*, and *Phytoma* (French and Spanish version), which are not included in the Web Of Science. For (iii), unpublished experiments were considered that were conducted by the co-authors of this report or that were collected from the archives of their institutions. To be included in the database, an experiment had to meet the following criteria: the experiment included disease severity assessment (*X*); the experiment had a suitable experimental design with at least 3 replicates and an untreated control (NT); and the experiment evaluated at least one fungicide treatment that was applied at timings A, B, C, or D, or at several timings, e.g., strategy AB. For source (i), 22 studies were selected from 5 papers published in *Food Additives & Contaminants*, *Journal of Environmental Science and Health*, *American Journal of Enology and Viticulture*, *European Journal of Plant Pathology*, *Phytopathologia Mediterranea* and *Plant Pathology*. For source (ii), 62 studies were selected from 22 papers published in *Giornate Fitopatologiche*, *Vitis*, *EPPO Bulletin*, *New Zealand Plant Protection*, and *Scientific Papers*. For source (iii), 32 studies were considered from experiments in France (Bordeaux), Spain (Logroño, Ourense, Fraisoro, Laguardia, and Zalla), and Italy (Piacenza, Ravenna, and Cormons). In total, 116 studies were included, and these were conducted between 1963 and 2016 in Australia, France, Italy, Luxemburg, New Zealand, Spain, Romania, Switzerland, and USA. Most studies were conducted with a randomized complete block design,

with 4 replicate blocks. The vine variety, the fungicide/s used (active ingredient/s), and application timing varied among studies. A Table describing the 116 studies included is provided as supplementary material (Table S1).

Fourteen treatment strategies were evaluated and were grouped into four types: one spray per season (applied at timings A, B, C, or D); two sprays per season (strategies AB, AC, BC, BD, or CD); three sprays per season (strategies ABC, ACD, ABD, or BCD); or four sprays per season (strategy ABCD). Strategy AD was excluded because it was assessed in only one study. To increase the number of studies for each strategy, a disease severity value (X) was calculated from other strategies included in the same experiment when possible (see Table S1). For example, if disease severities were available for strategies ABC and BC (X_{ABC} and X_{BC}, respectively) in one experiment, then the disease severity of strategy A in that experiment was calculated as $X_A = X_{ABC} - X_{BC}$.

2.2. Meta-analysis

2.2.1. Effect of fungicide treatments

A network meta-analysis was conducted to evaluate the effect of the different treatment strategies in reducing disease severity compared to the non-treated control[29,30]. For each study and treatment (included the non-treated control), disease severity data were extracted from the publication/report and used to conduct the analysis. A detailed explanation of the procedure is provided in the supplementary material (Analysis explanation). Briefly, the meta-analysis was conducted with the software R (v 3.4.0; package 'metafor')[31,32] by using a multivariate random effects model. Assumptions of residual heterogeneity and consistency were assessed[33]. For heterogeneity, residual heterogeneity (Q_E) was tested and I^2 statistic calculated[34,35]. The Q_E test evaluates whether the variability in the observed effect that is not accounted by the fungicide treatment strategy is larger than one would expect based on sampling variability only; I^2 was calculated for each treatment and indicates the proportion of total variation in the estimates of treatment effect that is due to heterogeneity between studies. An I^2 of 0% indicates that all of the variability in the

estimated effect is due to sampling error within trials, and that none is due to heterogeneity. An I^2 value near 100%, in contrast, indicates that most of the observed variance is due not to sampling error but to variance between studies[34]. For consistency, the hypothesis that the treatment effect from direct evidence is consistent with the treatment effect from indirect evidence (i.e., calculated cases; see Table S1) was tested[33].

Treatment effect is presented as *L*, the difference (in the log of the severity mean) for each treatment (T) relative to the untreated control (NT) in the form $L_T=ln(X_T)-ln(X_{NT})$; the log severity of treatments was used instead of *X* because its distribution is closer to the normal one, as requested by the analysis. Therefore, negative values of *L* indicate that BBR severity was lower in the treated plot than in the NT control, i.e., that the treatment reduced the disease severity compared to the untreated control. Standard errors, confidence intervals, and significant statistics were calculated as described in the supplementary material (Analysis explanation). A Wald-type test statistic was used to determine whether the treatment effects *L* were significantly different from zero, i.e., whether the disease severity in the treated plots $ln(X_T)$ differed from that in the untreated plots $ln(X_{NT})$. The percentage of disease reduction relative to the control was also estimated[29,36].

