horticulturae

MDPI

Review

Biological Control of the Cucumber Downy Mildew Pathogen *Pseudoperonospora cubensis*

Zhanbin Sun [1,*], Shufan Yu [1], Yafeng Hu [1] and Yanchen Wen [2,*]

[1] School of Light Industry, Beijing Technology and Business University, Beijing 100048, China; ysf18437906517@163.com (S.Y.); huyafengii@163.com (Y.H.)
[2] Key Laboratory of Plant Nutrition and Fertilizer, Ministry of Agriculture, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, China
* Correspondence: twins5616@126.com (Z.S.); wenyanchen@caas.cn (Y.W.)

Abstract: Cucumber downy mildew (CDM) is a destructive plant disease caused by the air-borne oomycete pathogen *Pseudoperonospora cubensis*. CDM causes severe yield reduction of cucumber and significant economic losses. Biocontrol is a promising method to control CDM with the advantage of being beneficial to sustainable agricultural development. However, until now, no reviews of biocontrol of CDM have been reported. The objective of this review is to more comprehensively understand the biocontrol of CDM. In this review, the biological characteristics of *P. cubensis* are introduced, and strategies for screening biocontrol agents to suppress CDM are recommended. Then the current biocontrol agents, including fungi such as *Trichoderma* and biocontrol bacteria such as *Bacillus*, which possess the ability to control CDM, and their control characteristics and ability against CDM are also summarized. The potential mechanisms by which these biocontrol agents prevent CDM are discussed. Finally, several suggestions for future research on the biocontrol of CDM are provided.

Keywords: *Pseudoperonospora cubensis*; cucumber downy mildew; biocontrol fungi; biocontrol bacteria

Citation: Sun, Z.; Yu, S.; Hu, Y.; Wen, Y. Biological Control of the Cucumber Downy Mildew Pathogen *Pseudoperonospora cubensis*. *Horticulturae* **2022**, *8*, 410. https://doi.org/10.3390/horticulturae8050410

Academic Editor: Harald Scherm

Received: 23 March 2022
Accepted: 2 May 2022
Published: 6 May 2022

1. Introduction

Cucumber downy mildew (CDM), caused by the oomycete *Pseudoperonospora cubensis* (Berk. et Curt.) Rostov., is an airborne foliar disease with characteristics of rapid spread and crop destruction [1–3]. CDM severely affects the quality and yield of cucumber in all growing areas and results in significant economic losses [4–8]. CDM is distributed worldwide and occurs in both the seedling and adult plant stages of cucumber [9–13]. When the cucumber plant is infected by *P. cubensis*, the upper side of the leaf surface exhibits yellowish-brown lesions with irregular form, and gray sporangium layers appear on the lower side of the leaf surface under high humidity. Multiple lesions merge after serious infection, which causes the leaves to turn yellow and wither [14–18]. The reduction in cucumber yield caused by CDM is detrimental if no control countermeasures are applied in the early infection stage of *P. cubensis*. The damage of CDM to cucumber yield and quality increases over time, and CDM has become an important factor limiting the yield and quality of cucumbers [19–23].

Effective control methods are important to reducing the damage caused by CDM. Currently, common methods used to control CDM include chemical control, planting of CDM-resistant cucumber varieties, and biocontrol. Chemical control is one of the most widely used and effective methods to control CDM. Different kinds of fungicides, including azoxystrobin, fenamidone, dimethomorph, pyraclostrobin, and cyazofamid, are commonly used to control CDM [24–28]. However, frequent use of fungicides is costly and may result in environmental and food-safety concerns [29–31]. Moreover, the long-term use of fungicides may result in resistance of *P. cubensis*, which could be solved by developing or rotating new types of fungicides [32–36]. One additional approach to control

CDM is to plant resistant varieties [37–41]. However, developing resistant varieties is time-consuming, and the existing resistant cultivars are limited and cannot satisfy market requirements. Biocontrol is another potential way to manage CDM. Biocontrol mainly utilizes mechanisms such as competition, antagonism, or the production of secondary metabolites by microorganisms to control CDM. Several kinds of biocontrol microorganisms have been reported to exhibit control efficacy against CDM. At present, many reviews of managing CDM by chemical control and cultivation of resistant cultivars have been reported. However, no reviews for biocontrol of CDM exist.

The aim of this review was to summarize and analyze the research by using biocontrol methods to suppress CDM. First, we introduce the biological characteristics of the CDM pathogen *P. cubensis*. Second, the review presents a strategy of how to effectively screen biocontrol agents to control CDM. Then biocontrol agents with the ability to control CDM are also introduced, as well as their potential control mechanisms. Finally, some suggestions are presented for improving the control ability of CDM by using biocontrol. Overall, this review provides a basis for the broader application of biocontrol to manage CDM.

2. *Pseudoperonospora cubensis*

Pseudoperonospora cubensis was first described by Berkeley in Cuba in 1868 [42]. Current taxonomic classification indicates that *P. cubensis* belongs to Stramenopiles (kingdom), Oomycota (phylum), Oomycetes (Class), Peronosporales (order), Peronosporaceae (family), and *Pseudoperonospora* (genus) [43]. Sporangiophores, sporangia, and zoospores are produced by *P. cubensis* through asexual reproduction and are essential for infection [44–47]. In addition, *P. cubensis* can also generate oospores according to sexual reproduction, but the role of oospores in the *P. cubensis* disease cycle remains unknown [48–53]. Sporangia of *P. cubensis* can be dispersed by wind, rain, insects, or farm implements [54–57]. *Pseudoperonospora cubensis* can infect more than 20 Cucurbit genera and 60 species [58–60]. As a strict obligate parasite, *P. cubensis* depends on living host tissue to grow, survive, reproduce, and disperse [61–63].

The infection cycle of *P. cubensis* starts when the sporangia touch the surface of the cucumber leaf (Figure 1). Sporangia can produce zoospores to achieve infection [64–66]. Zoospores can germinate and form germ tubes to infect cucumber leaves via stomata [67]. The mycelium grows intercellularly in cucumber leaves and, if environmental conditions are favorable, forms sporangiophores. Then sporangia are generated at the terminus of the sporangiophore branch [68]. Finally, the generated sporangia can continue to proceed with the infection cycle through wind or other dispersion mechanisms [69]. Temperature and humidity are the main factors that influence the infection efficiency of *P. cubensis*. Germination and infection occur at 5 to 28 °C, with 15 °C being the optimal infection temperature. A relative humidity higher than 90% is suitable for the occurrence of CDM [16,70–74].

Understanding the infection mechanism of *P. cubensis* is critical for further improving the control ability of CDM. A transcriptome analysis of *P. cubensis*–infected cucumber in different stages showed that numerous genes were differentially expressed during the process of *P. cubensis* infection. Among these differentially expressed genes, genes encoding hydrolytic enzymes, including proteinases, lipases, and carbohydrate active enzymes, might be involved in the damage of *P. cubensis* to the cucumber host [75]. In addition, genes encoding RXLR-type effectors, which play important roles in the virulence of *P. cubensis*, were also found [76]. Research showed that the RXLR effector protein-encoding gene *Psc*RXLR1 was upregulated at the early infection stages of *P. cubensis* [77]. Moreover, *P. cubensis* suppressed cucumber defense responses by reducing the expression levels of host-defense-response-related genes, such as lipoxygenase, cationic peroxidases, and cinnamate 4-hydroxylases in cucumber [75,78].

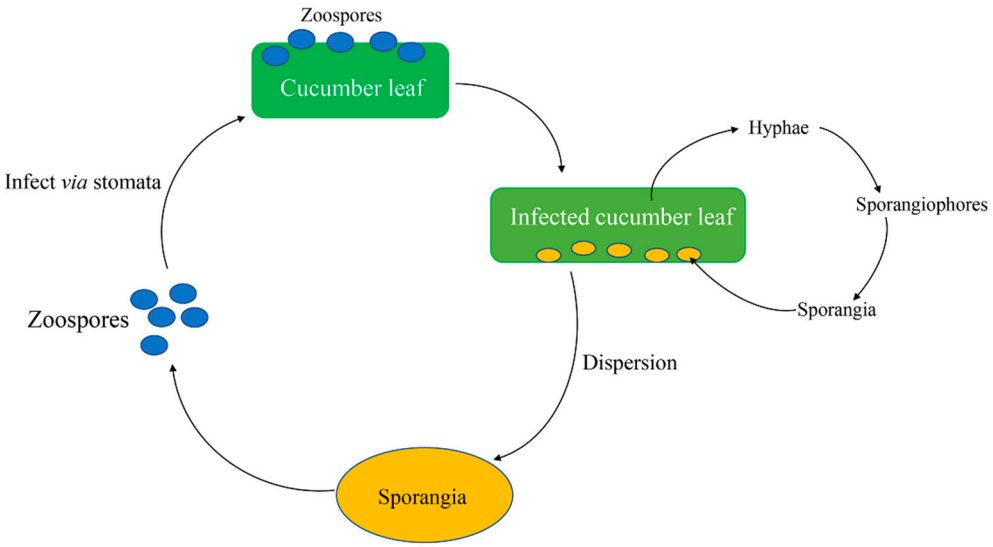

Figure 1. Infection cycle of *Pseudoperonospora cubensis*.

