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# A Meta-Analysis of 67 Studies on the Control of Grape Sour Rot Revealed Interesting Perspectives for Biocontrol

**Chiara Brischetto [1,2], Vittorio Rossi [1,2] and Giorgia Fedele [1,2,*]**

[1] Department of Sustainable Crop Production (DI.PRO.VE.S.), Università Cattolica del Sacro Cuore, 29100 Piacenza, Italy; chiara.brischetto@unicatt.it (C.B.); vittorio.rossi@unicatt.it (V.R.)
[2] Research Center on Plant Health Modelling (PHeM), Università Cattolica del Sacro Cuore, 29100 Piacenza, Italy
* Correspondence: giorgia.fedele@unicatt.it

**Abstract:** Sour rot (SR) is a disease complex that affects grape berries during ripening and can cause severe yield losses and deterioration of wine quality. The etiology and epidemiology of the disease remain uncertain, which has severely limited the development of specific, targeted management strategies. In this study, a network meta-analysis was applied to data collected through a previous systematic literature review for statistically comparing the efficacy of different methods for the control of SR and some filamentous fungi isolated from rotten berries. Use of either synthetic fungicides (CHEM) and natural compounds or biocontrol microorganisms (BIO) provided partial and variable control of SR; however, the efficacy of BIO was similar to, or higher than, that provided by CHEM. Agronomic practices (AGRO) had a significant but lower effect on SR. The integration of different control methods (IPM) provided better and less variable disease control than any single method. Natural compounds, such as zeolites and bicarbonates, and microorganisms (e.g., yeasts *Candida* and *Aureobasidium*) are also promising alternatives to synthetic fungicides in SR control.

**Keywords:** *Vitis vinifera*; synthetic fungicides; natural compounds; biocontrol microorganisms; integrated disease management

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## 1. Introduction

Sour rot (SR) is a grapevine disease complex caused by a community of microorganisms including yeasts, bacteria, and filamentous fungi [1–3]. Recently, this disease has received increased interest because of its effect on yield losses and reduced wine quality in several grape production regions of the world [4–7].

Commonly, the first symptoms of SR are oxidation of the grape skin, turning it light-brown in color in both red and white varieties [8,9], followed by softening and disaggregation of the internal tissues. Affected berries ooze fermented grape pulp that smells of acetic acid and drips onto other berries within the cluster [10–13]. The SR-associated microorganisms penetrate berries through cracks and wounds in the berry skin that are caused by both abiotic (e.g., rain, high humidity, hail, and berry abrasion) and biotic (e.g., pests and birds) factors, including fungal pathogens such as *Botrytis cinerea* [14] and *Erysiphe necator* [15], feeding activity of berry moths such as the tortricid *Lobesia botrana* [16] and wasps [17], or oviposition of *Drosophila suzuki* flies [18]. The typical smell of affected berries [19] is caused by the activity of acetic acid bacteria (AAB) such as *Acetobacter*, *Gluconobacter*, and *Gluconacetobacter*, which oxidate the ethanol produced by the fermentation to acetic acids of berry sugars by non-*Saccharomyces* yeasts [20]. Some filamentous spoilage fungi such as *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, and *Rhizopus* can finally grow on rotten berries [2,21].

The etiology and epidemiology of the disease are still not completely understood, which has severely limited the development of specific, targeted management strategies [9]. Recently, a systematic literature review was conducted by Brischetto et al. [22] with the aim of collecting and synthesizing the available literature on SR, focusing on its etiology, epidemiology, and control. Disease control strategies are mainly based on the integration of cultural and chemical control methods [23,24]. SR can be partially managed through practices focused on creating less conducive environment for the disease, reducing fruit fly infestations, preventing berry damages, choosing a trellis system that reduces canopy density, or managing the canopy to optimize air movement and reduce humidity. Leaf removal on the fruit zone is the most applied technique, able to decrease the incidence and severity of SR because it reduces the density of the canopy, increasing air movement, solar radiation, and temperature while reducing the relative humidity within the fruiting zone. Also, leaf removal increases the penetration of plant protection products (PPPs) into the fruit zone [23,25–27]. Chemical programs using PPPs can also contribute to the direct control of SR development. For instance, Tjamos et al. [24] proved that the treatments with fludioxonil were effective in controlling the incidence of SR caused by *Aspergillus* spp., while the treatments with cyprodinil were ineffective. However, the increase in restrictions of the use of PPPs determines the need of developing alternative SR management practices that can effectively control the disease.

In this work, we used the results of the above-mentioned systematic literature review [22] to statistically compare methods for the control of SR and of some filamentous fungi isolated from rotten berries through a network meta-analysis. Network meta-analysis allows for the integration and analysis of results obtained from different sources of information, considering all possible correlations [28], for determining the overall effectiveness of numerous disease control methods. This multi-strategy analysis permits the use of a large number of individual studies and is built on the principle that overall knowledge is based on the collection and combination of results from individual studies and observations [29–32].

## 2. Materials and Methods

### 2.1. Database of Studies

A database concerning studies of SR control was assembled based on the systematic literature review conducted by Brischetto et al. [22]. The research methodology for this systematic review was described by Brischetto et al. [22] and was conducted by using the three most relevant online bibliographic databases: (i) Scopus (https://www.scopus.com/, accessed on 17 October 2023); (ii) Web of Science Core Collection (http://webofknowledge.com/WOS, accessed on 17 October 2023); and (iii) Google Scholar (https://scholar.google.com/, accessed on 17 October 2023). Database searches were conducted in English. The search terms “sour rot”, “grape”, and “vitis vinifera” were combined into search strings using wildcards (*) and connectors (AND, OR) for identifying works for inclusion.

