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An Effect and Less Spraying Control Method Successfully Controls *Botrytis cinerea* on Grapes in China

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Abstract: Gray mold caused by *Botrytis cinerea* is a destructive disease in grapes. Although the preharvest use of pesticides can control it, fungicide resistance in *B. cinerea* is now common. We used an Effect and Less Spraying Control (ELSC) method for applying fungicides effective against *B. cinerea* on grapes. The spraying schedule was determined by exploring the key stages of *B. cinerea* invasion using field and in vitro inoculation tests. The results indicated that the stage most vulnerable to pathogen invasion is the full-bloom stage. The disease incidence/severity in this stage is highest compared with the pre-bloom, 10-days-after-full-bloom, bunch-closure and veraison stages. Given the inoculation results and the threat of residual infected petals, the ELSC method established an optimum spray schedule at full bloom and 10 days after full bloom. To evaluate the ELSC method, four kinds of fungicides were used in an experimental trial in Beijing in 2015 and 2017; Shanghai in 2016; and Hebei in 2019 and 2021. Fludioxonil was the most effective fungicide, followed by *Pythium oligandrum (bio-fungicide)*, difenoconazole + azoxystrobin and pyraclostrobin. ELSC was more effective against *B. cinerea* than the traditional control schedule, when comparing the disease severity (i.e., 0.07 ± 0.10% in ELSC and 0.49 ± 0.014% in the traditional practice when using fludioxonil). The average yield per hectare in ELSC confirmed that spraying during flowering does not have a deleterious effect on grape yield. It produced a 1224.37 00 kg/ha greater yield than the control group when fludioxonil was applied.

Keywords: pathogen invasion; *Botrytis cinerea*; full bloom; control strategy

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1. Introduction

Botrytis cinerea is the major bunch rot pathogen in temperate grape-growing regions with seasonal rains or high relative humidity conditions [1,2]. The pathogen causes heavy losses in grapes before and after harvest [3]. Conidia, produced in late winter and early spring on over-wintering mycelium and/or sclerotia on host tissues, is considered to be the most important infective unit of *B. cinerea* [4]. The traditional disease management strategy is to establish a spray program covering multiple growth stages [5]. On average, four sprays are made to target *B. cinerea* between flowering and harvest [6,7]. However, the risks of fungal resistance and pesticide residues encourage the search for an effective but reduced spraying schedule.

To determine the stages when fungicide application is required, the epidemiological development of the pathogen must be considered. This is driven by many factors, such as the genetic structure of the *B. cinerea* population [8], weather conditions [9,10], plant architecture [11,12] and grape susceptibility [13]. The susceptible level of grape growth stages to *B. cinerea* affects early infection and later disease expression. This has been

demonstrated with regard to the flowering stage [14,15]. Both the stigma and style provide places where pathogens can infect and remain latent [16]. *B. cinerea* also infects fruit via fruit pedicels. Infection appears when berries have wounds, and this can lead to high disease severity [4]. Ripening berries can also be infected through contact with infected berries [17]. Six infection pathways have been recognized: (1) conidial infection of the style and ovules; (2) conidial infection of stamens, petals or fruit pedicels; (3) conidial infection of the floral debris; (4) conidial accumulation within the developing bunch; (5) conidial infection of the ripening fruit; and (6) conidial accumulation on fruit and dispersal to wounds caused by picking [18]. These grape growth stages can all act as initial sites of *B. cinerea* colonization, leading to the establishment of latent infections [4,17].

Inoculation in different grape growth stages reflects the effect of pathogen infection on later disease incidence/severity. For example, bloom inoculation leads to more serious *B. cinerea* infection than pre-bloom and post-bloom stages [7]. Latent infections occur less frequently at the bunch-closure stage than the veraison stage [18]. We can set the fungicidal schedule on these stages that have more serious disease incidence/severity after inoculation. Based on the infection pathways of *B. cinerea*, one should consider the pre-bloom, full-bloom and after-full-bloom stages; bunch-closure stage; and veraison stage to determine the key stages of *B. cinerea* infection in which to apply fungicides. Such an approach is more conducive to finding a control strategy that is efficacious and that involves less frequent spraying when combined with a highly efficient fungicide.