3. Strategy of Screening Biocontrol Agents

In contrast to other phytopathogens, as a strict obligate parasite, *P. cubensis* cannot survive on artificial growth media. Therefore, screening biocontrol agents that are effective against *P. cubensis* cannot directly use the traditional plate confrontation assay. Investigating the control ability of biocontrol agents against *P. cubensis* can only be performed on living plants, including leaf disc assays, detached leaf assays, and field trials. A sporangium release inhibition method was also used to evaluate the control effect of biocontrol agents against *P. cubensis*. Compared with the convenience advantage brought by plate confrontation screening, it is difficult to screen biocontrol agents on a large scale by using living plants. Therefore, the selection of an appropriate screening strategy for biocontrol agents against *P. cubensis* is very important for improving screening efficiency.

Currently, there are two main strategies for screening biocontrol agents against *P. cubensis*. The first method is to isolate a range of microorganisms from environmental materials without restrictions. Then a criterion is established to narrow the quantity and species for the control assay of *P. cubensis*. The criterion for screening biocontrol microorganisms could be set as follows: investigating the production ability of enzymes or metabolites that are harmful to *P. cubensis* or evaluating the antagonistic effects on alternative oomycete pathogens, which have a close evolutionary relationship and similar pathogenesis mechanism, as does *P. cubensis*. Zheng et al. [79] isolated 163 bacterial strains from different microenvironments, including the leaf interior, phyllosphere, rhizosphere, and bulk soils, from healthy and diseased plants. Finally, 19 bacterial isolates were selected to further investigate the control efficacy of CDM. The selection criteria could be summarized as follows: these bacterial strains were representative, for which they were derived from all isolated samples, and the relationship among these bacterial strains had high genetic diversity. Furthermore, these bacterial strains produced extracellular hydrolytic enzymes, such as chitinase, protease, and cellulase, which may be involved in controlling *P. cubensis*.

Another method is to choose a field where CDM is present at a high incidence. Then microorganisms in plant tissues or soils from asymptomatic plant areas are screened. This is mainly because *P. cubensis* is an airborne pathogen, and most plants should be infected if no biocontrol agents existed. Therefore, uninfected plants or the surrounding soil might contain microorganisms that potentially control CDM. Sun et al. [80] isolated 81 leaf endophytic bacteria from healthy cucumber plants in areas where most plants were infected by *P. cubensis*. Then these isolates were used to evaluate their ability to control CDM. Similarly, *Bacillus licheniformis* HS10 originated from a healthy cucumber rhizosphere, from which the surrounding plants were seriously infected.

In this review, "Cucumber downy mildew" and "*Pseudoperonospora cubensis*" were set as keywords in searching the Web of Science and PubMed. Manuscripts about the biological characters, infection cycle, and mechanism of *P. cubensis* symptoms, damage, epidemiology, and control methods were reviewed, especially all the articles related to biocontrol of CDM, were included.

4. Biocontrol Agents

As a potentially sustainable control method, biological control has attracted widespread attention. To date, only a few fungal genera, including *Trichoderma*, *Pestalotiopsis*, and *Fusarium,* and numerous bacterial genera, including *Bacillus*, *Paenibacillus*, *Enterobacter*, *Streptomyces*, *Pseudomonas*, *Derxia*, and *Aneurinibacillus*, have exhibited biocontrol ability against CDM (Table 1).

4.1. Biocontrol Fungi

Trichoderma spp. are excellent biocontrol agents that are effective against numerous plant pathogens. The mechanism of action of *Trichoderma* against pathogens mainly depends on the secretion of cell-wall-degrading enzymes, including chitinase, chitosanase, glucanase, and proteases; the production of secondary metabolites; and the induction of plant resistance [81–85]. Many studies have shown that different *Trichoderma* species have the ability to control CDM. *Trichoderma harzianum* was the most reported species that could effectively control CDM. In some studies, the biocontrol ability of *T. harzianum* against CDM was similar to the chemical treatment in different cucumber growth stages. A single application of *T. harzianum* at the seventh week decreased the percentage of infection of cucumber plants with CDM (11.0%) compared with the control treatment (99.7%) [86]. Moreover, the growth and yield parameters of cucumber in the *T. harzianum* treatment were increased compared with the control, including plant length, leaf weight, leaf area, total chlorophyll, fruit number (19.3 vs. 33.3 per plant), and plant yield (1.5 vs. 3.4 kg) [87]. El-Khalily et al. investigated the ability of two *Trichoderma* species, *T. harzianum* and *T. viride*, to control CDM. Both agents exhibited control ability against CDM at 90 days after planting [88]. Differences in disease incidence were observed between the two fungi, ranging from 44.3 to 66.7%. Alvarado-Aguayo et al. recommend a 500 g/ha dose of *T. harzianum* for suitable control of CDM compared with other doses [89]. The application of *T. harzianum*/*P. fluorescens* decreased the percentage of CDM infection in cucumber plants. Treated plants showed only 7.4% CDM severity compared with 95% recorded in the untreated control at the seventh week and increased the yield (843.3 g in control and 1565.7 g in *T. harzianum*/*P. fluorescens* treatment), vegetative fresh weight (302.0 vs. 396.0 g), and plant length (143.3 vs. 148.7 cm) [86].

Other *Trichoderma* species also exhibited control ability against CDM. Applications of *T. asperellum* reduced the damage caused by *P. cubensis* [90]. *Trichoderma atroviride* TRS25 diminished the incidence of CDM and elicited a host defense response in cucumber plants. In addition, the usage of TRS25 was beneficial to cucumber growth, including increasing germination (65.9 $\pm$ 4.2 seeds per plot in control; 84.6 $\pm$ 2.5 in TRS25 treatment), and shoot fresh weight (19.2 $\pm$ 0.6 vs. 39.1 $\pm$ 1.4 g per plant) compared with the untreated control [91]. The mixture of *T. hamatum* and *T. harzianum* improved the control of CDM and enhanced the number of fruit (19 in mixture application; 18.3, 17, and 18 per plant in single use of each species, respectively) and weight of fruits (3.4 kg in mixture application; 3.1, 2.9, and 3.0 kg per plant in single use of each species, respectively) at 6 weeks after sowing [92].

Two endophytes, *Pestalotiopsis* and *Fusarium,* were isolated from the leaves of *Pyrethrum cineraryiifolium* Trev. Their fermentation products were then used to investigate the control ability of CDM both in protective and therapeutic application. The results showed that both species exhibited protective and therapeutic control ability against CDM [93,94]. Two *Fusarium* strains, FO47 and FO47B10, exhibited 64.0 and 61.8% control efficacy against CDM at the preliminary disease stage. In addition, FO47B10 improved the

growth and development of cucumber, including seeding survival, root length, chlorophyll content, and aboveground fresh weight of individual plants and single plant yield [95].

4.2. Biocontrol Bacteria

Bacillus is a promising biocontrol agent that can protect against many pathogens via the mechanisms of competition for nutrition and space, secretion of antimicrobial substances, and induced plant resistance [96–100]. Different *Bacillus* species exhibited biocontrol effects against CDM. Both *B. subtilis* (37.5 ± 2.6%) and *B. pumilus* (34.1 ± 2.1%) decreased the disease severity of CDM compared with the control (91.1 ± 1.4%). Moreover, the application of *B. subtilis* and *B. pumilus* increased the chlorophyll content and peroxidase and polyphenoloxidase activities of cucumber plants [101]. A similar phenomenon was found in another *B. subtilis* strain, and the disease severity of CDM was lower by using *B. subtilis* (17.2%) compared with the control (30.0%) at 5 weeks. Plant growth parameters, including plant length, total chlorophyll, leaf number, leaf weight, and leaf area; and yield parameters, including plant yield (1.5 vs. 3.6 kg), fruit length (13.0 vs. 17.7 cm), fruit diameter (1.9 vs. 2.7 cm), and shelf life, were also higher in response to the application of *B. subtilis* compared with the control [87].