The search string provided papers in which SR was associated with different microorganisms, including the yeasts *Pichia* spp., *Candida* spp., and *Hanseniaspora* spp., and/or the AAB *Gluconobacter* spp. and *Acetobacter* spp., which have been frequently associated with SR for their capability to produce ethanol, acetic acid, and other compounds [13,20]. Other papers reported the occurrence or co-occurrence of filamentous fungi such as *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp., *Alternaria* spp., and *Cladosporium* spp., which may worsen the berry rot [21], spoil wine [12], and/or produce mycotoxins under vineyard conditions [2,33]. As we were interested in the control of the disease complex, we did not select papers based on the microorganisms that have been associated with SR by different authors.

To be included in the database for meta-analysis, papers had to meet the following criteria: (i) at least one treatment type was applied once to several times in a season to control berry rot; (ii) treatments were applied in the field or laboratory with either natural or artificial inoculum; (iii) berry rot was assessed as incidence of affected bunches (referred to as $X$ for the meta-analysis); and (iv) the experiment had a suitable experimental design with at least three replicates and an untreated control (NT). Papers dealing with insecticides for controlling *Drosophila* spp. flies (Diptera: Dosophilidae) were not included in the analysis because we focused on methods for direct control of the disease rather than insect vectors.

Sixteen papers were considered in aggregate, for a total of 67 studies, with a study being a single independent experiment in which at least two treatments were compared, one of them being an untreated control (NT). Overall, 573 entries (i.e., combination of treatment and disease/causal agent) were considered [34]. These entries were split in three datasets: (i) 142 entries conducted on SR in field experiments between 1988 and 2022 in Canada, Greece, Italy, Spain, and USA; (ii) 66 entries conducted on *Aspergillus* spp. in field experiments between 2002 and 2006 in Greece; and (iii) 368 entries carried out in laboratory conditions with the following filamentous fungi: *Aspergillus* spp. (189 entries), *Rhizopus stolonifer* (47 entries), *Fusarium oxysporum* (44 entries), *Ulocladium* sp. (44 entries), and *Penicillium comune* (44 entries).

For field experiments, most of the studies were conducted with a randomized complete block design, with four replicate blocks. Grape variety and treatment (i.e., disease control methods) varied among studies, as summarized in Table 1 for datasets (i) and (ii) and in Table 2 for dataset (iii). The treatments were grouped into the four following categories: CHEM (chemical), based on the application of synthetic fungicides only (e.g., fludioxonil, cyprodinil, and carbendazim); BIO (biological), based on the application of biological control agents (BCAs, e.g., *Aureobasidium pullulans*, *Candida sake*, and *Ulocladium oudemansii*), natural products (e.g., zeolite, calcium chloride, and sodium bicarbonate), or their combination; AGRO (agronomical), based on leaf removal; and IPM (integrated pest management), based on the integration of AGRO, BIO, and/or CHEM.

**Table 1.** Main characteristics of the studies conducted in the field. Treatments were grouped into four categories: AGRO, based on agronomic practices; BIO, based on the application of biological control agents, natural products, or their combination; CHEM, based on the application of chemical fungicides only; and IPM, integrated (based on the integration of AGRO, BIO, and/or CHEM).

| Treatment Category | Type of Intervention | Grape Variety | Location, Years | Paper Source |
|---|---|---|---|---|
| AGRO | Leaf removal | Barbera (1) [1], Chenin blanc (3), Sauvignon (1) | USA, 1988, 1990 | [23] |
| | Leaf removal | Barbera (1), Thompson Seedless (1), Chardonnay (1), Zinfandel (1), Carignane (1) | USA, 1989–1992 | [25] |
| | Leaf removal | Chardonnay (1) | Canada, 2004 | [26] |
| | Leaf removal | Chardonnay (2) | USA, 2017–2018 | [27] |
| BIO | *Candida saitoana* + chitosan, antifungal lytic enzyme | Italia (1) | Italy, 2004 | [35] |
| | Calcium chloride, sodium bicarbonate, sodium carbonate, potassium carbonate | Italia (2) | Italy, 2006 | [5] |
| | *Aureobasidium pullulans* | Agiorgitiko (9), Grenache Rouge (6) | Greece, 2003–2006 | [36] [2] |
| | *Candida sake*, fatty acids, chitosan, *Ulocladium oudemansii* | Macabeu (2) | Spain, 2009–2010 | [37] |
| | Zeolitites | Montepulciano (2), Cococciola (2) | Italy, 2015–2016 | [38] |
| | *C. sake*, fatty acids, maltodextrin, potato starch | Macabeu (2) | Spain, 2015–2016 | [39] |
| | Oligochitosans, oligopectates, mycorrhizal fungi, copper, sulfur | Nero d’Avola (1), Inzolia (1) | Italy, 2002 | [40] |
| | Zeolite | Trebbiano d’Abruzzo (2) | Italy, 2018–2019 | [41] |

**Table 1.** *Cont.*

| Treatment Category | Type of Intervention | Grape Variety | Location, Years | Paper Source |
|---|---|---|---|---|
| CHEM | Mepanypirim | Italia (1) | Italy, 2004 | [35] |
| | Procymidone, fludioxonil, cyprodinil | Italia (2) | Italy, 2006 | [5] |
| | Fludioxonil, cyprodinil | Agiorgitiko (9), Grenache Rouge (6) | Greece, 2003–2006 | [36] [2] |
| | Cyprodinil, fludioxonil | Montepulciano (2), Cococciola (2) | Italy, 2015–2016 | [38] |
| | Fludioxonil, cyprodinil, carbendazim | Not available (6), Grenache Rouge (3),<br>Cabernet Sauvignon (3) | Greece, 2003–2004 | [24] [2] |
| IPM | Leaf removal, fenarimol, sulfur, cryolite, propargite | Barbera (1), Thompson Seedless (1), Chardonnay (1), Zinfandel (1), Carignane (1) | USA, 1989–1992 | [25] |
| | Cyprodinil, fludioxonil, zeolite | Montepulciano (2), Cococciola (2) | Italy, 2015–2016 | [38] |

[1] Number in brackets indicates the number of studies. [2] Studies that also include the effect of treatments on the incidence of affecting berries from which *Aspergillus niger* and *A. carbonarius* were isolated.