However, resistant fungal strains have been documented in China, and these have caused reduced fungicide efficacy. Some isolates are now highly resistant to carbendazim [19,20] and pyrimethanil [21]. Likewise, tests in California on boscalid, cyprodinil, fenhexamid and pyraclostrobin showed that they are now less effective against resistant strains of *B. cinerea* [22].

Pyraclostrobin is a member of the strobilurin group of fungicides. It controls Botrytis disease by inhibiting mitochondrial respiration [23]. Difenoconazole + azoxystrobin is a mixed fungicide used outside of China to control gray mold. This preparation belongs to the quinone outside inhibitors (QoIs, a class of agriculture fungicides) targeting germ tube elongation [24]. There is widespread QoI resistance in North America. Fludioxonil is also used for fungus control on grapes [25]. Fludioxonil belongs to the phenylpyrroles chemical class, affecting osmoregulation and inhibiting mycelium growth. The low resistance of *B. cinerea* to fludioxonil has been documented in several plants in China, such as the strawberry [26] and tomato [27].

Biocontrol is a welcome technology for mitigating the health and environmental problems caused by fungicide overuse. *Pythium oligandrum* is an active microbial and broad-spectrum fungicide. This organism is used in Europe for controlling fungal diseases of plants, including those affecting grapevine [28]. Although other biological agents were reported, such as Fungicover@ or *Ulocladium oudemansii*, that can significantly reduce *B. cinerea* incidence on aborted flowers and calyptras by 46–85%, there is no product in China [29]. Compared with other biological agents which have been used to make products, such as *Aureobasidium pullulans, Bacillus subtilis* or *Trichoderma atroviride, Pythium oligandrum* induces grapevine defense systems and kills pathogens. For this reason, it has received more attention as a potential biocontrol agent against *B. cinerea* [30]. The alternative fungicide has positive evaluations in regard to controlling Botrytis disease. However, the control effect of these fungicides when they are used in the ELSC method against *B. cinerea* on grapes still needs to be assessed and compared with that of traditional control methods.

The objective of this study was to develop an Effect and Less Spraying Control (ELSC) method. This was initiated by (1) assessing the key stage of *B. cinerea* invasion via in vitro and field inoculation in five grape growth stages (i.e., pre-bloom, full-bloom, 10-days-after-full-bloom, bunch-closure and veraison stages), which are stage 17, stage 23, stage 27, stage 33 and stage 35 based on a standardized way to describe phenological growth stages of grapevines (Eichhorn–Lorenz stage, [31]); (2) determining the requisite stages for *B. cinerea* control based on significant difference of disease incidence and severity

between stages; and (3) testing the control effect of the fungicides (i.e., pyraclostrobin, difenoconazole + azoxystrobin, fludioxonil and *Pythium oligandrum* (bio-fungicide)) against pathogen infection, using the proposed new control practice and a traditional fungicide schedule.

2. Materials and Methods

The workflow of this study is shown in Figure 1. The control practice was conducted in 2015 and 2017. It aimed to evaluate spraying at the full-bloom and 10-days-after-full-boom stages (ELSC) were vulnerable to pathogen invasion. Meanwhile, the control practice was compared to a traditional control method.