In addition to *B. subtilis*, other *Bacillus* species also exhibited biocontrol ability against CDM. *Bacillus licheniformis* HS10 was isolated from a healthy cucumber rhizosphere, and its crude protein extract exhibited 60.1% control efficacy against CDM [102]. The activity of the plant defense enzymes PAL and POD was increased after spraying HS10. An antifungal protein was purified from HS10 and suppressed *P. cubensis* on cucumber leaves [103]. *Bacillus pumilus* DS22 and *B. licheniformis* HS10 were isolated from soils of diseased and healthy cucumber plants, respectively. Both isolates reduced the disease severity of CDM at 8 dpt (days post treatment) and 21 dpt and increased the height (DS22: 28.6 ± 1.3 cm, HS10: 33.2 ± 4.2 cm, Control: 26.8 ± 4.5 cm) and dry weight of cucumber plants (DS22: 5.3 ± 0.1 g, HS10: 4.9 ± 0.4 g, Control: 4.2 ± 0.5 g) [79].

For other *Bacillus* species, both the fermentation broth and fermentation filtrate of *B. velezensis* achieved higher control levels against CDM than chemical pesticides. The concentration of the fermentation filtrate of *B. velezensis* without any dilution was more suitable for control of CDM compared with diluted concentrations [104]. *Bacillus chitinosporus* isolated from cucumber roots reduced the severity of CDM from 10.8 to 5.9% after 35 days. Further studies showed that metabolites from *B. chitinosporus* caused damage to the development of *P. cubensis*, including twisting, turgor loss, and sporangiophore collapse [105]. Separate leaf assays, sporangium release inhibition assays, and field experiments were conducted to investigate the biocontrol ability of *Bacillus* strains CE8, Z-X-3, and Z-X-10 against CDM, and all the strains exhibited control ability that was better than chemical pesticides and the control treatment [80,106].

Pseudomonas, *Streptomyces*, and *Paenibacillus* are also very important for controlling plant diseases [107–111]. A single application of *Ps. fluorescens* exhibited lower percentage of disease severity of cucumber plants with CDM (6%) than the control (95%) in the greenhouse. *Pseudomonas fluorescens* was beneficial for the improvement of yield and biomass of cucumber plants compared with the control [86]. The application of other *Ps. fluorescens* strains also reduced the incidence of CDM and improved the growth and yield parameters [87,92,107,108]. *Streptomyces* can produce fungicidal metabolites that may be harmful to pathogens. A *Streptomyces* strain was isolated from soil, and the fermentation broth inhibited the germination of *P. cubensis* sporangia and improved the control ability against CDM (63.9%) [109]. Another spent forest mushroom compost-derived strain, *S. padanus* PMS-702, reduced the severity of CDM compared with the control, and the 10-fold dilution suspension inhibited the germination of *P. cubensis* sporangia [112]. In addition, the usage of *Pa. polymyxa* effectively controlled CDM. The concentration of *Pa. polymyxa* P1 was positively correlated with the control ability of CDM in the field experiment and was higher (89.9 ± 2.2% for P1 in the highest application dose) than that of chemical pesticides (74.9 ± 2.7%) [111].

Other bacterial biocontrol agents are seldom reported to be involved in CDM control. *Aneurinibacillus migulanus* reduced disease severity of CDM (54%) compared with the control (94%) [113]. Another *A. migulanus* strain reached 92% efficacy to control CDM [114]. For *Enterobacter cloacae*, application improved seed germination at the 3rd day (82% in treatment and 39% in control) and cucumber yield (6200 kg in treatment and 4550 kg in control). Both the single usage of *E. cloacae* (88%) or a mixture of *E. cloacae* strains and fungicides (96.6%) had control ability against CDM [115]. *Enterobacter* strain DP14 controlled CDM in two sequential years. DP14 also promoted cucumber plant growth, including height, leaf area, fresh and dry weight, and single fruit weight [79]. *Derxia gummosa* reduced the disease severity of CDM (18.1%) compared with the control (30.0%) and increased the growth characteristics of plant length, total chlorophyll, leaf weight and area, and fresh and dry weight, as well as yield parameters of plant yield, fruit length, and diameter [87].

Table 1. Overview of biocontrol agents showing control ability against cucumber downy mildew.

Biocontrol Microorganisms	Strain Name	Application Types	Application Scale	Application Manner	Application Frequency	Investigated Time	Disease Severity 2	Disease Severity 1	Disease Index Scale	Disease Index 2	Disease Index 1
Fungi											
Trichoderma harzianum	1 [92]	Live organism	Greenhouse	Spray	Every three weeks	—	12	65.7	—	—	—
	2 [92]	Live organism	Greenhouse	Spray	Every three weeks	—	12.7	65.7	—	—	—
	— [86]	Live organism	Plastic house	Spray	Successive	Seven weeks	7.7	95	—	—	—
	— [87]	Live organism	Greenhouse	Spray	Every week	Five weeks after application	17.2	30	—	—	—
	— [88]	Live organism	Greenhouse	Spray	Every week	90 days from planting	19.3	100	—	—	—
Trichoderma atroviride	TRS25 [91]	Live organism	Field	Seed treatment	Once	Harvest	40% lower than CK	—	—	—	—
Trichoderma viride	— [88]	Live organism	Greenhouse	Spray	Every week	90 days from planting	40.8	100	—	—	—
Trichoderma hamatum	2 [92]	Live organism	Greenhouse	Spray	Every three weeks	—	11.7	65.7	—	—	—
Fusarium oxysporum	FO47 [95]	Live organism	Greenhouse	Soil mix	Once	7 days after inoculation of *P. cubensis*	—	—	0–4	6.7	18.6
	FO47B10 [95]	Live organism	Greenhouse	Soil mix	Once	7 days after inoculation of *P. cubensis*	—	—	0–4	7.1	18.6
	Y2 [94]	Fermentation supernatant	Greenhouse	Spray	Once	7 days after application	—	—	0–4	27.8	67.3
Pestalotiopsis microspora	Y1 [93]	Fermentation supernatant	Greenhouse	Spray	Once	—	—	—	0–4	13.9	42.6
Bacteria											
Bacillus subtilis	4 [92]	Live organism	Greenhouse	Spray	Every three weeks	—	15.7	65.7	—	—	—
	— [87]	Live organism	Greenhouse	Spray	Every week	Five weeks after application	17.2	30	—	—	—
	— [101]	Live organism	Greenhouse	Spray	Every 10 days	One week after the last spray	32.8	88.3	—	—	—
Bacillus pumilus	DS22 [79]	Live organism	Field	Spray	Every 10 days	15 days after application	4.6	20.2	—	—	—
	— [101]	Live organism	Greenhouse	Spray	Every 10 days	One week after the last spray	35.2	88.3	—	—	—
Bacillus licheniformis	HS10 [79]	Live organism	Field	Spray	Every 10 days	15 days after application	5.5	20.2	—	—	—
Bacillus asahii	CE8 [80]	Live organism	Field	Spray	Once	7–12 days after application	—	—	0–9	41.1	70.9
Bacillus velezensis	HMQAU19044 [104]	Live organism	Pot experiment	Spray	Once	7 days after application	—	—	0–9	31.3	77.8
	HMQAU19044 [104]	Fermentation filtrate	Pot experiment	Spray	Once	7 days after application	—	—	0–9	15.98	77.8
Bacillus chitinosporus	— [105]	Metabolites	Plastic house	Spray	Every week	35 days after application	5.9	10.8	—	—	—