**Table 2.** Main characteristics of studies conducted using biological control agents to control filamentous fungi that were associated with berries affected by sour rot through artificial inoculation of berries under laboratory conditions.

| Biocontrol Agent | Target Fungus | Country; Grape Variety | Paper Source |
|---|---|---|---|
| *Candida guilliermondii*, *Kloeckera apiculata* | *Aspergillus niger* (1) [1], *Rizhopus stolonifer* (1) | Israel; Thompson Seedless | [42] |
| *Saccharomyces cerevisiae*, *S. chevalieri*, *S. kluyveri*, *Candida catenulata*, *C. famata*, *C. rugosa*, *C. sake*, *C. versatilis*, *Debaryomyces vanrijiae*, *Dekkera anomala*, *Issatchenkia orientalis*, *Kluyveromyces marxianus*, *Pichia mebranifaciens*, *Sporobolomyces roseus*, *Torulaspora delbrueckii* | *A. caelatus* (1), *A. carbonarius* (1), *A. terreus* (1), *A. versicolor* (1), *Fusarium oxysporum* (1), *Penicillium comune* (1), *R. stolonifer* (1), *Ulocladium* sp. (1) | Argentina; Redglobe | [3] |
| *Candida intermedia*, *C. friedrichii*, *Cyberlindnera jadinii*, *Lachancea thermotolerans* | *A. carbonarius* (2) | Italy; Italia | [43] |

[1] Number in brackets indicates the number of studies.

The laboratory studies, which included three replicates, evaluated the efficacy of BCAs (Table 2). In these studies, intact berries were taken from the vineyard during the ripening period and rinsed in water, surface-disinfected, washed again to remove disinfectants, and finally wounded with a sterile needle. The treatment methods with BCAs were different, comprising the immersion of wounded berries in a suspension of each BCA ($10^8$ CFU/mL) [42,43] or pipetting the suspension of each yeast (containing $10^6$ CFU/mL) into the wound [3]. Following treatment, berries were inoculated with fungal spore suspensions (containing $10^4$–$10^7$ cells/mL) pipetted into each wound [3] or sprayed by using a hand sprayer [42,43]. After inoculation, berries were air-dried, placed in plastic boxes or bags, and kept at temperatures of 22–25 °C for six days depending on the study.

### 2.2. Meta-Analysis

A network meta-analysis was conducted to evaluate the effect of the treatment in reducing disease incidence compared to the non-treated control [44,45]. For each study and treatment (including the non-treated control), mean disease incidence data were extracted from the publication and used to conduct the analysis. For each study, disease incidence ($X$) from treated (T) and non-treated plots (NT) were used to estimate treatment effect ($L$)

according to the following equation: $L_T = ln(X_T) - ln(X_{NT})$. The log incidence of treatments was used because the distribution of $ln(X)$ is closer to a normal distribution with respect to the distribution of $X$.

The within-study variance was estimated for each treatment of a single study as $s^2 = V/(n\overline{X}^2)$, where $V$ is the residual error component of the study, $n$ is the number of replicates, and $\overline{X}$ is the mean disease incidence. To calculate $V$ for each study, different approaches were used [45,46]: (i) when the original data were available, $V$ was extracted directly from the ANOVA table of the study (i.e., the residual variance or mean square error); (ii) when the study did not include the ANOVA table but did include the least significant difference (LSD), $V$ was calculated as ($n$ (LSD/1.96)$^2$/2); (iii) when only the significant mean separation was provided (i.e., significant differences between means denoted by letters in a graph or a table), the estimated LSD was computed as the average between the smallest observed significant difference and the largest observed non-significant difference.

The meta-analysis was conducted with the software R (v. 3.4.0; package "metafor") [47,48]. A multivariate random effects model was fitted via linear (mixed-effects) models by using the rma.mv function. The model was fitted in the form $Y \sim N(\mu, \Sigma + S)$, where $\sim N$ indicates a multivariate normal distribution, $\mu$ is the expected value for the different treatments, $\Sigma$ is the between-study variance–covariance matrix, and $S$ is the within-study variance–covariance matrix. The restricted maximum-likelihood estimation (REML) method was used for model fitting. Random effects were specified in the form ~inner | outer, with the outer factor corresponding to the study identification and the inner factor corresponding to the treatment type (i.e., the disease control method).

To assess the nature of the residual heterogeneity, $I^2$ statistics were calculated as proposed by [49] and [50] by also running a similar model but with fixed effects; the $I^2$ statistic was based on the relation between the variance–covariance matrix of models with fixed and random effects in the following form: (vcov(random)[1] − vcov(fixed)[1])/vcov(random)[1]).

Treatment effects were presented as $L$, with negative values of $L$ indicating that SR incidence was lower in the treated plot than in the NT control (i.e., the treatment reduced the disease incidence compared to the untreated control). Standard errors and confidence intervals were likewise calculated for these values of $L$. A Wald-type test statistic was used to determine whether the mean differences in $L$ were significantly different from zero, namely, whether the disease incidence 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 as $(1 - \exp(L)) \times 100$, and the 95% confidence intervals were calculated as by [44,51].

## 3. Results

In dataset (i), there were 54 entries for BIO, which considered the efficacy on SR of different BCAs (i.e., *Candida* spp., *Aureobasidium pullulans*, and *Ulocladium oudemansii*) applied in the vineyard alone and/or in combination with substances of natural origin (e.g., chitosan, sodium bicarbonate, fatty acids, and zeolites), or with fungicides (specifically, copper or sulfur). For AGR, there were 24 entries dealing with leaf removal for reducing moisture of the bunch during ripening. In particular, the leaf removal was applied pre-bloom in 20.8% of these studies (<BBCH 61), after bloom in 37.5% (>BBCH 61), in full bloom in 37.5%, and during veraison in 4.2% (BBCH 81).