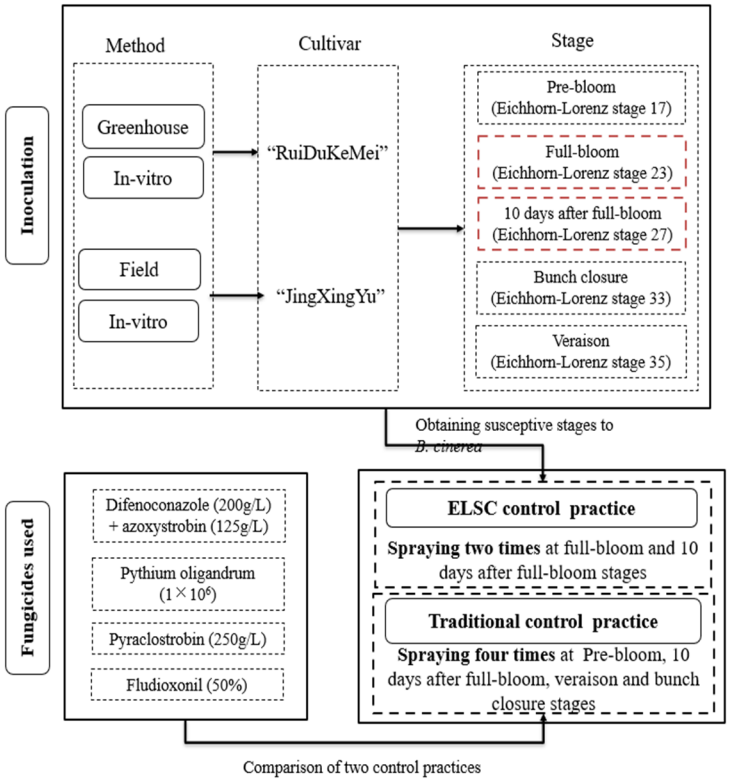

Figure 1. Inoculation and control experiment design.

The validity of the method in ELSC was proved through an inoculation experiment in vitro, in the field and in the greenhouse at the pre-bloom (Eichhorn–Lorenz stage 17), full-bloom (Eichhorn–Lorenz stage 23), 10-days-after-full-bloom (Eichhorn–Lorenz stage 27), bunch-closure (Eichhorn–Lorenz stage 33) and veraison stages (Eichhorn–Lorenz stage 35) in 2017, using two table grape cultivars which have different susceptibility to *B. cinerea*.

The cultivars used for inoculation tests were JingXiangYu (*Vitis vinifera*) from Wenyi Farm (115.91° N, 40.46° E) and RuiDuKeMei (*Vitis vinifera*) from the Beijing Grape and Wine Research Institute in the Yanqing District (115.98° N, 40.47° E), Beijing. The former was planted in a field with 0.45 plants per square meter, and it has high susceptibility to *B. cinerea* [32]; the latter was planted in a plastic greenhouse (600 m^2) with 240 plants, and it has low susceptibility to *B. cinerea*.

2.1. Botrytis cinerea Inoculum

The *B. cinerea* isolate came from Wenyi Farm (115.91° N, 40.46° E) in Beijing, 2015. It was stored at −70 °C in 15% glycerol (v/v) until required for use. To gather the conidia and adjust the concentration, the *B. cinerea* isolate was grown on potato dextrose agar (PDA) cultures in darkness at 20 °C for 10 d. These cultures were immersed in sterile distilled water containing Tween 20 (0.01% v/v), and the subsequent suspensions were then filtered

through sterile cell strainers (Falcon, 100 µm mesh). The accession number of the Botrytis cinerea used is JZB350035.

2.2. Inoculation Experiment

The inoculation method used was detailed in inoculation experiments by Kell et al. [7]. Table 1 lists the in vitro and field inoculation date of each stage.

Table 1. Inoculation dates of in vitro and field experiments at each stage.

Cultivars/Periods		Pre-Bloom	Full Bloom	10 Days after Full Bloom	Bunch Closure	Veraison
RuiDuKeMei	In vitro	18 May	25 May	3 June	20 June	21 July
	Greenhouse	18 May	27 May	7 June	26 June	26 July
JingXiangYu	In vitro	7 June	15 June	23 June	5 July	21 July
	Field	7 June	16 June	26 June	4 July	26 July

The shoots of the grapes were taken back to the laboratory and inserted into a triangular bottle flask filled with 75% alcohol for 30 s to disinfect them (Supplementary Materials Table S1). They were then transferred to another flask full of tap water for clearing, and this was replicated 3 times. Afterward, they were air-dried. The inoculations were carried out by dipping whole plant materials in glass beakers containing a 100 mL of conidial suspension (5×10^5 conidia/mL^{-1} of water). For the control group, whole plant materials were dipped in sterile water. Following inoculation, they were covered with a plastic bag to preserve moisture, and the branches were inserted into a triangular bottle flask full of tap water to keep the materials fresh. Subsequently, they were cultured in an artificial climate box at 23 °C and 70% humidity for 24 h. The plastic bag was taken off after 24 h. Four individual inflorescences or fruit clusters were sampled per stage, and each stage was replicated 3 times.

In the field, the grape shoots were dipped into a glass bottle containing a conidial suspension (5×10^5 conidia $\times$ mL^{-1} of water). The whole shoots were covered with a plastic bag to maintain moisture. The plastic bag was removed after 24 h. Six individual inflorescences or fruit clusters were sampled per stage, and each stage was replicated 3 times.