Table 1. *Cont.*

Biocontrol Microorganisms	Strain Name	Application Types	Application Scale	Application Manner	Application Frequency	Investigated Time	Disease Severity 2	Disease Severity 1	Disease Index Scale	Disease Index 2	Disease Index 1
Bacillus sp.	HP4 [79]	Live organism	Field	Spray	Every 10 days	15 days after application	11.6	20.2	—	—	—
	Z-X-3 [106]	Fermentation supernatant	Greenhouse	Spray	Every 6 days	—	—	—	0–4	0.5	0.8
	Z-X-10 [106]	Fermentation supernatant	Greenhouse	Spray	Every 6 days	—	—	—	0–4	0.5	0.8
Streptomyces sp.	NO.24 [109]	—	Pot experiment	Spray	Once	7 days after application	—	—	—	13.8	38.3
Pseudomonas fluorescens	4 [92]	Live organism	Greenhouse	Spray	Every three weeks	Five weeks after application	20.7	65.7	—	—	—
	Pf1 [107]	Filtrate product	Field	Spray	—	—	—	—	0–5	8.9	44.6
	— [86]	Live organism	Plastic house	Spray	Successive	Seven weeks	6	95	—	—	—
	— [87]	Live organism	Greenhouse	Spray	Every week	Five weeks after application	16.7	30	—	—	—
Enterobacter cloacae	— [115]	Live organism	Greenhouse	Spray	—	—	—	—	—	10.3	85.6
Enterobacter sp.	DP14 [79]	Live organism	Field	Spray	Every 10 days	15 days after application	5.6	20.2	—	—	—
Paenibacillus polymyxa	P1 [111]	Live organism	Field	Spray	Every 7 days	7 days after the last application	—	—	0–9	2.5	24.7
Derxia gummosa	— [87]	Live organism	Greenhouse	Spray	Every week	Five weeks after application	18.1	30	—	—	—

Note: "—" represents not available. Disease Index 1 and Index 2 mean disease index in CK group and treatment group, respectively. Disease Severity 1 and Disease Severity 2 mean disease severity (%) in CK group and treatment group, respectively. Representative data from each work in the literature are shown.

5. Biocontrol Mechanisms against CDM

Understanding the biocontrol mechanism is critical for further improving the control ability of CDM. Currently, several mechanisms have been reported to be involved in the biocontrol of CDM (Figure 2). The production of hydrolytic enzymes or metabolites, and induced plant resistance are two main mechanisms involved in controlling CDM. The biocontrol agents *T. harzianum* and *Ps. fluorescence* secrete degrading enzymes such as glucanase or produce secondary metabolites, which are crucial for antifungal effects [86]. *Bacillus licheniformis* HS10 has the ability to produce the hydrolytic enzymes protease, chitinase, and cellulase, which are important for controlling CDM [103]. Metabolites from *B. chitinosporus* resulted in the collapse of sporangiophores of *P. cubensis* [105]. Similarly, secondary metabolites produced by *S. padanus* affected the germination of *P. cubensis* sporangia [112].

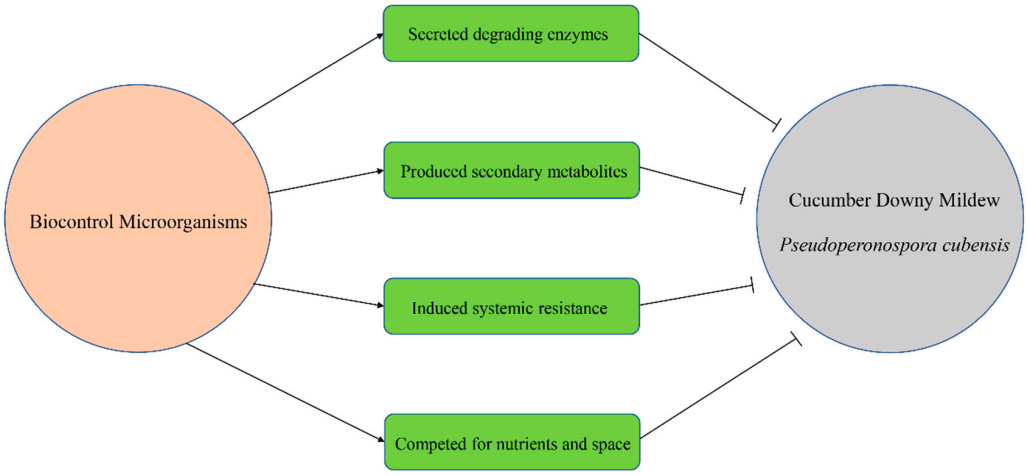

Figure 2. Mechanisms of biocontrol agents to suppress cucumber downy mildew caused by *Pseudoperonospora cubensis*.

Trichoderma atroviride TRS25 exhibited control ability against CDM. The activities of plant defense enzymes such as guaiacol peroxidase, ascorbate peroxidase, and phenylalanine ammonia lyase in cucumber leaves were increased after the application of TRS25 [91]. One reason for the high control ability of TRS25 might be the stimulation of host resistance by TRS25. The activity of phenylalanine ammonia lyase was increased by the usage of *B. licheniformis* HS10 in cucumber plants, which might enhance plant defenses, thus reducing the damage caused by *P. cubensis* [79]. Similar results were reported for *B. subtilis*, *Ps. fluorescens*, *T. harzianum*, *D. gummosa*, and *B. pumilus*, the application of which increased the activities of some defense enzymes in cucumber, peroxidase, and polyphenoloxidase [101,108].

Competition for space or nutrients with *P. cubensis* is another important mechanism for biocontrol agents to suppress CDM. The ability of *B. subtilis* to control CDM is mainly caused by its strong space-occupying capacity on plants, thereby preventing pathogen colonization [92,116]. In addition, *Ps. fluorescens* has a powerful competitive ability to utilize iron, which is important for pathogen growth and pathogenic capacity [92,117]. Moreover, the supernatant of *A. migulanus* accelerated the drying period of leaves, along with the inhibition of zoospore release and dispersion of pathogens [113]. Sometimes, a mixture of different biocontrol agents provided higher control ability against CDM, which might be due to synergistic effects. Functions from different biocontrol agents include inducing host resistance, producing antifungal compounds, or mycoparasitism [92].

6. Conclusions and Prospects

CDM is a destructive oomycete plant disease that causes a serious reduction in cucumber production and economic loss. Biocontrol may be an effective way to control CDM because it has the advantage of being environmentally friendly and contributing to

sustainable agricultural development. Numerous biocontrol agents, such as *Trichoderma*, *Bacillus*, *Paenibacillus*, *Enterobacter*, *Streptomyces*, and *Pseudomonas*, exhibited control of CDM. To comprehensively understand the biocontrol of CDM, this review focuses on the strategy of screening biocontrol agents, control characteristics, and efficacy against CDM by different biocontrol agents, as well as the potential biocontrol mechanisms. This review should provide useful information for the further application of biocontrol agents to suppress CDM.

Although the application of biocontrol agents to prevent fungal and oomycete plant diseases has been widely reported, controlling CDM by using biocontrol methods has seldom been studied. Future suggestions are provided as follows for further extensive application of biocontrol agents to prevent CDM:

(1) Study the molecular mechanism by which biocontrol agents prevent CDM. This could include investigating the transcriptome of biocontrol agents and analyzing the differentially expressed genes in biocontrol agents during the process of controlling CDM. The functions of differentially expressed genes in biocontrol agents preventing CDM can be studied through gene knockout, silencing, or overexpression.

(2) Expand the variety and amount of biocontrol agents that effectively control CDM. Compared with controlling fungal plant diseases, the variety and amount of biocontrol agents that suppress CDM are small. The selection of a suitable screening method could be an effective way to expand the variety and amount of biocontrol agents. Another way is to investigate the control ability of CDM by known biocontrol agents with significant control ability against other fungal and oomycete plant diseases, e.g., other *Bacillus* strains, *Clonostacys rosea*, and *Coniothyrium minitans* [118–120].

(3) Improve the control efficacy of existing biocontrol agents suppressing CDM. Several methods could be applied. One approach is to optimize the culture and inoculation conditions. In addition, genetic manipulations such as mutagenesis or genetic engineering could be used to improve the control ability of biocontrol agents suppressing CDM.

(4) Study the synergistic effect of integrating multiple biocontrol agents or combining biocontrol agents and fungicides. We can expect to reach an ideal control efficacy for CDM by optimizing the proportion of each component.

(5) Develop biocontrol agent products. Although many potential biocontrol agents were screened, commercially available products are very few. Therefore, developing biocontrol agent products is crucial for the wide application of biocontrol agents to control CDM.