2.2.2. Differences between pairwise combinations

Differences between treatment strategies were tested for all pairwise treatment combinations (i.e., the 14 strategies of 1, 2, 3, or 4 sprays). In total, 98 pairwise combinations were tested by a contrast analysis between the values of *L*.

To assess the across-studies variability, the frequency of studies was determined when (i) X_1 was significantly higher than X_2, (ii) X_2 was significantly higher than X_1, and (iii) no significant differences were observed between X_1 and X_2.

2.2.3. Effect of publication type and fungicide class

A multivariate meta-analysis model was also used to evaluate the effect of two categorical variables: (i) publication type, and (ii) fungicide class. For publication type, studies were categorized as no-JCR, JCR, and experimental report (as described in 2.1). For fungicide class, a new database was created that excluded the untreated control, and studies were categorized into 18 groups based on the combination of fungicides used (listed in Table S1). Fungicides were grouped based on the chemical classes defined by the Fungicide Resistance Action Committee (FRAC)[37]. The fungicide class E3 (dicarboximides, including chlozolinate, dimethachlone, iprodione, procymidone, and vinclozolin) was used as reference in the meta-analysis because the fungicides in this class were used, alone or in combination, in 69 of the 116 studies. E3 fungicides have been extensively applied against *B. cinerea* worldwide[38].

A separate analysis was performed for each of the two categorical variables. The interaction between the treatment strategy and these two factors was not evaluated because of the complexity of the models obtained (45 interactions for publication type, and 111 interactions for fungicide class) and because of the low number of cases for some of these interactions.

2.3 Data and code availability

All data and the R scripts used are provided as Supporting Information (Table S1.csv and Network_MA_code.R).

3. RESULTS

3.1 Database overview

BBR severity in the untreated plots of the 116 studies ranged from 0.1 to 87.4%, with 90% of the values ranging from 3.9 to 64.8%, indicating that the database included a wide range of epidemics (Fig. 1). The average disease severity in the untreated controls was 32.5% (s.e. 2.1%) with some asymmetry (0.55) and a

negative kurtosis (-0.65). Disease severity also showed high variability among plots treated with fungicides; this variability generally decreased with increasing number of sprays (from 1 to 4) per season (Fig. 1). For example, with the 1 spray in A, 90% of the disease severity values ranged from 0.9 to 54.8%; with the 2 sprays in AC, the values ranged from 1.5 to 43.6%; with 3 sprays in ACD, the values ranged from 1.6 to 26.8%; and with 4 sprays in ABCD, the values ranged from 0.2 to 13.2% (Fig. 1).

3.2. Treatment effects and pairwise comparisons

Both heterogeneity and consistency tests indicated that the results of the meta-analysis can be considered robust. The test for residual heterogeneity rejected the null hypothesis of homogeneity across studies (Q_E= 47672; df = 585; $P < 0.0001$) and the values of I^2 were >80% for all strategies except BC and ABC (Table 1). Therefore, the heterogeneity in the estimated *L* values was mainly due to the among-studies variability and not to the sampling errors in each study. Based on the Wald test, no significant interaction between treatment effect and the nature of the case (i.e., if they were calculated) was found (P > 0.1), suggesting lack of inconsistency within the dataset used.

The average values of *L* were significantly < 0 for all 14 strategies (i.e., estimated BBR severity was lower in the treated than in the untreated plots; Table 1). A value of *L* close to 0 (i.e., the treatment had no/low effect) was estimated for strategy B, whereas *L* values were approximately -0.5 for strategies A, C, and D (Table 1). Pairwise comparison by linear contrasts showed that *L* values estimated for strategies A, C, and D were not significantly different from but were lower than the *L* value estimated for strategy B (Table 2); therefore, strategies A, C, and D provided better disease control than strategy B, with the percentage of disease reduction ranging from 36.7% to 41.7% (Fig. 2).

Estimated values of *L* were lower compared to the untreated control when 2 sprays were applied instead of 1, except for strategy AB (Table 1). The value of *L* for strategy AB was not significantly different than those for strategies A, C, and D, and was higher only than the value for strategy B (P=0.002; Table 2). Estimated values of *L* were not significantly different for strategies AC and BC (P=0.082) and were lower than for the single sprays (P<0.05), except when strategy AC was compared with strategy C (P=0.12). When 2 sprays

were applied, the lower values of *L* were estimated for strategies BD and CD, which caused with an average disease reduction of 70.5 and 68.4%, respectively (Fig. 2). These strategies were not significantly different from each other (P=0.717), and provided better control than all other 1- and 2-spray strategies (P<0.05; Table 2).