The gray mold assessments were carried out 7 d after the in vitro inoculation and each 7 days after until at harvest (22 August) in the field inoculation. The disease incidence (the percentage of diseased inflorescences or fruit clusters in samples) and the disease severity (the percentage of diseased flowers or grapes in a cluster) were determined on 6 samples per growth stage replicated 3 times.

2.3. Fungicide Application

Based on the ELSC method, four fungicides were used to test the fungicide control efficacy against gray mold on the grape cultivars in Beijing, 2015 and 2017. The four fungicides were 325 g/L difenoconazole + azoxystrobin suspension agent SC (Switzerland Syngenta Crop Protection Co., Ltd., Basel, Switzerland), 250 g/L pyraclostrobin EC (BASF SE), 1×10^6 spore/g *Pythium oligandrum* WP (Czech Biologics Co., Ltd., Jesenice u Prahy, Czech Republic) and 50% fludioxonil (Switzerland Syngenta Crop Protection Co., Ltd.). The four formulated fungicides and their application rates are described in Table 2. Their application doses were used at label rates. No surfactants/adjuvants were used in the sprays. They used a conic nozzle, and the spray PSI is 43.5. For all the treatments, 1500 L/ha of water was used for the spraying suspension. The treatments were arranged in a randomized block design, consisting of 3 replicates each with 10 vines. The sprayer is the "MATABI" manual sprayer manufactured in Spain. The spraying quantity is 457 mL/min.

Table 2. Fungicides, dilution ratios, and application rates used in the studies.

Treatments	Fungicides	Formulation	Dilution Ratio	Application Rate (gai/ha [x])
1	Difenoconazole (200 g/L) + azoxystrobin (125 g/L)	SC	1500	325
2	Pyraclostrobin (250 g/L)	EC	1875	200
3	*Pythium oligandrum* (1×10^6)	WP	1000	--
4	Fludioxonil (50%)	WP	5000	150
5	Control group			

[x] gai/ha represents grams of active ingredient per hectare.

After this work, other cultivars were tested in the next years with two or three fungicides because of some limitation in vineyard (Table 3).

Table 3. Other control trials in three years.

Year	Site	Cultivar	Pattern
2016	Shanghai	ShenFeng	Greenhouse
		Summerblack	Greenhouse
2019	Hebei	Riesling	Field
2021	Hebei	Riesling	Field

The proposed ELSC schedule was conducted in 2015 and 2017 based on the result of the inoculation experiment to obtain the key stages of pathogen invasion, which determined the two sprays applied during full bloom (Eichhorn–Lorenz stage 23) and 10 days after full bloom (Eichhorn–Lorenz stage 27). The traditional routine spraying schedule was compared with ELSC in 2017. The traditional schedule specifically targets *B. cinerea* during the pre-bloom (Eichhorn–Lorenz stage 17), 10-days-after-full-bloom (Eichhorn–Lorenz stage 27), bunch-closure (Eichhorn–Lorenz stage 33) and veraison stages (Eichhorn–Lorenz stage 35), a total of four sprays [6].

2.4. Weather Condition

The main conditions influencing *B. cinerea* mycelial growth and sporulation are shown in Supplementary Materials Figure S1 (JingXiangYu at Wenyi Farm) and Figure S2 (RuiDuKeMei at Beijing Grape and Wine Research Institute). These conditions include the maximum daily air temperature, the daily rainfall and the wetness duration per day after the inoculation of each stage. In each stage, the daily maximum temperature was below the maximum temperature for mycelial growth (40 °C; [33]). Sufficient humidity for mycelial growth was present in all the stages during hours where there was at least 0.2 mm of rainfall or greater than 90% relative humidity [17].