Author Contributions: Conceptualization, Z.S. and Y.W.; investigation, Z.S. and S.Y.; data curation, S.Y. and Y.H.; writing—original draft preparation, Z.S.; writing—review and editing, Z.S. and Y.W.; supervision, Z.S.; funding acquisition, Z.S. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by Science and Technology general project of Beijing municipal education commission (KM202110011002).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Lebeda, A.; Urban, J. Temporal changes in pathogenicity and fungicide resistance in *Pseudoperonospora cubensis* populations. *Acta Hortic.* **2007**, *731*, 327–336. [CrossRef]
2. Miao, J.Q.; Dong, X.; Chi, Y.D.; Lin, D.; Chen, F.R.; Du, Y.X.; Liu, P.F.; Liu, X.L. *Pseudoperonospora cubensis* in China: Its sensitivity to and control by oxathiapiprolin. *Pestic. Biochem. Physiol.* **2018**, *147*, 96–101. [CrossRef] [PubMed]
3. Sun, S.L.; Lian, S.; Feng, S.; Dong, X.; Wang, C.; Li, B.; Liang, W. Effects of temperature and moisture on sporulation and infection by *Pseudoperonospora cubensis*. *Plant Dis.* **2017**, *101*, 562–567. [CrossRef] [PubMed]
4. Colucci, S.J.; Webner, T.C.; Holmes, G.J. The downy mildew epidemic of 2004 and 2005 in the eastern United States. In Proceedings of the Cucurbitaceae 2006, Asheville, NC, USA, 17–21 September 2006; pp. 403–411.

5. Palti, J.; Cohen, Y. Downy mildew of cucurbits *Pseudoperonospora.cubensis*. The fungus and its hosts, distribution, epidemiology and control. *Phytoparasitica* **1980**, *8*, 109–147. [CrossRef]
6. Thomas, C.E. Downy mildew. In *Compendium of Cucurbit Diseases*; Zitter, T.A., Ed.; Cornell University Press: Ithaca, NY, USA, 1996; pp. 25–27.
7. Wang, H.C.; Zhou, M.G.; Wang, J.X.; Chen, C.J.; Li, H.X.; Sun, H.Y. Biological mode of action of dimethomorph on *Pseudoperonospora cubensis* and its systemic activity in cucumber. *Agric. Sci. China* **2009**, *8*, 172–181. [CrossRef]
8. Yang, X.T.; Li, M.; Zhao, C.J.; Zhang, Z.; Hou, Y.L. Early warning model for cucumber downy mildew in unheated greenhouses. *N. Z. J. Agric. Res.* **2007**, *50*, 1261–1268. [CrossRef]
9. Ahamad, S.; Narain, U.; Prajapati, R.K.; Lal, C. Management of downy mildew of cucumber. *Ann. Plant Prot. Sci.* **2000**, *8*, 254–255.
10. Burkhardt, A.; Day, B. A genomics perspective on cucurbit-oomycete interactions. *Plant Biotechnol.* **2013**, *30*, 265–271. [CrossRef]
11. Holmes, G.J.; Main, C.E.; Keever, Z.T. Cucurbit downy mildew: A unique pathosystem for disease forecasting. In *Advances in Downy Mildew Research*; Spencer-Phillips, P.T.N., Jeger, M., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2004; Volume 2, pp. 69–80.
12. Lebeda, A.; Schwinn, F.J. The downy mildews–an overview of recent.research progress. *J. Plant Dis. Prot.* **1994**, *101*, 225–254.
13. Shetty, N.V.; Wehner, T.C.; Thomas, C.E.; Doruchowski, R.W.; Shetty, K.V. Evidence for downy mildew races in cucumber tested in Asia, Europe, and North America. *Sci. Hortic.* **2002**, *94*, 231–239. [CrossRef]
14. Lebeda, A.; Pavelková, J.; Urban, J.; Sedláková, B. Distribution, host.range and disease severity of *Pseudoperonospora cubensis* on cucurbits in the Czech Republic. *J. Phytopathol.* **2011**, *159*, 589–596. [CrossRef]
15. Oerke, E.C.; Steiner, U.; Dehne, H.W.; Lindenthal, M. Thermal imaging of.cucumber leaves affected by downy mildew and environmental conditions. *J. Exp. Bot.* **2006**, *57*, 2121–2132. [CrossRef]
16. Savory, E.A.; Granke, L.L.; Quesada-Ocampo, L.M.; Varbanova, M.; Hausbeck, M.K.; Day, B. The cucurbit downy mildew pathogen *Pseudoperonospora cubensis*. *Mol. Plant Pathol.* **2011**, *12*, 217–226. [CrossRef] [PubMed]
17. Xu, S.C.; Wang, H.B.; Feng, J.J.; Xiang, H.F.; Wu, M.D.; Wang, Z.M.; Wei, D.Y.; Zhang, H.C.; Tang, Q.L. Progress in cucumber downy mildew and mechanisms of host resistance. *Chin. J. Biotechnol.* **2022**. [CrossRef]
18. Zhao, X.J.; Lin, Z.M.; Zhao, Z.J. Studies on diversity of symptoms of cucumber downy mildew. *J. Shanxi Agric. Sci.* **2000**, *28*, 72–74.
19. Cohen, Y.; Van Den Langenberg, K.M.; Wehner, T.C.; Ojiambo, P.S.; Hausbeck, M.; Quesada-Ocampo, L.M.; Lebeda, A.; Sierotzki, H.; Gisi, U. Resurgence of *Pseudoperonospora cubensis*: The causalagent of cucurbit downy mildew. *Phytopathology* **2015**, *105*, 998–1012. [CrossRef]
20. Granke, L.L.; Morrice, J.J.; Hausbeck, M.K. Relationships between airborne *Pseudoperonospora cubensis* sporangia, environmental conditions, and cucumber downy mildew severity. *Plant Dis.* **2014**, *98*, 674–681. [CrossRef]
21. Holmes, G.J.; Ojiambo, P.S.; Hausbeck, M.K.; Quesada-Ocampo, L.; Keinath, A.P. Resurgence of cucurbit downy mildew in the United States: A watershed event for research and extension. *Plant Dis.* **2015**, *99*, 428–441. [CrossRef]
22. Lebeda, A.; Urban, J. Distribution, harmfulness and pathogenic variability of cucurbit downy mildew in the Czech Republic. *Acta Fytotech. Zootech.* **2004**, *7*, 170–173.
23. Rondomański, W.; Żurek, B.; Sekuł, S. Downy mildew of cucumber a new problem on cucumber in Poland Roczn. *Nauk. Roln seria ET* **1987**, *17*, 200–209.
24. Guan, A.; Wang, M.; Yang, J.; Wang, L.; Xie, Y.; Lan, J.; Liu, C. Discovery of a new fungicide candidate through lead optimization of pyrimidinamine derivatives and its activity against cucumber downy mildew. *J. Agric. Food Chem.* **2017**, *65*, 10829–10835. [CrossRef] [PubMed]
25. Ishii, H.; Fraaije, B.A.; Sugiyama, T.; Noguchi, K.; Nishimura, K.; Takeda, T.; Amano, T.; Hollomon, D.W. Occurrence and molecular characterization of strobilurin resistance in cucumber powdery mildew and downy mildew. *Phytopathology* **2001**, *91*, 1166–1171. [CrossRef] [PubMed]
26. Keinath, A.P. Utility of a cucumber plant bioassay to assess fungicide efficacy against *Pseudoperonospora cubensis*. *Plant Dis.* **2016**, *100*, 490–499. [CrossRef] [PubMed]
27. Mitani, S.; Kamachi, K.; Sugimoto, K.; Araki, S.; Yamaguchi, T. Control of cucumber downy mildew by cyazofamid. *J. Pestic. Sci.* **2003**, *28*, 64–68. [CrossRef]
28. Salas, S.E.; Shepherd, C.P.; Ngugi, H.K.; Genet, J.L. Disease control attributes of oxathiapiprolin fungicides for management of cucurbit downy mildew. *Plant Dis.* **2019**, *103*, 2812–2820. [CrossRef]
29. Chen, Z.J.; Zhang, S.L.; Zhang, F.; Liang, Y.L.; Xu, F.L. Present situateion of chemical pesticides controlling cucumber pests in sunlight greenhouse and its controlling countermeasures. *Chin. J. Eco-Agric.* **2006**, *14*, 141–143.
30. Zhang, F.; Qin, Z.; Zhou, X.; Xin, M.; Li, S.; Luan, J. Expression and functional analysis of the propamocarb-related gene *CsMAPEG* in cucumber. *BMC Plant Biol.* **2019**, *19*, 371. [CrossRef]
31. Zhang, B.; Zhao, W.; Zhang, Y.L.; Ma, L.G.; Qi, J.S.; Li, C.S. Control effect of five kinds of preparations and combinations of cucumber downy mildew and its evaluation. *Agrochemicals* **2015**, *54*, 456–457.