When 3 sprays were applied, estimated values of *L* were sometime not significantly different from those values obtained when only 2 sprays were applied. Estimated values of *L* for strategies ABC and ABD were close to -0.7 (48.5 and 53.6% disease reduction, respectively; Table 1 and Fig. 2). Based on estimated values of *L*, strategies ABC and ABD were only better than strategy AB but were not better than the other 2-spray strategies (Table 2). The estimated value of *L* was larger for strategy ABC than for BC, BD, or CD, and the estimated value of *L* was larger for ABD than for BD or CD (P<0.011; Table 2). A *L* value of -0.92 was estimated for strategy BCD (60.0% disease reduction), but when compared with 2- and 3-spray strategies, the estimated effect of strategy BCD was significantly lower only than those of AB and ABC (P<0.05; Table 2). The lowest value of *L* for the 3-spray strategies was estimated for ACD (*L*=-1.23; 70.7% disease reduction), which was lower than for all other 3- and 2-spray strategies (P<0.05), except for BD and CD (Table 2).

Finally, the value of *L* estimated for the 4-spray strategy ABCD (-1.69) was significantly lower than those for all other strategies (P≤0.007; Tables 1 and 2). The average disease reduction with strategy ABCD was 81.6% (Fig. 2).

The frequency distribution of studies in which the mean severity of one strategy was higher than, equal to, or lower than that of the second strategy revealed substantial variability among individual studies (Fig. 3). For instance, when strategy A was compared with D, BBR severity did not significantly differ in 62% of the studies, was significantly lower for D than for A in 35% of the studies, and was significantly lower for A than for D in 3% of the studies (Fig. 3A). Comparisons concerning the 4-spray strategy ABCD showed that, even though the average (all studies considered) mean severity was significantly higher for ABCD than for all of the other strategies (Table 2), the frequency of studies in which ABCD was not significantly different from that of a second strategy was sometimes high (Fig. 3B).

No significant differences were observed between the different publication types (P=0.556). In contrast, significant differences were observed for fungicide groups (Table 3); the fungicide combinations D1/E2 (anilino-pyrimidines/phenylpyrroles) and E3/MS (dicarboximides/multi-site) significantly (P<0.001) reduced BBR severity compared to the E3 group (dicarboximides), which was used as the reference.

4. DISCUSSION AND CONCLUSION

Since the 1970s, several experiments have been carried out to assess the effectiveness of different fungicides and timings for controlling BBR of grapevines. In these experiments, fungicide strategies were based on the application of sprays during four grape growth stages: A, end of flowering; B, pre-bunch closure; C, veraison; and D, 1 to 3 weeks before harvest[16,23]. Most of these experiments were published in national technical journals or were conducted by local institutions with the objective of developing practical recommendations for viticulturists; other experiments remained unpublished (details in Table S1). To our knowledge, this paper is the first to summarize the information from those experiments (from 116 studies) with the aim of drawing robust conclusions[27,33,39].

Meta-analysis was used in the current study, and researchers have expressed the concern that the results of meta-analyses may not be robust because of "publication bias". The concern is that negative results often remain unpublished or are not included in JCR-indexed journals and are therefore less likely to be included in a meta-analysis[27,39]. In the current research, publication bias was unlikely for two reasons. First, only 19% of the studies were obtained from JCR-indexed journals; the other 81% were either published in journals/reports not accessible by a systematic review of the main scientific databases or were unpublished. Second, publication source (i.e., JCR-indexed journal, non-JCR journals, or experimental reports) did not affect the results of this work.

There are two additional reasons for considering the findings of this paper robust. First, the database included a wide range of BBR epidemics; disease severity ranged from 0.01% to approximately 90%. Second, the variability in disease severities was caused by among-studies variability rather than by sampling

errors within experiments, as indicated by the I^2 statistic[27,34]; among-studies variability may be mainly related to different environmental conditions that promoted or restricted BBR development[27,28].