2.5. Statistical Analysis

The experimental data were statistically analyzed using R software (R Development Core Team 2009, version 3.6.2). Fisher's least significant difference (LSD) was calculated for disease incidence/severity between each treatment, and this was used to determine the key stages of *B. cinerea* invasion. The control effect was calculated using an equation for the final disease severity for each fungicide (CE_f) and water control treatment (CE_w, Equation (1)). The control effect and the average yield per hectare were used as indictors to assess the effectiveness of the fungicides against *B. cinerea*. The average yield per hectare (Yield) was calculated by an average yield of 10 vines ($Yield_{10}$) in three replicates and the number of vines per hector (N, Equation (2)).

$$CE = (CE_w - CE_f) \times 100/CE_w \quad (1)$$

$$\text{Yield} = \text{Yield}_{10} \times \text{N} \quad (2)$$

3. Results

3.1. Inoculation In Vitro and in Field

In the in vitro inoculation with *B. cinerea* conidia suspension, no symptoms occurred after the stages of 7 d in pre-bloom, 10 days after full bloom, bunch closure and veraison (Figure 2). At the full-bloom stage, inflorescence infection caused withered symptoms, with the pedicel and rachis turning black and portions of the cluster shriveling and dropping off (Supplemental Materials Table S1). The control group, exposed to sterile water at the full-bloom stage, had no symptoms. The results of in vitro inoculation showed that, for the JingXiangYu and RuiDuKeMei cultivars, there was a significant difference (* $p < 0.05$) between the full-bloom stage and the other four stages, with a disease incidence of 58.33% ± 10% (JingXiangYu) and 75% ± 10% (RuiDuKeMei); and a disease severity of 16.67% ± 14.16% (JingXiangYu) and 17.08% ± 10.25% (RuiDuKeMei). For the field inoculation experiment, the disease incidence and severity of the pre-bloom, full-bloom and 10-days-after-full-bloom stages were higher than that of the bunch-closure and veraison stage for the JingXiangYu cultivar; the inoculation test at the full-bloom stage produced the highest disease incidence/severity for the two cultivars (Supplemental Materials Figures S3 and S4).

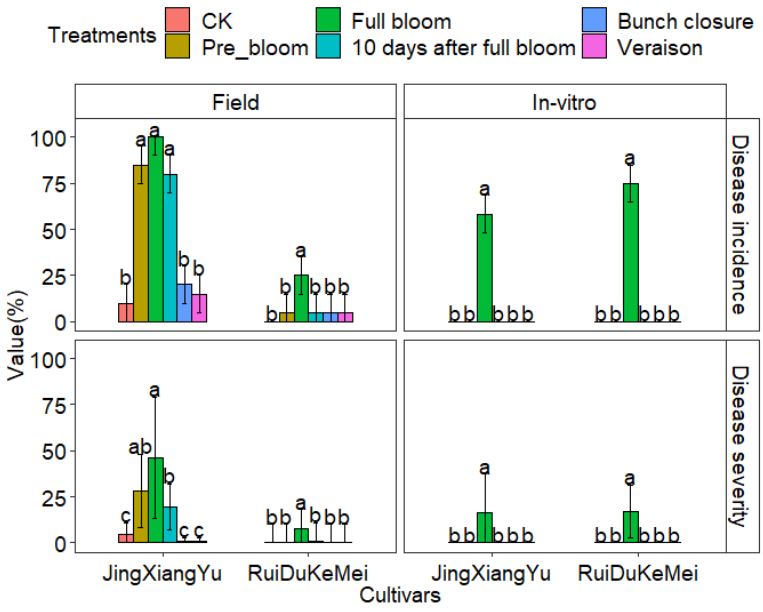

Figure 2. Disease incidence/severity at harvest of each treatment of JingXiangYu and RuiDuKeMei cultivars in field and in vitro inoculation. Lowercase letters, a, b and c, are significantly different across histogram ($p < 0.05$).

The results of the field inoculation also showed that the pre-bloom and 10-days-after-full-bloom stages were important times to control gray mold on grapes, with the disease severity of the two stages significantly different (* $p < 0.05$) from that of the control group. For this reason, the spray schedule for the ELSC method involves two stages: full bloom and 10 days after full bloom.

3.2. Control Experiments

The ELSC method and a traditional control practice were applied to control *B. cinerea* on one cultivar in 2015 and 2017 with four treatments (Table 4). A heavy grape gray mold happened in 2015 (Supplemental Materials Figure S5).

Table 4. Comparison of disease incidence/severity caused by *B. cinerea*, control effect and yield of ELSC and the traditional control practices in two-year and one-year cultivar ("JingXiangYu") experiment trial [x].