32. Blum, M.; Waldner, M.; Olaya, G.; Cohen, Y.; Gisi, U.; Sierotzki, H. Resistance mechanism to carboxylic acid amide fungicides in the cucurbit downy mildew pathogen *Pseudoperonospora cubensis*. *Pest Manag. Sci.* **2011**, *67*, 1211–1214. [CrossRef]
33. Okada, K.; Furukawa, M. Occurrence and countermeasure of fungicide-resistant pathogens in vegetable field of Osaka prefecture. *J. Pestic. Sci.* **2008**, *33*, 326–329. [CrossRef]
34. Reuveni, M.; Eyal, M.; Cohen, Y. Development of resistance to metalaxyl in *Pseudoperonospora cubensis*. *Plant Dis.* **1980**, *64*, 1108–1109. [CrossRef]
35. Urban, J.; Lebeda, A. Fungicide resistance in cucurbit downy mildew-methodological, biological and population aspects. *Ann. Appl. Biol.* **2006**, *149*, 63–75. [CrossRef]
36. Zhu, S.S.; Liu, X.L.; Wang, Y.; Wu, X.H.; Liu, P.F.; Li, J.Q.; Yuan, S.K.; Si, N.G. Resistance of *Pseudoperonospora cubensis* to flumorph on cucumber in plastic houses. *Plant Pathol.* **2007**, *56*, 967–975. [CrossRef]
37. Call, A.D.; Criswell, A.D.; Weimer, T.C.; Klosinska, U.; Kozik, E.U. Screening cucumber for resistance to downy mildew caused by *Pseudoperonospora cubensis* (Berk. and Curt.) Rostov. *Crop Sci.* **2012**, *52*, 577–592. [CrossRef]
38. Holdsworth, W.L.; Summers, C.F.; Glos, M.; Smart, C.D.; Mazourek, M. Development of downy mildew-resistant cucumbersfor late-season production in the northeastern United States. *HortScience* **2014**, *49*, 10–17. [CrossRef]
39. Horejsi, T.; Staub, J.E.; Thomas, C. Linkage of random amplified polymorphic DNA markers to downy mildew resistance in cucumber (*Cucumis.sativus* L.). *Euphytica* **2000**, *115*, 105–113. [CrossRef]
40. Innark, P.; Ratanachan, T.; Khanobdee, C.; Samipak, S.; Jantasuriyarat, C. Downy mildew resistant/susceptible cucumber germplasm (*Cucumis sativus* L.) genetic diversity assessment using ISSR markers. *Crop Prot.* **2014**, *60*, 56–61. [CrossRef]
41. Olczak-Woltman, H.; Marcinkowska, J.; Niemirowicz-Szczytt, K. The genetic basis of resistance to downy mildew in *Cucumis* spp.—latest developments and prospects. *J. Appl. Genet.* **2011**, *52*, 249–255. [CrossRef]
42. Cohen, Y. The combined effects of temperature, leaf wetness, and inoculum concentration on infection of cucumbers with *Pseudoperonospora cubensis*. *Can. J. Bot.* **1977**, *55*, 1478–1487. [CrossRef]
43. Göker, M.; Voglmayr, H.; Riethmüller, A.; Oberwinkler, F. How do obligate parasites evolve? A multi-gene phylogenetic analysis of downy mildews. *Fungal Genet. Biol.* **2007**, *44*, 105–122. [CrossRef]
44. Lange, L.; Eden, U.; Olson, L.W. Zoosporogenesis in *Pseudoperonospora cubensis*, the causal agent of cucurbit downy mildew. *Nord. J. Bot.* **1989**, *8*, 497–504. [CrossRef]
45. Ojiambo, P.S.; Holmes, G.J. Spatiotemporal spread of cucurbit downy mildew in the eastern United States. *Phytopathology* **2011**, *101*, 451–461. [CrossRef] [PubMed]
46. Runge, F.; Thines, M. Host matrix has major impact on the morphology of *Pseudoperonospora cubensis*. *Eur. J. Plant Pathol.* **2011**, *129*, 147–156. [CrossRef]
47. Salcedo, A.F.; Purayannur, S.; Standish, J.R.; Miles, T.; Thiessen, L.; Quesada-Ocampo, L.M. Fantastic downy mildew pathogens and how to find them: Advances in detection and diagnostics. *Plants* **2021**, *10*, 435. [CrossRef]
48. Bedlan, G. Erstmaliger nachweis von oosporen von *Pseudoperonospora cubensis* (Berk. et Curt.) Rost. an Gewächshausgurken in Österreich. *Pflanzenschutzberichte* **1989**, *50*, 119–120.
49. Cohen, Y.; Meron, I.; Mor, N.; Zuriel, S. A new pathotype of *Pseudoperonospora cubensis* causing downy mildew in cucurbits in Israel. *Phytoparasitica* **2003**, *31*, 458–466. [CrossRef]
50. Cohen, Y.; Rubin, A.E.; Galperin, M. Host preference of mating type in *Pseudoperonospora cubensis*, the downy mildew causal agent of cucurbits. *Plant Dis.* **2013**, *97*, 292. [CrossRef]
51. Runge, F.; Thines, M. Reevaluation of host specificity of the closely related species *Pseudoperonospora humuli* and *P. cubensis*. *Plant Dis.* **2012**, *96*, 55–61. [CrossRef]
52. Runge, F.; Thines, M. A potential perennial host for *Pseudoperonospora cubensis* in temperate regions. *Eur. J. Plant Pathol.* **2009**, *123*, 483–486. [CrossRef]
53. Zhang, X.; Chen, Y.; Zhang, Y.J.; Zhou, M.G. Occurrence and molecular characterization of azoxystrobin resistance in cucumber downy mildew in Shandong province in China. *Phytoparasitica* **2008**, *36*, 136–143. [CrossRef]
54. Forsberg, A.S. Downy mildew-*Pseudoperonospora cubensis* in Swedish cucumber fields. *Växtskyddsnotiser* **1986**, *50*, 17–19.
55. Neufeld, K.N.; Isard, S.A.; Ojiambo, P.S. Relationship between disease severity and escape of *P.cubensis* sporangia from a cucumber canopy during downy mildew epidemics. *Plant Pathol.* **2013**, *62*, 1366–1377. [CrossRef]
56. Niu, D.; Fu, J.; Wang, L.J. Latest research development of cucumber downy mildew. *J. Northeast F. Univ.* **2008**, *9*, 94–98.
57. Spring, O.; Gomez-Zeledon, J.; Hadziabdic, D.; Trigiano, R.N.; Thines, M.; Lebeda, A. Biological characteristics and assessment of virulence diversity in pathosystems of economically important biotrophic oomycetes. *Crit. Rev. Plant. Sci.* **2018**, *37*, 439–495. [CrossRef]
58. Kitner, M.; Lebeda, A.; Sharma, R.; Runge, F.; Dvorak, P.; Tahir, A.; Choi, Y.J.; Sedlakova, B.; Thines, M. Coincidence of virulence shifts and population genetic changes of *Pseudoperonospora cubensis* in the Czech Republic. *Plant Pathol.* **2015**, *64*, 1467–1470. [CrossRef]
59. Lebeda, A.; Widrlechner, M. A set of Cucurbitaceae taxa for differentiation of *Pseudoperonospora cubensis* pathotypes. *J. Plant Dis. Prot.* **2003**, *110*, 337–349.
60. Lebeda, A.; Cohen, Y. Cucurbit downy mildew (*Pseudoperonospora cubensis*)-biology, ecology, epidemiology, host-pathogen interaction and control. *Eur. J. Plant Pathol.* **2011**, *129*, 157–192. [CrossRef]