For CHEM, the 21 entries considered the efficacy of synthetic fungicides used alone, specifically aminopyrimidines (mepanypirin, cyprodinil), phthalates (procymidone), benzodioxoles (fludoxionil), and benzimidazoles (carbendazim). Finally, for IPM, the six entries combined leaf removal, fungicides, and/or natural compounds.

SR incidence in the NT plots of the studies conducted in the field ranged from 0.3% to 97.5%, with 90% of the values ranging from 4.4% to 73.8%, indicating a wide range of epidemiological situations (Figure 1).

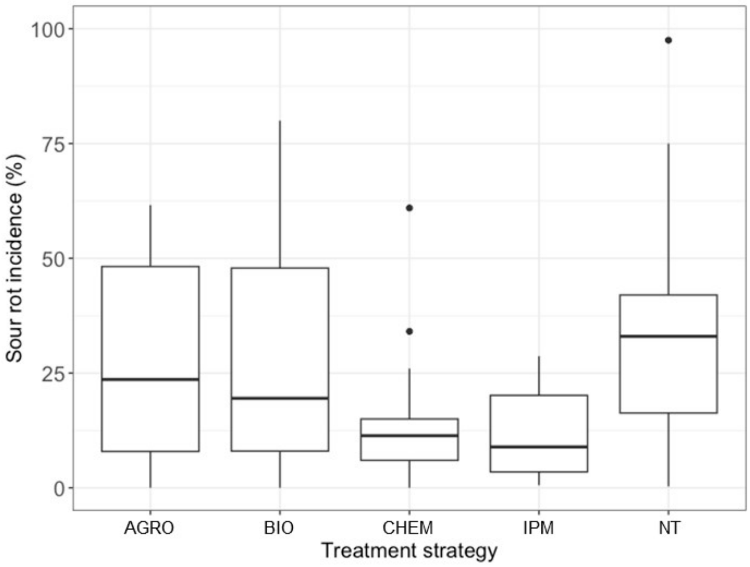

**Figure 1.** Box plot representing the distribution of grape sour rot incidence in different field studies in which disease control treatments were grouped as follows: AGRO, based on agronomic practices; BIO, based on the application of biological control agents, natural products, or their combination; CHEM, based on the application of chemical fungicides only; and IPM, integrated (based on the integration of AGRO, BIO, and/or CHEM). NT means non-treated control.

The average disease incidence was 33.9% (±3.7%) in the NT plots and 24.4% (±2.2%) in the treated plots. Disease incidence showed high variability among treatments (Figure 1). For example, with the AGRO and BIO treatments, 90% of disease incidence values ranged from 0.4% to 59.8% and from 1.8% to 70.7%, respectively. Lower variability was observed for CHEM, where 90% of the disease incidence values ranged from 0% to 34%.

Heterogeneity testing indicated that the results of the meta-analysis were consistent and robust. The test for residual heterogeneity rejected the null hypothesis of homogeneity across studies ($Q_E = 5253$, df = 136, $p < 0.0001$), and the values of $I^2$ were >70% for all treatments except for the integrated one (Table 3). Therefore, the heterogeneity in the estimated *L* values was mainly due to the among-studies variability and not the sampling errors in each study. The average values of *L* were significantly less than zero for all treatments (i.e., estimated SR incidence was lower in the treated than in the untreated plots; Table 3). Pairwise comparison by linear contrast showed that the *L* value estimated for IPM (−1.15) was significantly lower than those for all other treatment categories ($p \leq 0.025$; Table 4), with an average disease reduction of 68.6% with respect to NT (Figure 2).

**Table 3.** Effect on reduction in sour rot incidence compared to the untreated control of four treatment categories: CHEM (application of chemical fungicides only); BIO (application of biological control agents, natural products, or their combinations); AGRO (application of agronomical practices only); or IPM (integration of chemical fungicides with strategies from the other categories).

| Treatment Category | K ł | $I^{2\,\S}$ | Estimated Effect in Disease Reduction | | | | |
|---|---|---|---|---|---|---|---|
| | | | $L^{¥}$ | Se of *L* | 95% Confidence Interval of *L* | | *p* |
| AGRO | 24 | 93.7 | −0.53 | 0.136 | −0.80 | −0.26 | <0.001 |
| BIO | 54 | 93.7 | −0.78 | 0.138 | −1.05 | −0.51 | <0.001 |
| CHEM | 21 | 95.6 | −0.66 | 0.126 | −0.91 | −0.41 | <0.001 |
| IPM | 6 | 71.4 | −1.15 | 0.169 | −1.49 | −0.83 | <0.001 |

ł Total number of entries included in the analysis. § $I^2$ indicates the percentage of total variation in the estimates of treatment effects that were due to heterogeneity between studies. An $I^2$ value near 100% indicates that most of the observed variance was real (i.e., not due to sampling error, but to variance between studies). ¥ Summary of the estimated effect for each treatment category relative to the untreated control NT, in the form $L_T = ln(X_T) - ln(X_{NT})$, where *X* is the disease incidence at harvest.

**Table 4.** Pairwise comparison of the effect on reduction in sour rot incidence compared to the non-treated control for different treatment categories: CHEM (application of chemical fungicides only); BIO (application of biological control agents, natural products, or their combinations); AGRO (application of agronomical practices only); or IPM (integration of chemical fungicides with strategies from the other categories).

| | **BIO** | **CHEM** | **IPM** |
|---|---|---|---|
| AGRO | 0.25 (0.196) | 0.13 (0.447) | 0.63 (0.007) |
| BIO | | −0.12 (0.406) | 0.38 (0.025) |
| CHEM | | | 0.50 (0.002) |

The values in each cell correspond to *L* (AGRO) − *L* (BIO) = −0.53 − (−0.78) = −0.25, where *L* is the estimated effect; a negative value indicates that the incidence of sour rot estimates in a row is lower than those in a column; the probability value of the comparison is in parentheses.