With a 1-spray strategy, BBR control was, on average, more effective when fungicides were applied at timing A, C, or D rather than B. Based on the *B. cinerea* infection pathways defined by Elmer and Michailides (2), spraying in A (flowering) would simultaneously affect various infection pathways: i) conidial infection of the style and ovules; ii) conidial infection of the stamens or petals; iii) fruit infection via the fruit pedicel; and iv) colonisation of floral debris. Treatments in B (pre-bunch closure), when berries are not susceptible to *B. cinerea* infection[6], have the main aim of disinfesting the colonised floral debris before the debris is enclosed in the growing bunch[2]. In this meta-analysis, it is therefore not surprising that fungicide sprays were more effective at timing A than B. Later during the season, sprays at timings C and D would reduce the infection of ripening berries caused by both conidial and berry-to-berry infection[2,8].

With a 2-spray strategy, strategy AB provided similar disease control as A but better control than B. This result shows that spraying in B after having sprayed in A is not convenient when a 2-spray strategy is used, probably because the two sprays affect the same infection pathway (i.e., the production of inoculum on bunch trash). Recent results of Calvo-Garrido et al.[40] (not included in this meta-analysis) confirmed that treatments applied with strategy A vs. AB did not differ in their control of *B. cinerea* on bunch trash and of latent infections at veraison, indicating that a treatment in B did not provide additional control if a treatment had been applied in A. Control was better with strategy AC than AB; unfortunately, AD was not included in this work because only few studies with this strategy were retrieved with the literature search. When the spray in A was missed, strategies BC, BD, and CD provided good control. Therefore, combining treatments affecting both early infection pathways (at timing A or B) and late infection pathways (at timing C or D) results in effective disease control. With a 3-spray strategy, disease control was consistently better with strategy ACD than with BCD and this may be explained based on the effect of A or B on the infection pathways, as described before.

Although the 4-spray strategy, ABCD, provided the best control, it often led to unjustified fungicide applications. The latter inference is supported by Figure 3B, which shows that spraying 4 times in ABCD did

not always provide better disease control than spraying 1, 2, or 3 times. Therefore, recommending a routine BBR control strategy based on 4 sprays is not justified; it is not profitable for the grower and has negative consequences on human health, environmental pollution, and fungicide resistance management[18-20, 41].

The inferences and conclusions presented in the previous paragraphs can be considered relevant regardless of the specific fungicides used. Even though fungicide class (defined based on the chemical classes from FRAC[37]) had a significant effect on BBR control, only 2 of 18 classes (or combinations of classes) were significantly different from the reference class, the dicarboximides (E3). Control was better with the fungicide combinations D1/E2 (anilino-pyrimidines/phenylpyrroles) and E3/MS (dicarboximides/multi-site) than with E3. Results concerning E3/MS should be interpreted cautiously, because only 3 studies were considered, and all were carried out by the same research group; in those studies, E3/MS may have been more effective than E3 because the *B. cinerea* population may have been resistant to E3, which is a well-known problem[18, 41, 42]. Results for D1/E2 (cyprodinil/fluxodinil) may be considered more consistent than those for E3/MS because results for D1/E2 were from 14 studies that were conducted by different research groups. Investigating the efficacy of single fungicides or fungicide combinations against *B. cinerea* was not the aim of the present research.

Given the results of this study and irrespective of the fungicides used, practical recommendations for BBR control should be based on the following findings: i) strategy A provides better control than B; ii) strategy AC provides similar control as BC (there are no data for a robust comparison of AD vs BD); iii) strategy ACD is slightly better than BCD; and iv) strategy ABCD is useful only when severe epidemics are expected. Therefore, spraying at timing A seems to be very useful for achieving efficient and flexible BBR control in vineyards. Spraying at timing B instead of A does not provide the same flexibility because, if the grower initially decides to adopt a 1-spray strategy and the season subsequently becomes highly favourable for *B. cinerea*, the grower would no longer be able to adopt strategy ABCD. Similarly, the BC or BCD strategies, which are still possible if a spray is not applied at timing A, provide the same control as AC or less control than ACD; strategy BD provides good average control, but comparison with AD was not possible because

the latter strategy was not evaluated in this work. If a spray is applied in A, spraying in B is useful only if the grower decides to adopt the ABCD strategy; otherwise, AC or ACD provide satisfactory solutions for 2- or 3-spray strategies, respectively.

In some viticultural areas, spraying at timing A has been considered much less effective than spraying in B. After conducting a 2-year experiment in which strategy ABCD provided the same control as BCD, Corvi and Tullio[43] proposed to eliminate the spray in A; however, in both years of that experiment, the environmental conditions during flowering were unfavourable for BBR development. Pérez-Marín[25] recommended strategy BC based on a 4-year experiment in the same vineyard, but no statistical analysis was provided. In Italy and Spain, most of the regional public sanitary services recommend spraying in B rather than in A to their viticulturists[44-47]. On the other hand, some recent papers emphasize the importance of spraying at timing A rather than B[7,8,40].