Treatment		1	2	3	4	CK
				Disease incidence (%)		
ELSC	2015	93.33 ± 4.44 ab	86.66 ± 7.70 b	93.33 ± 4.44 ab	66.67 ± 4.44 c	100 ± 0.47 a
	2017	14.33 ± 4.08 a	17.00 ± 6.76 a	2.50 ± 1.66 b	3.33 ± 3.30 b	17.50 ± 2.04 a
Trad[x]	2017	4.06 ± 1.96 b	11.25 ± 3.51 ab	5.00 ± 2.89 b	4.50 ± 2.89 b	
				Disease severity (%)		
ELSC	2015	47.12 ± 32.97 a	28.85 ± 29.12 b	16.44 ± 22.86 c	6.32 ± 11.38 c	56.69 ± 29.62 a
	2017	2.39 ± 1.41 a	2.46 ± 1.95 a	0.68 ± 0.65 a	0.07 ± 0.10 a	2.78 ± 1.92 a
Trad	2017	1.18 ± 1.54 a	1.85 ± 2.03 a	0.50 ± 0.34 a	0.49 ± 0.014 a	
				Control effect (%)		
ELSC	2015	16.88	49.11	71.00	88.85	
	2017	14.03	11.51	75.54	97.48	
	Ave	15.46	30.31	73.27	93.16	
Trad	2017	57.55	33.45	82.01	82.37	
				Average yield per hectare (1×10^3kg/ha)		
ELSC	2017	12.38 ± 0.28 b	11.69 ± 0.22 ab	12.29 ± 0.09 b	12.9 ± 0.98 c	11.6 ± 0.29 a
Trad	2017	12.33 ± 0.55 b	11.96 ± 0.57 ab	12.5 ± 0.16 b	12.6 ± 0.14 b	

[x] 1,2,3 and 4 represent four kinds of fungicides used in ELSC and the traditional control method (Trad), these fungicides are difenoconazole + azoxystrobin (1), pyraclostrobin (2), *Pythium oligandrum* (3) and fludioxonil (4). Ave is the abbreviation of average value. The lowercase letters, a, b and c, are significantly different across columns ($p < 0.05$).

In ELSC method, the JingXiangYu cultivar (2015 and 2017) had the lowest disease incidence when fludioxonil was applied (66.67% ± 4.44% in 2015 and 3.33% ± 3.30% in 2017) compared to the other treatments. The disease severity in each treatment in 2017 showed no significant difference.

The average control effect (unitless) of fludioxonil (93.16) proved that the ELSC method is effective against *B. cinerea*, followed by *Pythium oligandrum* (73.27), pyraclostrobin (30.31) and difenoconazole + azoxystrobin (15.46). Compared to the traditional control practice (four sprays in 2017), fludioxonil also had a better control effect (82.37) than the other fungicides.

The application of all four fungicides produced higher yields than the control group for both control methods. The yield and control effect were 230.00 kg/ha and 10.79 (unitless) greater than the traditional control approach when fludioxonil was applied.

Except for the "JingXiangu" cultivar, we performed the ELSC method on more cultivars to ensure its effective on *B. cinerea* control (Figure 3). The disease incidences 14.2% and 4.41% for the "Riesling" cultivar (Hebei) in 2019 and 2021; and 2.17% and 1.88% for the "ShenFeng" and "summer black" cultivars (Shanghai) in 2016 when fludioxonil was applied were significantly higher than the control group under the $p = 0.05$ significance level.