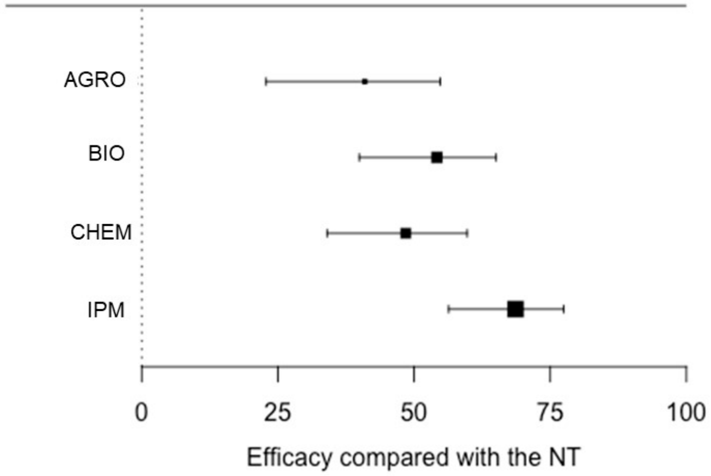

**Figure 2.** Efficacy of different treatments on the control of grape sour rot in the field expressed as the percentage of disease reduction relative to the non-treated control (NT), as estimated using the meta-analysis. Treatments were grouped as follows: AGRO, based on agronomic practices; BIO, based on the application of biological control agents, natural products, or their combination; CHEM, based on the application of chemical fungicides only; and IPM, integrated (based on the integration of AGRO, BIO, and/or CHEM). Whiskers show 95% confidence intervals. Dots represent the average efficacy of treatments, and dot size increases with the precision of estimates.

The *L* value estimated for BIO was not significantly different from, but was lower than, those estimated for both AGRO and CHEM (Table 4); therefore, BIO provided better disease control than both AGRO and CHEM, with an average disease reduction of 54.1% (Figure 2).

In dataset (ii), 10 entries and 38 were conducted to evaluate the effect of *A. pullullans* (BIO) and fludioxonil, cyprodinil, or carbendazim (CHEM) on the reduction in *Aspergilli* isolated from affected bunches. Aspergillus rot incidence ranged from 12% to 100% (Figure 3), with an average of 58.9% (±8.1%) in NT bunches.

Disease incidence showed higher variability among plots treated with CHEM (Figure 3), with values ranging from 0% to 100%. The test for residual heterogeneity rejected the null hypothesis of homogeneity across studies ($Q_E$ = 693, df = 63, $p < 0.0001$), and the value of $I^2$ was >95% for CHEM only (Table 3). The average values of *L* were significantly less than zero for BIO, but not for CHEM (Table 5). The *L* value estimated for BIO (−0.24) was not significantly different from, but was lower than, that estimated for CHEM (−0.12; $p = 0.213$). Therefore, BIO provided slightly better efficacy than CHEM, with an average reduction of 21.1% compared to NT (Figure 4).

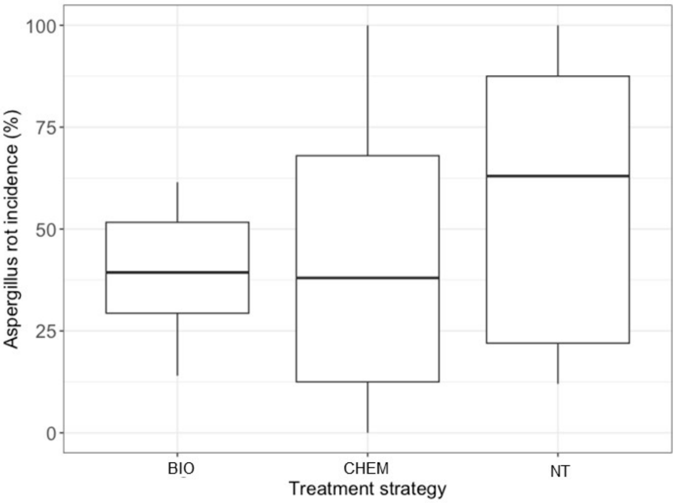

**Figure 3.** Box plots representing the distribution of the incidence of *Aspergilla* isolated from rotten berries that had been treated in the field to control sour rot. Treatments were grouped as follows: BIO, based on the application of biological control agents, natural products, or their combination; and CHEM, based on the application of chemical fungicides only. NT means non-treated control.

**Table 5.** Effect in the reduction in *Aspergillus* spp. incidence compared to the untreated control of two treatment categories: CHEM (application of chemical fungicides only); and BIO (application of biological control agents, natural products, or their combinations).

| Fungicide Treatment Strategy | K [ł] | $I^2$ [§] | Estimated Effect in Disease Reduction | | | | |
|---|---|---|---|---|---|---|---|
| | | | $L$ [¥] | Se of $L$ | 95% Confidence Interval of $L$ | | $p$ |
| BIO | 10 | 62.8 | −0.24 | 0.092 | −0.417 | −0.058 | 0.009 |
| CHEM | 38 | 95.6 | −0.12 | 0.142 | −0.395 | −0.163 | 0.414 |

[ł] Total number of entries 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., not due to sampling error, but to variance between studies). [¥] Summary of the estimated effect for each treatment categories relative to the untreated control NT in the form $L_T = ln(X_T) - ln(X_{NT})$, where $X$ is the disease incidence at harvest.