61. Bains, S.S.; Jhooty, J.S. Over wintering of *Pseudoperonospora cubensis* causing downy mildew of muskmelon. *Indian Phytopathol.* **1976**, *29*, 213–214.
62. Hall, G.S. Modern approaches to species concepts in downy mildews. *Plant Pathol.* **1996**, *45*, 1009–1026. [CrossRef]
63. Thines, M. Phylogeny and evolution of plant pathogenic oomycetes: A global overview. *Eur. J. Plant Pathol.* **2014**, *138*, 431–447. [CrossRef]
64. Cohen, Y. Downy mildew of cucurbits. In *The Downy Mildews*; Spencer, D.M., Ed.; Academic Press: London, UK, 1981; pp. 341–354.
65. Sun, Y.J. Occurrence regularity, environmental influencing factors and control techniques of cucumber downy mildew. *J. Seed Ind. Guide* **2021**, *6*, 35–38.
66. Zhu, J.X.; Wang, Y.P.; Guo, P.Y.; Gao, F.J. Progress of study on downy mildew in cucumber. *Nor. Horticul.* **2008**, *4*, 74–78.
67. Bouwmeester, K.; van Poppel, P.M.J.A.; Govers, F. Genome biology cracks enigmas of oomycete plant pathogens. *Annu. Plant Rev.* **2009**, *34*, 102–133.
68. Choi, Y.J.; Hong, S.B.; Shin, H.D. A re-consideration of *Pseudoperonospora cubensis* and *P. humuli* based on molecular and morphological data. *Mycol. Res.* **2005**, *109*, 841–848. [CrossRef] [PubMed]
69. Granke, L.L.; Hausbeck, M.K. Dynamics of *Pseudoperonospora cubensis* sporangia in commercial cucurbit fields in Michigan. *Plant Dis.* **2011**, *95*, 1392–1400. [CrossRef] [PubMed]
70. Arauz, L.F.; Neufeld, K.N.; Lloyd, A.L.; Ojiambo, P.S. Quantitative models for germination and infection of *Pseudoperonospora cubensis* in response to temperature and duration of leaf wetness. *Phytopathology* **2010**, *100*, 959–967. [CrossRef]
71. Cohen, Y.; Rubin, A.E.; Galperin, M. Formation and infectivity of oospores of *Pseudoperonospora cubensis*, the causal agent of downy mildew in cucurbits. *Plant Dis.* **2011**, *95*, 874. [CrossRef]
72. Kanetis, L.; Holmes, G.J.; Ojiambo, P.S. Survival of *Pseudoperonospora cubensis* sporangia exposed to solar radiation. *Plant Pathol.* **2009**, *59*, 313–323. [CrossRef]
73. Neufeld, K.N.; Ojiambo, P.S. Interactive effects of temperature and leaf wetness duration on sporangia germination and infection of cucurbit hosts by *Pseudoperonospora cubensis*. *Sci. Hortic.* **2011**, *96*, 345–353. [CrossRef]
74. Shi, Y.X.; Li, B.J.; Liu, X.M. The study of cucumber downy mildew. *J. Northeast Agric. Univ.* **2002**, *33*, 391–395.
75. Adhikari, B.N.; Savory, E.A.; Vaillancourt, B.; Childs, K.L.; Hamilton, J.P.; Day, B.; Buell, C.R. Expression profiling of *Cucumis sativus* in response to infection by *Pseudoperonospora cubensis*. *PLoS ONE* **2012**, *7*, e34954. [CrossRef] [PubMed]
76. Savory, E.A.; Adhikari, B.N.; Hamilton, J.P.; Vaillancourt, B.; Buell, C.R.; Day, B. mRNA-Seq analysis of the *Pseudoperonospora cubensis* transcriptome during cucumber (*Cucumis sativus* L.) infection. *PLoS ONE* **2012**, *7*, e35796. [CrossRef] [PubMed]
77. Savory, E.A.; Zou, C.; Adhikari, B.N.; Hamilton, J.P.; Buell, C.R.; Shiu, S.H.; Day, B. Alternative splicing of a multi-drug transporter from *Pseudoperonospora cubensis* generates an RXLR effector protein that elicits a rapid cell death. *PLoS ONE* **2012**, *7*, e34701. [CrossRef]
78. Zhang, P.; Zhu, Y.; Luo, X.; Zhou, S. Comparative proteomic analysis provides insights into the complex responses to *Pseudoperonospora cubensis* infection of cucumber (*Cucumis sativus* L.). *Sci. Rep.* **2019**, *9*, 9433. [CrossRef] [PubMed]
79. Zheng, L.; Gu, C.; Cao, J.; Li, S.M.; Wang, G.; Luo, Y.M.; Guo, J.H. Selecting bacterial antagonists for cucurbit downy mildew and developing an effective application method. *Plant Dis.* **2018**, *102*, 628–639. [CrossRef]
80. Sun, Z.B.; Yuan, X.F.; Zhang, H.; Wu, L.F.; Liang, C.; Feng, Y.J. Isolation, screening and identification of antagonistic downy mildew endophytic bacteria from cucumber. *Eur. J. Plant Pathol.* **2013**, *137*, 847–857. [CrossRef]
81. Alfiky, A.; Weisskopf, L. Deciphering *Trichoderma*-plant-pathogen interactions for better development of biocontrol applications. *J. Fungi* **2021**, *7*, 61. [CrossRef] [PubMed]
82. Karlsson, M.; Atanasova, L.; Jensen, D.F.; Zeilinger, S. Necrotrophic mycoparasites and their genomes. *Microbiol. Spectr.* **2017**, *5*. [CrossRef]
83. Mukherjee, M.; Mukherjee, P.K.; Horwitz, B.A.; Zachow, C.; Berg, G.; Zeilinger, S. *Trichoderma*-plant-pathogen interactions: Advances in genetics of biological control. *Indian J. Microbiol.* **2012**, *52*, 522–529. [CrossRef]
84. Sood, M.; Kapoor, D.; Kumar, V.; Sheteiwy, M.S.; Ramakrishnan, M.; Landi, M.; Araniti, F.; Sharma, A. *Trichoderma*: The "secrets" of a multitalented biocontrol agent. *Plants* **2020**, *9*, 762. [CrossRef]
85. Tyśkiewicz, R.; Nowak, A.; Ozimek, E.; Jaroszuk-Ściseł, J. *Trichoderma*: The current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth. *Int. J. Mol. Sci.* **2022**, *23*, 2329. [CrossRef] [PubMed]
86. Al-Aswad, R.M.A.; Al-Azzawi, Q.K.Z. Control of downy mildew disease on cucumber caused by the fungus *Psuedoperonospora cubensis* by using environmentally friendly materials. *Euphrates J. Agric. Sci.* **2021**, *13*, 98–110.
87. Elsharkawy, M.M.; Kamel, S.M.; El-Khateeb, N.M.M. Biocontrol control of powdery and downy mildews of cucumber under greenhouse conditions. *Egypt. J. Biol. Pest Co.* **2014**, *24*, 301–308.
88. El-khalily, M.R.; Gazar, T.A.; EL-Sheshtawi, M. Ability of some antagonistic fungi for controlling cucumber downy mildew disease caused by *Pseudoperonospora cubensis*. *J. Plant Protect. Pathol.* **2021**, *12*, 67–69.
89. Alvarado-Aguayo, A.; Pilaloa-David, W.; Torres-Sánchez, S.; Torres-Sánchez, K. Efecto de *Trichoderma harzianum* en el control de mildiu (*Pseudoperonospora cubensis*) en pepino. *Agron. Costarric.* **2019**, *43*, 101–111. [CrossRef]