Results of this work provide information on the efficacy of different BBR control strategies based on 1, 2, 3, or 4 sprays per season. How many sprays are necessary to control BBR in a vineyard may depend on several factors, including weather conditions, the susceptibility level of the variety, the microclimate as influenced by the canopy structure and density, and presence of powdery mildew and berry moth insects[1]. This decision can clearly be made easier by use of a mathematical model that is able to predict the risk of the disease development. A recently published mechanistic model for *B. cinerea*[8] predicts, on a daily basis, the relative infection severity during two infection windows corresponding to the two grape-growing periods relevant for *B. cinerea* infection: i) between "inflorescences clearly visible" and "berries groat-sized, bunches begin to hang"; and ii) ripening berries[2]. The model, which is based on relative infection severity values, predicts the final BBR as light, intermediate, or severe. The model has been integrated in a Decision Support System (DSS) for the sustainable management of vineyards and is therefore available for growers[48]. The findings of the current study, combined with the model predictions, should improve BBR management in vineyards.

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FIGURE LEGENDS

Figure 1. Box plots representing the distribution of Botrytis bunch rot severity in different studies in which a fungicide treatment strategy (T) was compared to an untreated control (NT). Strategies are combinations of treatments applied in A (end of flowering, growth stage 69 of Lorenz et al.[11]), B (pre-bunch closure, growth stage 77), C (veraison, growth stage 83), and/or D (before harvest, before growth stage 89).

Figure 2. Efficacy of different fungicide treatment strategies for the control of Botrytis bunch rot expressed as the percentage of disease reduction relative to the untreated control as estimated by the meta-analysis; whiskers show the 95% confidence interval; the dot size increases with the precision of estimates. Strategies are combinations of treatments applied in A (end of flowering, growth stage 69 of Lorenz et al.[11]), B (pre-bunch closure, growth stage 77), C (veraison, growth stage 83), and/or D (before harvest, before growth stage 89).

Figure 3. Frequency distribution of the studies in which the differences of disease severity between two fungicide treatment strategies for the control of Botrytis bunch rot were significant; white, grey, and black bars indicate the frequency of studies in which severity was less in the first strategy than in the second strategy, equal in both strategies, or greater in the first than in the second strategy, respectively. Strategies are combinations of treatments applied in A (end of flowering, growth stage 69 of Lorenz et al.[11]), B (pre-bunch closure, growth stage 77), C (veraison, growth stage 83), and/or D (before harvest, before growth stage 89).

Table 1. Effect in the reduction of Botrytis bunch rot severity compared to the untreated control of fourteen fungicide treatment strategies based on 1, 2, 3, or 4 fungicide sprays applied at timings A (end of flowering, growth stage 69 of Lorenz et al.[11]), B (pre-bunch closure, growth stage 77), C (veraison, growth stage 83), and/or D (before harvest, before growth stage 89).

Fungicide treatment strategy	K[⍰]	$I^{2,\S}$	Estimated effect in disease reduction				
			$L^{¥}$	se of *L*	95% confidence interval of L		*P*
A	55	93.9	-0.46	0.076	-0.61	-0.31	<0.001
B	44	86.1	-0.25	0.042	-0.33	-0.17	<0.001
C	50	93.9	-0.54	0.060	-0.66	-0.42	<0.001
D	34	89.0	-0.45	0.059	-0.57	-0.34	<0.001
AB	34	97.1	-0.54	0.089	-0.72	-0.37	<0.001
AC	32	93.0	-0.71	0.100	-0.90	-0.51	<0.001
BC	36	66.0	-0.89	0.057	-1.01	-0.78	<0.001
BD	23	90.7	-1.22	0.131	-1.48	-0.96	<0.001
CD	26	94.0	-1.15	0.095	-1.34	-0.97	<0.001
ABC	25	78.1	-0.66	0.075	-0.81	-0.51	<0.001
ABD	26	86.3	-0.77	0.094	-0.95	-0.58	<0.001
ACD	24	84.7	-1.23	0.126	-1.47	-0.98	<0.001
BCD	31	94.8	-0.92	0.102	-1.11	-0.72	<0.001
ABCD	44	81.1	-1.69	0.132	-1.95	-1.43	<0.001

[⍰] total number of studies included in the analysis.