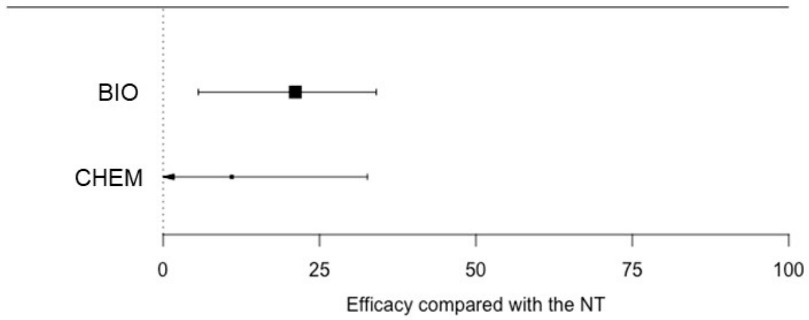

**Figure 4.** Efficacy (%) of different treatments on the incidence of *Aspergilli* isolated from rotten berries that had been treated to control sour rot, relative to the non-treated control (NT), as estimated with the meta-analysis. Treatments were grouped as follows: BIO, based on the application of biological control agents, natural products, or their combination; and CHEM, based on the application of chemical fungicides only. Whiskers show 95% confidence intervals. Dots represent the average efficacy of treatments, and dot size increases with the precision of estimates.

Incidence of berry rot caused by artificial inoculation of berries with fungi isolated from sour rot-damaged grapes (dataset (iii)) ranged from 10% to 100% in NT, with an average of 86.9% (±7.6%). The average rot incidence was 86.7% (±1.6%) in berries that had been treated with BCAs, with a wide range of 17.9% to 100%. The test for residual heterogeneity rejected the null hypothesis of homogeneity across the studies ($Q_E = 1607$, df = 366, $p < 0.0001$), and the heterogeneity in the estimated $L$ value was mainly due to the among-studies ($I^2 = 94.6\%$). The average values of $L$ for the treated groups was significantly less than zero (−0.265; confidence interval 95%: −0.502, −0.029; $p = 0.028$). Overall, the treatment provided a disease reduction of 23.3% compared to NT.

When dataset (iii) was explored for *Aspergillus* spp. only, the average disease incidence was 81.8% (±12.5%) in NT, with values ranging from 10% to 100%. The test for residual heterogeneity rejected the null hypothesis of homogeneity across studies ($Q_E = 1231$, df = 176, $p < 0.0001$), and the values of $I^2$ were >80% for all the tested microorganisms, except for *Kloeckera* sp. ($I^2 = 39.4\%$) and *Lachancea* sp. ($I^2 = 48.9\%$). The average values of $L$ for the treated groups were significantly less than zero for *Candida* spp., *Debaryomyces* sp., *Dekkera* sp., *Kloeckera* sp., *Kluyveromyces* sp., *Pichia* sp., and *Saccharomyces* spp. (Table 6). The highest disease reduction was observed with *Kloeckera* sp. (94.3%) (Figure 5).

**Table 6.** Effect of biocontrol microorganisms on the reduction in grape berry rot incidence caused by *Aspergilli* isolated from berries showing sour rot symptoms, as compared to the untreated control under laboratory conditions.

| **Biocontrol Microorganism** | **K** [ł] | $I^2$ [§] | **Estimated Effect in Berry Rot Reduction** | | | | |
|---|---|---|---|---|---|---|---|
| | | | $L$ [¥] | **Se of** $L$ | **95% Confidence Interval of** $L$ | | $p$ |
| *Candida* | 41 | 97.1 | −0.43 | 0.22 | −0.86 | −0.01 | 0.044 |
| *Cyberlindnera* | 2 | 80.7 | 0.07 | 0.26 | −0.43 | 0.58 | 0.772 |
| *Debaryomyces* | 8 | 90.6 | −0.76 | 0.22 | −1.19 | −0.32 | 0.001 |
| *Dekkera* | 8 | 94.9 | −0.45 | 0.22 | −0.87 | −0.02 | 0.041 |
| *Issatchenkia* | 4 | 81.6 | −0.45 | 0.24 | −0.91 | 0.01 | 0.055 |
| *Kloeckera* | 1 | 39.4 | −2.86 | 1.21 | −5.23 | −0.50 | 0.018 |
| *Kluyveromyces* | 8 | 90.1 | −0.68 | 0.22 | −1.12 | −0.24 | 0.002 |
| *Lachancea* | 2 | 48.9 | −0.62 | 0.34 | −1.28 | 0.04 | 0.065 |
| *Pichia* | 8 | 95.2 | −0.47 | 0.22 | −0.90 | −0.05 | 0.029 |
| *Saccharomyces* | 64 | 97.3 | −0.45 | 0.22 | −0.87 | −0.02 | 0.038 |
| *Sporobolomyces* | 4 | 94.7 | −0.38 | 0.22 | −0.81 | 0.04 | 0.078 |
| *Torulaspora* | 32 | 97.2 | −0.40 | 0.22 | −0.82 | 0.03 | 0.066 |

[ł] Total number of entries 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., not due to sampling error, but to variance between studies). [¥] Summary of the estimated effect for each genera relative to the untreated control NT, in the form $L_T = \ln(X_T) - \ln(X_{NT})$, where $X$ is the disease incidence at harvest.