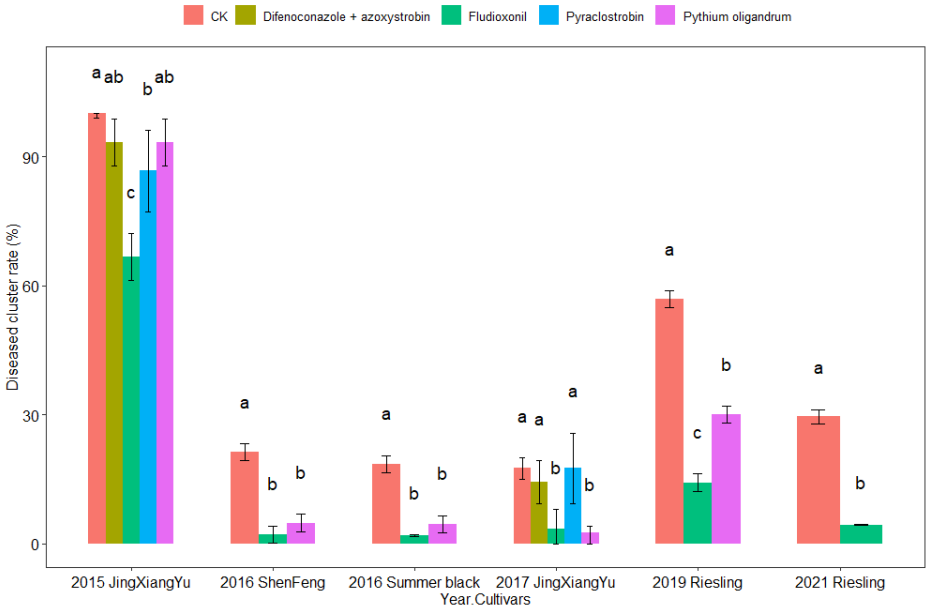

Figure 3. Diseased incidence of four cultivars with the ELSC method and different fungicides over several years. Lowercase letters, a, b and c, are significantly different across histogram ($p < 0.05$).

4. Discussion

Various researchers have shown, by means of inoculation [34] and electron microscopical studies [35], that bloom infection of grapes is closely related to disease expression at harvest. This study supplied further evidence demonstrating the effect of full-bloom infection on disease incidence and yield by comparing field and in vitro inoculations from the pre-bloom to veraison stages. Based on these results, we tested and validated an Effect and Less Spraying Control (ELSC) method against *B. cinerea* on grapes.

The pre-bloom and 10-days-after-full-bloom stages are important to *B. cinerea* invasion; however, the disease severity of the two stages (28.07% and 19.75%) were significantly different compared to the full-bloom stage (46.29%). During the pre-bloom period, the grape flowers are covered by the calyptra, which reduces the risk of infection by fungal pathogens. After blooming, the young berries are more resistant to *B. cinerea* than the grape flowers. However, flower debris still poses a danger to the young berries, remaining for several weeks and becoming trapped in the interior parts of the clusters. Removing the flower debris manually has been considered in several studies [36,37] as a partial alternative to pesticides; however, the time and labor costs were not taken into account.

The field inoculation experiment at the pre-bloom stage produced a higher disease incidence/severity compared to that of the 10-days-after-full-bloom stage. Pathogens in the air may cause infections during the blooming stage based on the high disease incidence/severity of the control group on the JingXiangYu cultivar. For the RuiDuKeMei cultivar, the disease incidence/severity of the two stages had similar values, thus supported the above inference, as no infection occurred in the control group.

The bunch-closure and veraison stages have lower disease severity than the other periods. The chemical constituents of immature grapes provide natural resistance to *B. cinerea*, but the berries become highly susceptible at maturity. Hill et al. [38] identified proanthocyanins-condensed tannins composed of cyanidin and delphinidin subunits as the substances that inhibit infection. Pezet and Pont [39] also showed that crude extracts from young clusters can strongly inhibit the germination of *B. cinerea* conidia. High levels of catechin also contribute to the period, helping young clusters fight off *B. cinerea* infection [40].

In this study, the in vitro experiment showed that only the full-bloom stage was infected by *B. cinerea*. The pre-bloom, 10-days-after-full-bloom, bunch-closure and veraison stages exhibited no symptoms. An optimal temperature of 15–25 °C is required for the

germination and subsequent infection of *B. cinerea* with free water [41]. Under the same concentration of pathogen infection, the full-bloom stage was more easily invaded than other stages [7]. A possible reason for asymptomatic infection might be that the pathogens are in a latent state. A similar situation occurs with *Puccinia striiformis*, which was unable to infect wheat seedlings in the laboratory [18] at constant temperatures at 21 °C, whereas infections occurred in the field when temperatures fluctuated between 18 °C and 30 °C [42].

Moreover, in field inoculation experiments, there were high standard errors in disease severity at harvest (Figure 2). It is known that six infection stages have been recognized. Two of them are conidial infection of the ripening fruit and conidial accumulation on fruit and dispersal to wounds caused by picking [18]. The conidial accumulation on fruit or farm operation randomly increasing fruits' infection could be the cause of the high standard errors in disease severity.