[§] I^2 indicates the percentage of total variation in the estimates of treatment effect that was due to heterogeneity between studies. An I^2 value near 100% indicates that most of the observed variance was real, i.e., was not due to sampling error but was due to variance between studies.

[¥] summary estimated effect for each treatment strategy relative to the untreated control NT, in the form $L=\ln(X_T)-\ln(X_{NT})$, where X is the disease severity at harvest.

Table 2. Pairwise comparison of the effect in the reduction of Botrytis bunch rot severity compared to the non-treated control for 14 fungicide treatment strategies based on 1, 2, 3, or 4 sprays applied at timings A (end of flowering, growth stage 69 of Lorenz et al.[11]), B (pre-bunch closure, growth stage 77), C (veraison, growth stage 83), and/or D (before harvest, before growth stage 89).

Fungicide treatment strategy	B	C	D	AB	AC	BC	BD	CD	ABC	ABD	ACD	BCD	ABCD
A	-0.21[1] (0.020)	0.08 (0.333)	-0.01 (0.980)	0.08 (0.142)	0.25 (<0.001)	0.44 (<0.001)	0.76 (<0.001)	0.69 (<0.001)	0.21 (0.018)	0.31 (0.002)	0.77 (<0.001)	0.46 (0.001)	1.23 (<0.001)
B		0.29 (<0.001)	0.21 (0.002)	0.30 (0.002)	0.46 (<0.001)	0.65 (<0.001)	0.97 (<0.001)	0.90 (<0.001)	0.41 (<0.001)	0.52 (<0.001)	0.98 (<0.001)	0.67 (<0.001)	1.44 (<0.001)
C			-0.09 (0.228)	0.01 (0.960)	0.17 (0.120)	0.36 (<0.001)	0.68 (<0.001)	0.61 (<0.001)	0.12 (0.201)	0.23 (0.046)	0.69 (<0.001)	0.38 (0.002)	1.15 (<0.001)
D				0.09 (0.423)	0.25 (0.039)	0.44 (<0.001)	0.76 (<0.001)	0.70 (<0.001)	0.21 (0.049)	0.31 (0.002)	0.77 (<0.001)	0.46 (<0.001)	1.24 (<0.001)
AB					0.16 (0.036)	0.35 (0.001)	0.67 (<0.001)	0.61 (<0.001)	0.12 (<0.001)	0.22 (0.034)	0.68 (<0.001)	0.37 (0.012)	1.14 (<0.001)
AC						0.19 (0.082)	0.51 (<0.001)	0.44 (<0.001)	-0.04 (0.618)	0.06 (0.562)	0.52 (0.001)	0.21 (0.171)	0.98 (<0.001)
BC							0.32 (0.030)	0.26 (0.007)	-0.23 (0.011)	-0.13 (0.264)	0.33 (0.014)	0.02 (0.863)	0.79 (<0.001)
BD								-0.07 (0.717)	-0.55 (<0.001)	-0.45 (0.002)	0.01 (0.971)	-0.30 (0.098)	0.47 (0.007)
CD									-0.49 (<0.001)	-0.38 (0.001)	0.07 (0.558)	-0.24 (0.032)	0.53 (<0.001)
ABC										0.10 (0.270)	0.56 (<0.001)	0.25 (0.034)	1.03 (<0.001)
ABD											0.46 (<0.001)	0.15 (0.167)	0.92 (<0.001)
ACD												-0.31 (0.033)	0.46 (<0.001)
BCD													0.77 (<0.001)

[1] value in the cell corresponds to *L* (A) - *L* (B) = -0.46 – (-0.25) =-0.21, where *L* is the estimated effect; a negative value indicates that the severity of Botrytis bunch rot estimates in the row is lower than that estimates in the column; the probability value of the comparison is in parenthesis.

Table 3. Effect in the reduction of Botrytis bunch rot severity of different fungicide classes.