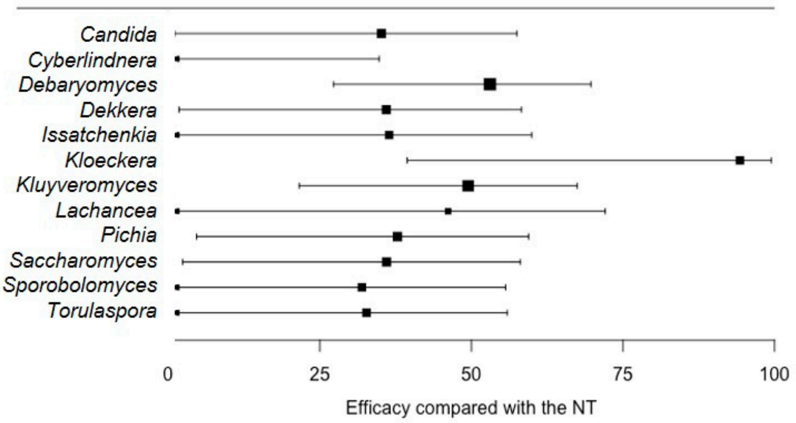

**Figure 5.** Efficacy of different microorganisms on the reduction in the incidence of grape berry rot caused by *Aspergilli* (% of berries) relative to the non-treated control (NT), as estimated with the meta-analysis. *Aspergilli* had been isolated from rotten berries showing sour rot symptoms, and were artificially inoculated on healthy, wounded berries. Whiskers show 95% confidence intervals. Dots represent the average efficacy of treatments, and dot size increases with the precision of estimates.

The *L* value estimated for *Kloeckera* sp. (−2.86) was significantly lower than that estimated for *Candida* spp. (−0.43; $p = 0.049$), *Cyberlindnera* sp. (0.07; $p = 0.018$), *Sporobolomyces* sp. (−0.38; $p = 0.045$), and *Torulaspora* sp. (−0.40; $p = 0.046$) (Table 6).

## 4. Discussion

In this work, a network meta-analysis was conducted using 16 papers (for a total of 67 studies and 573 entries) on the control of SR and some filamentous fungi isolated from rotten berries, which were retrieved through a previous systematic literature review [22]. The size of this dataset ensured the representativeness of the results. We used the term "sour rot" in the literature search because we were more interested in the control of the disease complex (which is mainly caused by a consortium of non-*Saccharomyces* yeasts and AAB that usually evolve during the disease) rather than on single microorganisms of the complex. Since some papers mentioned some filamentous fungi (e.g., *Aspergillus* spp., *Cladosporium* spp., *Rhizopus* spp., etc.) as components of SR, usually as final invaders of spoiled bunches, we dedicated a specific analysis to these fungi. Studies on the control of these fungi as single rotting agents have not been considered; for instance, studies on *Aspergillus* spp. for their ability to accumulate the mycotoxin ochratoxin A in grape berries with no reference to SR were not considered [52,53].

Overall, our meta-analysis showed partial and variable control of SR with either synthetic fungicides (CHEM) and natural compounds or biocontrol microorganisms (BIO). This result may be related to the etiology of SR, which involves microbial communities that include non-Saccharomyces, AAB, and eventually saprophytic or weak pathogenic filamentous fungi [2,20,21]. The composition of these microbial communities changes in different grape-growing areas, agricultural contexts, and seasons [54]. Clearly, plant protection products may be more or less effective depending on their activity against the specific microorganisms involved in the SR etiology in a specific agricultural situation.

The complexity of SR's etiology is likely the reason why the integration of different control methods (IPM) provided better and less variable disease control than other methods, with different plant protection products likely able to differently control different microorganisms. The integration of different methods in an IPM approach also meets the request of switching from synthetic fungicides to low-impact options for disease control [55,56]. Indeed, it is already known that the combination of agronomic practices and BCAs guarantees the greater efficacy of the latter [57].

Synthetic fungicides of different chemical groups were used in studies included in our meta-analysis. The most-used active ingredients belong to the anilino-pyrimidines and phenylpyrroles groups (e.g., cyprodinil, fludioxonil), commonly applied to control gray mold worldwide [45] and secondary bunch rots caused by filamentous fungi (EU pesticide database). Some active ingredients evaluated for SR control are no longer approved for their use in agriculture (procymidone, carbendazim, fenarimol) and, based on our dataset, several active ingredients currently authorized for the control of gray mold have not been tested for their efficacy in controlling SR. These active ingredients include the multisite folpet, succinate dehydrogenase inhibitors (e.g., boscalid), uncouplers of oxidative phosphorylation, and others. Their evaluation may enlarge the fist of synthetic fungicides useful for SR management in vineyards, leading to possible improvements in chemical control, even though the use of synthetic fungicides late in ripening leads to negative impacts and possible residues in the final products [2].

Leaf removal, which was the most investigated agronomic practice used in the selected studies (AGRO), had a significant but low effect on SR in our meta-analysis. This practice, consisting in the removal of basal leaves of shoots in the cluster zone, creates a less conducive environment for SR by facilitating air movement and reducing humidity in the bunch zone [9,10,23,58,59].

Leaf removal was also used as a component of IPM. When combined with CHEM or BIO, leaf removal may improve the distribution of BCA on bunches [60] in addition to modifying the grape's microclimate. However, microclimatic conditions can influence the survival, growth, and efficacy of BCAs [61,62]. For example, Calvo-Garrido et al. [63] found that leaf removal reduced the population of the BCA *C. sake* on grapes under the concentration threshold for effective SR suppression by exposing the yeast to abiotic factors constraining survival in field conditions, such as temperature, relative humidity, and sunlight. Therefore, integration of leaf removal and BCAs should be carefully evaluated.

In our meta-analysis, the efficacy of BIO (which includes natural compounds and biocontrol microorganisms) was similar to, or higher than, that provided by synthetic fungicides. This is a very interesting result because disease control with natural alternatives usually provides less and more variable control than with chemical pesticides in several pathosystems [64].

Zeolites and carbonates were among the most considered natural compounds for SR control; they were used in eight studies, while chitosan or fatty acids were used in only four. Zeolites are inorganic crystalline minerals characterized by a structure based on an infinitely extended three-dimensional framework, with cations bonded within this framework by electrostatic forces; these cations are exchangeable through diffusion in a medium containing another cation and add antimicrobial properties to the substance [65].