The well-timed application of fungicides was discussed earlier. A single application of spraying towards flowering (Eichhorn–Lorenz stage 23) resulted in a higher efficacy than towards the beginning of veraison in the Champagne region on the cultivar Pinot [43]. Confirming data were obtained in New South Wales vineyards in Australia [44]. This is consistent with our results in China on the four cultivars, but in contrast to observations in Luxembourg on Riesling, where the highest treatment efficacy occurred at bunch closure stage (Eichhorn–Lorenz stage 33, [45]). Petit et al. [46] proved a control practice of spraying at the end of flowering (Eichhorn–Lorenz stage 23), bunch closure (Eichhorn–Lorenz stage 33) and the beginning of veraison (Eichhorn–Lorenz stage 35) to be more effective to control *B. cinerea* on grape compared to a single fungicide application. Our work showed that two sprays, one during the full-bloom stage (Eichhorn–Lorenz stage 23) and the other during the 10-days-after-full-bloom stage (Eichhorn–Lorenz stage 27), result in a significant difference in the disease severity when applying fludioxonil (kg/ha), compared to using the traditional practice of four sprays.

Fludioxonil is currently registered for use in combination with cyprodinil, in the form of Switch 62.5WG (Syngenta Crop Protection, Greensboro, NC, USA), for *B. cinerea* control on small fruits in the USA [47]. It is also registered with pydiflumetofen under the trademark Miravis Prime in the USA for *B. cinerea* control on grapes. The potential of this fungicide to control *B. cinerea* on other hosts, such as in lettuce [48], strawberry [26] and peach [49], has been reported. It has also been documented that it carries a low risk of *B. cinerea* resistance [50]. Our results verify its effectiveness in controlling *B. cinerea*. Of the four fungicides tested, fludioxonil demonstrated the best control effect (97.48) and highest average yield ($12.9 \pm 0.98 \times 103$ kg/ha). Our results agree with Fedele G.'s finding, which that proved fludioxonil could significantly reduce *B. cinerea* sporulation on bunch debris [51].

Among the fungicide control treatments, biocontrol using microorganisms is an environmentally friendly treatment with respect to the issues of pesticide residues and environmental pollution. However, some works proved that *Pythium oligandrum* is a useful biological control agent against *B. cinerea* [52,53]. On the contrary, disease incidences in 2015 and 2017 were 86.66 ± 7.70 and 17.00 ± 6.76 in our work, showing minor differences with the check test.

5. Conclusions

In summary, the present results of our study indicated that ELSC is an Effect and Less Spraying control schedule and has no direct effect on grape yield. Although only two spray sessions were used at the flowering stage, the better control effect and higher average yield per hectare proved that the ELSC method has a better performance than the traditional control method. In addition, fludioxonil has a decided advantage in controlling gray mold. Thus, we suggested that the application of fludioxonil could be a worthwhile strategy for the control of *B. cinerea* on grapes as part of a management program based on the ELSC method.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy13102578/s1, Figure S1: Climate factors for the inoculations at Wenyi Farm in the five stages: Figure S2: Climate factors for the inoculations at Beijing Grape and Wine Research Institute in the five stages; Figure S3: Inoculated fruit clusters (A–E) and water control group (F) of JingXiangYu cultivar at harvest (22 August) in field inoculation experiment over five grape growth stages ((A) pre-bloom, (B) full bloom, (C) 10 days after full bloom, (D) bunch closure and (E) veraison) in 2015; Figure S4: Inoculated fruit clusters (A–E) and water control group (F) of RuiDuKeMei cultivar at harvest (22 August) in field inoculation experiment over five grape growth stages ((A) pre-bloom, (B) full bloom, (C) 10 days after full bloom, (D) bunch closure and (E) veraison) in 2015; Figure S5: Control effect of JingXiangYu cultivar applied ELSC method at harvest (27 August 2015) with four kinds of fungicides ((A) difenoconazole + azoxystrobin, (B) pyraclostrobin, (C) Pythium oligandrum and (D) fludioxonil) and water control group (E); Table S1: The treated fruit clusters and inflorescences of two cultivars (JingXiangYu and RuiDuKeMei) by conidial suspension (1) and water (2) in the in vitro inoculation experiment over five grape growth stages ((A) pre-bloom, (B) full bloom, (C) 10 days after full bloom, (D) bunch closure and (E) veraison) in 2015.

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Data Availability Statement: The data presented in this study are available upon request from the corresponding author. The data are not publicly available.

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