Fungicide class[a]	K[§]	Estimated effect			
		L[¥]	se(*L*)	95% CI (*L*)	*P*
E3 (intercept)	287	2.86	0.154	2.56 / 3.16	<0.001
C2/G3	6	-0.17	0.802	-1.74 / 1.40	0.829
C5	4	-1.20	0.721	-2.61 / 0.21	0.095
D1	3	-1.09	0.766	-2.59 / 0.41	0.156
D1/C2	6	0.12	0.801	-1.45 / 1.69	0.879
D1/C5	7	-1.35	0.803	-2.92 / 0.22	0.093
D1/C5/G3	7	-0.34	0.800	-1.90 / 1.23	0.675
D1/E2	75	-1.68	0.350	-2.37 / -1.00	<0.001
D1/G3	16	-0.64	0.530	-1.68 / 0.39	0.224
E0	14	0.65	1.115	-1.54 / 2.83	0.561
E3/C5/G3	3	0.37	1.118	-1.82 / 2.56	0.742
E3/D1	5	0.15	0.814	-1.45 / 1.74	0.857
E3/D1/B2	6	0.41	1.115	-1.78 / 2.59	0.714
E3/G1	9	-0.32	0.665	-1.62 / 0.98	0.628
E3/MS	8	-2.33	0.657	-3.61 / -1.04	<0.001
G3	21	-0.73	0.408	-1.53 / 0.07	0.073
MS	3	0.63	1.118	-1.56 / 2.82	0.573
MS/D1/C2	5	0.96	1.116	-1.22 / 3.15	0.387

[a] fungicides were grouped based on the chemical classes defined by the Fungicide Resistance Action Committee[47].

[§] total number of studies included in the analysis for each combination of fungicide class.

[¥] Results are presented as the difference (L) in the log mean of disease severity for each fungicide class relative to class E3 (intercept). *SE*= standard error; *CI*= confidence interval; *P* = probability value (significance of the effect in the reduction of the disease).