Zeolites, which also absorb water and reduce moisture on plant surfaces, show broad-spectrum antimicrobial activity and long-lasting active periods when modified with metal ions such as $Ag^{+}$, $Cu^{2+}$, $Fe^{2+}$, and $Zn^{2+}$ [66–71], and are capable of bonding with mycotoxins [72]. Modified natural zeolites show multiple modes of microbicidal action, including disruption of the cell wall, cell membrane damage, and the alteration of multiple cellular functions through the interaction with proteins, lipides, and DNA, as well as inducing of oxidative stress [73]. Thus, zeolites have potential for large use in crop protection [74]. In viticulture, zeolites have also been used to control downy and powdery mildews and Botrytis bunch rot [75–78].

Carbonates and bicarbonates are inorganic salts with well-known broad-spectrum activity in controlling a wide range of fungi, including food spoilage organisms and plant pathogens [79,80]. For example, ammonium, potassium, and sodium bicarbonates are able to directly inhibit fungal colony growth and spore development, leading to collapse and shrinkage most likely because of changes in the pH and osmotic pressure of cells [5,81–83]. Bicarbonates can also induce resistance, as demonstrated in citrus fruit [84]. Interestingly, sodium bicarbonate increases the efficacy of the biocontrol yeast *Hanseniaspora uvarum* against *B. cinerea* in table grapes [85]. Sodium bicarbonate also inhibits the growth of bacteria and yeasts [86].

The yeasts *Candida* spp. and *A. pullulans* were the most-used microorganisms for biocontrol in the field, and species of *Candida*, *Pichia*, *Kloeckera*, and *Saccharomyces* were tested under laboratory conditions. It is important to note that these yeasts are among the most common inhabitants of the grape berry surface during ripening [20,87–89], meaning that they can stably occupy this particular ecological niche, which is a prerequisite for BCA effectiveness. Stable colonization of the grape berry surface by yeasts (e.g., *A. pullulans*), microcracks and wounds (the pathway for SR microorganisms entering the berry pulp), high tolerance to different ecological stresses (e.g., desiccation and irradiation) [90,91], competition for space and nutrients [92–94], and antagonistic activity through the production of extracellular chitinases and β-1-3-glucanases [95] have been proven to be effective against *B. cinerea*, *Penicillium* spp., and *Aspergillus* spp. [96–99], as well as other microorganisms [100–102].

In contrast with yeasts, no studies were retrieved in the international literature on the effect of bacteria on SR control [22]. However, two Italian papers published in proceedings of annual technical meetings on crop protection (called Giornate Fitopatologiche) showed that *Bacillus subtilis* [103] and *B. amyloliquefaciens* [104] exhibited similar efficacy to fungicides (specifically, a mixture of fludioxonil and cyprodinil) in controlling SR. As *Bacillus* spp. have been largely investigated as biocontrol agents for *B. cinerea* control in grapes [90,105] through the production of secondary metabolites [106] and induction of resistance [107], research on the use of *Bacillus*-based biofungicides against SR should be further investigated. Spore-forming *Bacillus* species also have the advantages of a long shelf-life, a wide spectrum of activity, and a generally high compatibility with most synthetic fungicides [108].

The efficacy of BCAs in our meta-analysis is encouraging, considering that a previous meta-analysis on the biocontrol of main grapevine diseases highlighted the difficulty of drawing practical guidelines for BCA use against main grape diseases because of the great variability of microorganisms, application timing and frequency, methods for efficacy assessment, and varying environmental conditions [109]. However, further research is needed to better define the following key aspects: (i) the efficacy of BCAs against the main causal agents of SR; (ii) the biocontrol mechanisms (competition, parasitism, antibiosis, or induced resistance) that are more likely to be effective against SR-related microorganisms; and (iii) application timing under vineyard conditions. Research on the biocontrol of *B. cinerea* showed that the efficacy of different BCAs is strictly related to the timing of application, the plant substrate to be colonized in relation to the plant growth stage, the environmental conditions in relation to BCA requirements, and their modes of action [110].

Our meta-analysis did not include studies on the control *Drosophila* spp. flies in vineyards. However, *Drosophila melanogaster*, *D. suzukii*, and other species play a key role in SR development through a multitrophic interaction in which insect vectors of SR microorganisms, both epiphytically and in the gut, cause skin opening through the ovipositor, modify pulp composition, and hinder wound healing trough larval activities [13,18,20,22]. Therefore, control of *Drosophila* spp. can be a component of an integrated SR control scheme. However, the use of insecticides has promoted rising insecticide resistance, has negative effects on natural enemies and outbreaks of secondary pests, and has pre-harvest interval restriction [111–113]. Therefore, the alternative control of trough predators, parasitoids, and/or entomopathogens, mass trapping, attract and kill techniques, and repellents that have been applied to reduce direct damage to grapes caused by *D. suzukii* [112,114–116] should be explored for the control of fly populations in relation to SR.

## 5. Conclusions

The results of this study show that the use of alternatives to synthetic fungicides are promising for the control of SR in vineyards; these results may be related to the broad spectrum and multiple modes of action of these compounds, which may act against the multiple yeasts, bacteria, and filamentous fungi that together and/or in succession are involved in the SR complex. Among these alternatives, zeolites and bicarbonates were used more frequently than other natural compounds. These alternatives also include BCAs based on selected strains of those microorganisms (e.g., yeasts *Candida* and *Aureobasidium*) that usually colonize the grape berry surfaces, including microcracks and wounds, during ripening and are characterized by competition and antagonistic capacities against multiple microorganisms. Potential synergism between natural compounds and BCAs, which has been proven in other systems, should be investigated. Finally, an integration of direct SR control with the reduction in *Drosophila* spp. populations through non-chemical alternatives should be explored further.

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