90. Martínez, B.; González, E.; Infante, D. Nuevas evidencias de la accion Antagonista de *Trichoderma asperellum* Samuels. *Rev. Protección Veg.* **2011**, *26*, 131–132.
91. Szczech, M.; Nawrocka, J.; Felczynski, K.; Malolepsza, U.; Sobolewski, J.; Kowalska, B.; Maciorowski, R.; Jas, K.; Kancelista, A. *Trichoderma atroviride* TRS25 isolate reduces downy mildew and induces systemic defence responses in cucumber in field conditions. *Sci. Hortic.* **2017**, *224*, 17–26. [CrossRef]
92. Abd-El-Moity, T.H.; Tia, M.M.M.; Aly, A.Z.; Tohamy, M.R.A. Biological control of some cucumber diseases under organic agriculture. In Proceedings of the International Symposium on the Horizons of Using Organic Matter and Substrates in Horticulture, Cairo, Egypt, 6–9 April 2002; Volume 608, pp. 227–236.
93. Yi, X.H.; Feng, J.T.; Fang, X.L.; Li, Y.P.; Zhang, X. Biological characteristics and biological activity of *Pestalotiopsis* sp. obtained from leaf of *Pyrethrum cinerariafolium* Trey. *J. Zhejiang Univ.* **2008**, *34*, 516–524.
94. Yi, X.H.; Feng, J.T.; Wang, Y.H.; Guo, X.W.; Zhang, X. The preliminary study on screening and identification of endophytic fungi Y2 from *Pyrethrum cinerariifolium* and antifungal activity of Y2 fermentation products. *Chin. J. Pestic. Sci.* **2007**, *9*, 193–196.
95. Yang, M.; Zong, Z.F.; Guo, X.F.; Yao, J.; Claude, A. Effect of biocontrol agents FO47 and FO47B10 on watermelon and cucumber. *J. Northwest Sci. Tech. Univ. Agric. For.* **2005**, *33*, 57–60.
96. Andrić, S.; Meyer, T.; Ongena, M. *Bacillus* responses to plant-associated fungal and bacterial communities. *Front. Microbiol.* **2020**, *11*, 1350. [CrossRef] [PubMed]
97. Fan, B.; Wang, C.; Song, X.; Ding, X.; Wu, L.; Wu, H.; Gao, X.; Borriss, R. *Bacillus velezensis* FZB42 in 2018: The gram-positive model strain for plant growth promotion and biocontrol. *Front. Microbiol.* **2018**, *9*, 2491. [CrossRef] [PubMed]
98. Patel, R.R.; Patel, D.D.; Thakor, P.; Patel, B.; Thakkar, V.R. Alleviation of salt stress in germination of *Vigna radiate* L. by two halotolerant *Bacillus* sp. isolated from saline habitats of Gujarat. *Plant Growth Regul.* **2014**, *76*, 51–60. [CrossRef]
99. Fira, D.; Dimkić, I.; Berić, T.; Lozo, J.; Stanković, S. Biological control of plant pathogens by *Bacillus* species. *J. Biotechnol.* **2018**, *285*, 44–55. [CrossRef]
100. Miljaković, D.; Marinković, J.; Balešević-Tubić, S. The significance of *Bacillus* spp. in disease suppression and growth promotion of field and vegetable crops. *Microorganisms* **2020**, *8*, 1037. [CrossRef]
101. Mohamed, A.; Hamza, A.; Derbalah, A. Recent approaches for controlling downy mildew of cucumber under greenhouse conditions. *Plant Protect. Sci.* **2016**, *52*, 1–9. [CrossRef]
102. Zheng, L.; Xu, L.; Luo, Y.M.; Liu, H.X.; Guo, J.H. Preliminary study on the mechanism of a srain *Bacillus licheniformis* HS10 against cucumber downy mildew. *Guangdong Agric. Sci.* **2020**, *47*, 81–87.
103. Wang, Z.X.; Wang, Y.P.; Zheng, L.; Yang, X.N.; Liu, H.X.; Guo, J.H. Isolation and characterization of an antifungal protein from *Bacillus licheniformis* HS10. *Biochem. Biophys. Res. Commun.* **2014**, *454*, 48–52. [CrossRef]
104. Xiao, Q.; Li, S.W.; Liang, C.; Li, W.Y.; Mao, Y.W. Action mode and duration of *Bacillus velezensis* against cucumber downy mildew. *Agrochemicals* **2021**, *60*, 829–831.
105. Ketta, H.A.; Kamel, S.M.; Ismail, A.M.; Ibrahem, E.S. Control of downy mildew disease of cucumber using *Bacillus chitinosporus*. Egypt. *J. Biol. Pest Control* **2016**, *26*, 839–845.
106. Li, X.; Zhang, D.; Yang, W.X.; Dong, L.; Liu, D.Q. Studies on the effect of *Bacillus* on downy mildew of cucumber. *Plant Protect.* **2003**, *29*, 25–27.
107. Anand, T.; Chandrasekaran, A.; Kuttalam, S.; Raguchander, T.; Samiyappan, R. Management of cucumber (*Cucumis sativus* L.) mildews through azoxystrobin-tolerant *Pseudomonas fluorescens*. *J. Agric. Sci. Technol.* **2009**, *11*, 211–226.
108. Anand, T.; Raguchander, T.; Karthikeyan, G.; Prakasam, V.; Samiyappan, R. Chemically and biologically mediated systemic resistance in cucumber (*Cucumis sativus* L.) against *Pseudoperonospora cubensis* and *Erysiphe cichoracearum*. *Phytopathol. Mediterr.* **2007**, *46*, 259–271.
109. Tian, X.W.; Long, J.Y.; Bai, H.J.; Wu, W.J. Studies on the fungicidal activity of secondary metabolic products of actinomycetes. *Plant Protect.* **2004**, *30*, 51–54.
110. Patel, R.R.; Thakkar, V.R.; Subramanian, B.R. A *Pseudomonas guariconensis* strain capable of promoting growth and controlling collar rot disease in *Arachis hypogaea* L. *Plant Soil* **2015**, *390*, 369–381. [CrossRef]
111. Ye, N.W.; Wang, C.F.; Gan, H.L.; Wu, Z.Y.; Mi, F.; Mao, W.L. Control effect of *Paenibacilus polymyxa* strain P1 against cucumber downy mildew. *Plant Protect.* **2021**, *47*, 271–275.
112. Fan, Y.T.; Chung, K.R.; Huang, J.W. Fungichromin production by *Streptomyces padanus* PMS-702 for controlling cucumber downy mildew. *Plant Pathol. J.* **2019**, *35*, 341–350. [CrossRef]
113. Schuster, C.; Schmitt, A. Efficacy of a bacterial preparation of *Aneurinibacillus migulanus* against downy mildew of cucumber (*Pseudoperonospora cubensis*). *Eur. J. Plant Pathol.* **2018**, *151*, 439–450. [CrossRef]
114. Scherf, A.; Schuster, C.; Marx, P.; Gärber, U.; Konstantinidou-Doltsinis, S.; Schmitt, A. Control of downy mildew (*Pseudoperonospora cubensis*) of greenhouse grown cucumbers with alternative biological agents. Commun. *Agric. Appl. Biol. Sci.* **2010**, *75*, 541–554.
115. Georgieva, O.A.; Georgiev, G.A. Biological control of diseases on main vegetables-researches and practice in maritsa vegetable crops institute. In Proceedings of the IV Balkan Symposium on Vegetables and Potatoes, Plovdiv, Bulgaria, 17 June 2009; Volume 830, pp. 511–517.
116. Wolk, M.; Sorkar, S. Antagonism in vivo of *Bacillus* spp. against *Rhizoctoniasolani* and *Pythium* spp. *Anz. Fur Schadl. Skundey Pflanzenschutz Umweltschutz* **1993**, *66*, 121–125.

117. Loper, J.E. Role of fluorescent siderophore production in biological control of *Pythium ultimum* by a *Pseudomonas fluorescens* strain. *Phytopathology* **1988**, *78*, 166–172. [CrossRef]
118. Abbas, A.; Khan, S.U.; Khan, W.U.; Saleh, T.A.; Khan, M.H.U.; Ullah, S.; Ali, A.; Ikram, M. Antagonist effects of strains of *Bacillus* spp. against *Rhizoctonia solani* for their protection against several plant diseases: Alternatives to chemical pesticides. *Comptes Rendus. Biol.* **2019**, *342*, 124–135. [CrossRef] [PubMed]
119. Sun, Z.B.; Li, S.D.; Ren, Q.; Xu, J.L.; Lu, X.; Sun, M.H. Biology and applications of *Clonostachys rosea*. *J. Appl. Microbiol.* **2020**, *129*, 486–495. [CrossRef] [PubMed]
120. Zhao, H.; Zhou, T.; Xie, J.; Cheng, J.; Chen, T.; Jiang, D.; Fu, Y. Mycoparasitism illuminated by genome and transcriptome sequencing of *Coniothyrium minitans*, an important biocontrol fungus of the plant pathogen *Sclerotinia sclerotiorum*. *Microb. Genom.* **2020**, *6*, e000345. [CrossRef] [PubMed]