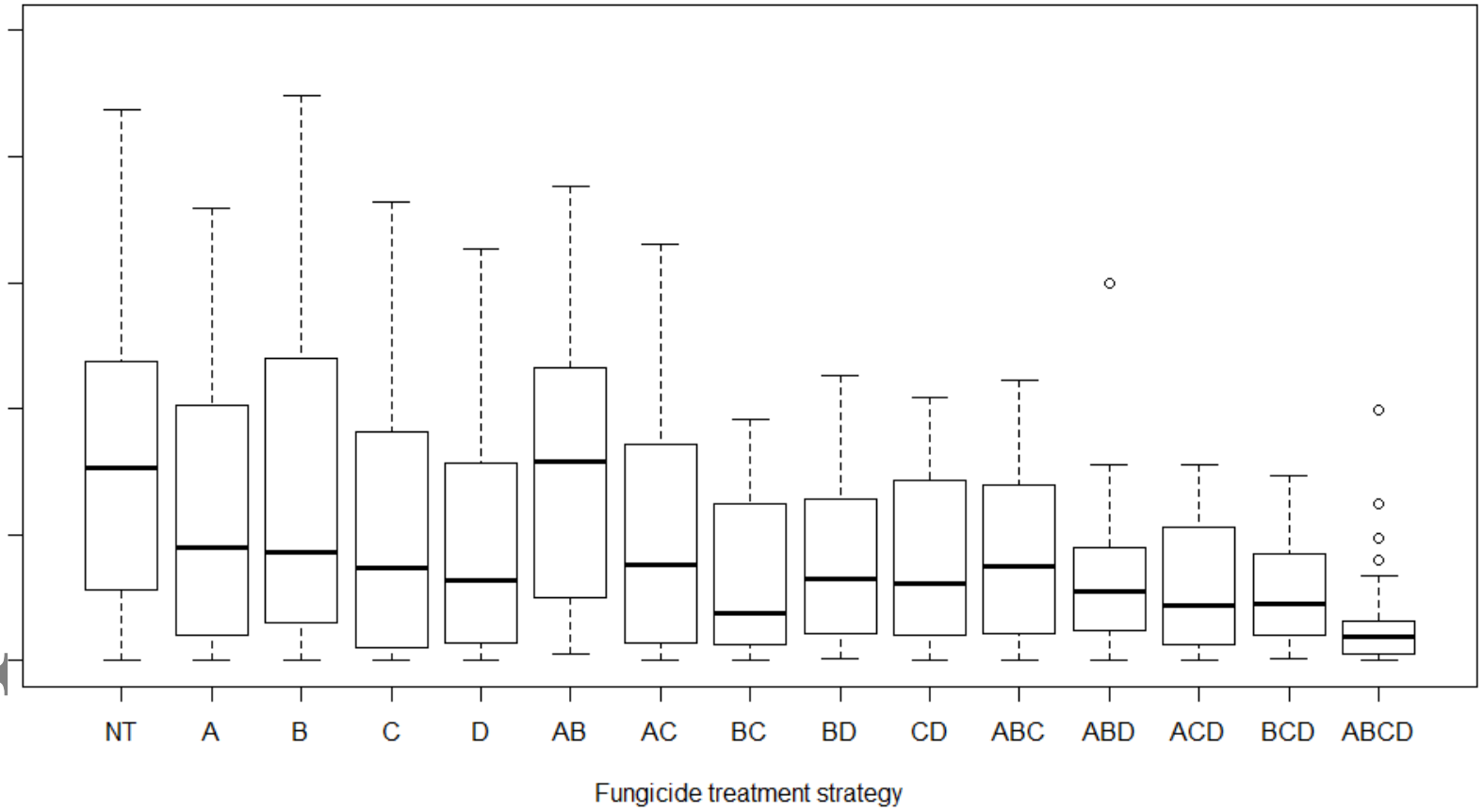

Fig 1

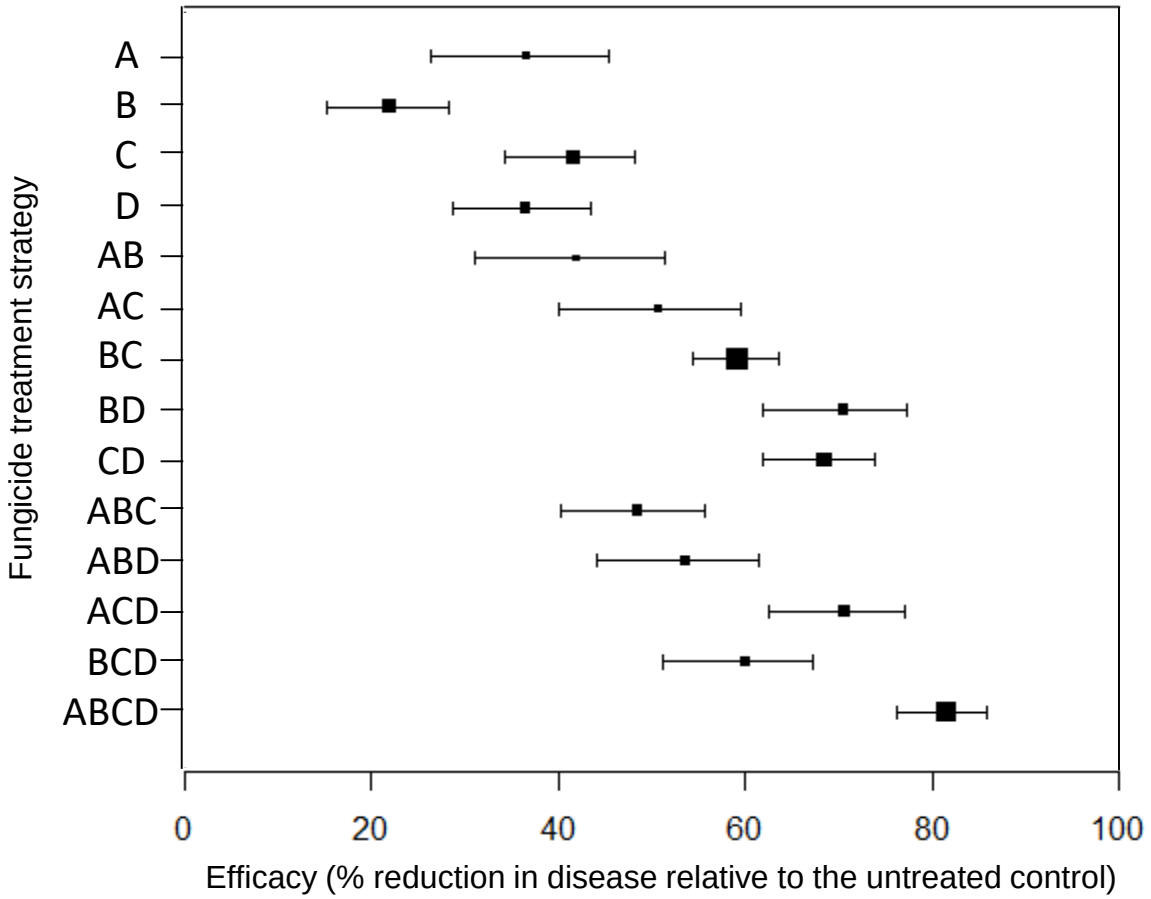

Fig 2