agrochemicals

MDPI

Review

Integrating Biological Control Agents for Enhanced Management of Apple Scab (*Venturia inaequalis*): Insights, Risks, Challenges, and Prospects

Chisom Augusta Okoro *, Abbas El-Hasan * and Ralf T. Voegele

Department of Phytopathology, Institute of Phytomedicine, Faculty of Agricultural Sciences, University of Hohenheim, 70599 Stuttgart, Germany; ralf.voegele@uni-hohenheim.de

* Correspondence: chisom.okoro@uni-hohenheim.de (C.A.O.); aelhasan@uni-hohenheim.de (A.E.-H.)

Abstract: Apple scab incited by the ascomycete *Venturia inaequalis* poses a significant threat to apple cultivation, necessitating a reassessment of existing disease management strategies. Attempts to manage apple scab include diverse approaches like developing disease forecasting models and the extensive application of synthetic chemical fungicides. However, the efficacy of these methods is compromised by inconsistencies, environmental concerns, and the pathogen's resistance, necessitating the exploration of alternative sustainable strategies. Addressing the challenges associated with apple scab management, this review strongly supports a shift towards the integration of biological control agents (BCAs). Emphasising the transformative synergy between BCAs and their bioactive secondary metabolites, we highlight their efficacy in advancing precision disease control through innovative and sustainable solutions. The review effectively presents a strong justification for the integration of BCAs and their by-products into apple scab management, offering insights into associated benefits, risks, and challenges while outlining promising prospects. Ultimately, it is expected to drive the adoption of environmentally conscious practices for effective apple scab management.

Keywords: biological control agents; bioactive secondary metabolites; disease control; bio-fungicides; apple scab

Citation: Okoro, C.A.; El-Hasan, A.; Voegele, R.T. Integrating Biological Control Agents for Enhanced Management of Apple Scab (*Venturia inaequalis*): Insights, Risks, Challenges, and Prospects. *Agrochemicals* **2024**, *3*, 118–146. https://doi.org/10.3390/agrochemicals3020010

Academic Editor: Christos G. Athanassiou

Received: 31 January 2024
Revised: 19 March 2024
Accepted: 20 March 2024
Published: 25 March 2024

1. Introduction

Apple scab incited by the ascomycete *Venturia inaequalis* (anamorph *Spilocea pomi* Fr.) poses a significant threat to apple cultivation and is notably challenging and expensive to manage [1]. This disease has emerged as a major concern in apple-growing regions worldwide, particularly in temperate areas characterized by cool and moist spring weather [2]. Despite continuous research efforts, apple scab remains remarkably resilient, emerging as the most economically damaging pathogen on apple trees globally [3]. The disease causes substantial economic damage on apple trees, affecting the vitality of leaves and fruits, and leading to both immediate and indirect losses [3–5]. Its detrimental effects extend to tree vigour, yield, and fruit quality, making it a paramount concern for growers [3,6]. Even a minor scab lesion renders an apple non-marketable, and the incidence of scab invariably results in significant losses [7]. Since the emergence of synthetic fungicides in the 1940s, fungicides have become the sole means to control apple scab [4]. Despite numerous attempts over decades to establish biocontrol strategies, it was only recently that a few of them have successfully reached the stage of commercial viability [1].

1.1. Overview of Apple Scab (*Venturia inaequalis*)

The precise time of emergence of scab on cultivated apples is unknown [2]. The initial documented report of apple scab was published by Fries in Sweden in 1819 [6]. However, the earliest indication of scab's incidence dates back to 1600, observed in a painting by Michelangelo Caravaggio titled 'The Supper at Emmaus' held at the National Gallery in

London [2]. The Simple Sequence Repeat (SSR) analysis of *V. inaequalis* samples from 28 orchards across five continents suggests a Central Asian origin, and its current global prevalence reveals significant genotypic diversity in almost all orchards [8].

Apple scab is caused by a pathogenic fungus *V. inaequalis*, representing the teleomorph or saprophytic state, and *S. pomi* Fr., representing the anamorph or parasitic state [6]. The manifestation of *V. inaequalis* infection on apple trees is a complex process linked to various factors. The fungus primarily targets young plant tissue and exhibits enormous adaptability [7]. It is most noticeable and severe on leaves and fruits, but it can also be seen on sepals, petals, young shoots, and bud scales [5]. In early spring, the initial signs of apple scab become apparent as dark green velvety spots on the lower surfaces of expanding leaves. As these lesions advance on the upper surfaces of mature leaves, they grow, leading to leaf splintering, swelling, scaliness, and eventual leaf drop [6]. Late-season infections, initially inconspicuous, may become apparent post-storage, leading to the phenomenon of pin-point scab [7]. Despite extensive research on its biology and control, apple scab remains a significant challenge, resulting in substantial economic consequences. The extent of losses varies with disease severity and prevailing weather conditions, establishing apple scab as a critical limitation to apple production on a global scale [7].

1.2. Infection Mechanism

V. inaequalis employs a sophisticated infection mechanism that adapts to the host's phenology and environmental conditions [2]. It thrives as a successful pathogen by accumulating specific genes that facilitate infection and reproduction without causing significant harmful effects to the host [2]. Investigations on *V. inaequalis* infection mechanism have revealed a unique infection strategy. Unlike typical host cell penetration, the fungus resides in the subcuticular space before sporulation, effectively neutralizing the host's defence mechanisms, possibly through specialized effectors [9]. Genetic evidence strongly supports the secretion of effectors by *V. inaequalis.* Several candidate effector genes have been identified, characterized by various features common to effector genes in filamentous fungi [9–11]. Additionally, Thakur et al. [12] identified crucial genes in *V. inaequalis* associated with biological processes such as metabolism, transport, and response to stimuli.

The disease cycle consists of two phases: the sexual or primary phase occurring mainly in winter, and the asexual or secondary phase which takes place during the vegetative period in spring/summer (Figure 1) [6,13].

During the dormant phase of the host tree, *V. inaequalis* strategically resides on leaves and fruit debris as pseudothecial initials or conidia in twig lesions. It adapts to temperature fluctuations to precisely synchronize with the developmental stage of the host tree, thus maximizing its possibility for future colonization [2,13]. As host plants break dormancy in early spring, heterothallic mating between different mating types takes place in the debris to initiate sexual reproduction. This process involves the fusion of a male hypha (antheridium) and a female receptive hypha (trichogyne) [5,13]. Subsequently, fertilization leads to the development of a pseudothecium within a dense mat of fungal mycelium (stroma), where asci and ascospores are formed. The diploid stage is brief, with meiosis giving rise to haploid ascospores within the pseudothecium [13]. The negatively geotropic nature of pseudothecium development causes the ostiole to face the air when leaves fall. This positioning increases the likelihood of ascospores being dispersed upward in spring, aiding their anemochoric dissemination [2]. The pathogen strategically discharges ascospores when host susceptibility is at its highest, optimizing the likelihood of successful establishment in the host canopy, and, thus, ensuring efficient disease progression [2]. When ascospores land on susceptible host tissue, in conditions of sufficient humidity, they germinate and attach to the leaf surface through adhesive mucilage, which acts as a bonding agent, allowing the spore to firmly adhere to the leaf and establish an infection site [7].

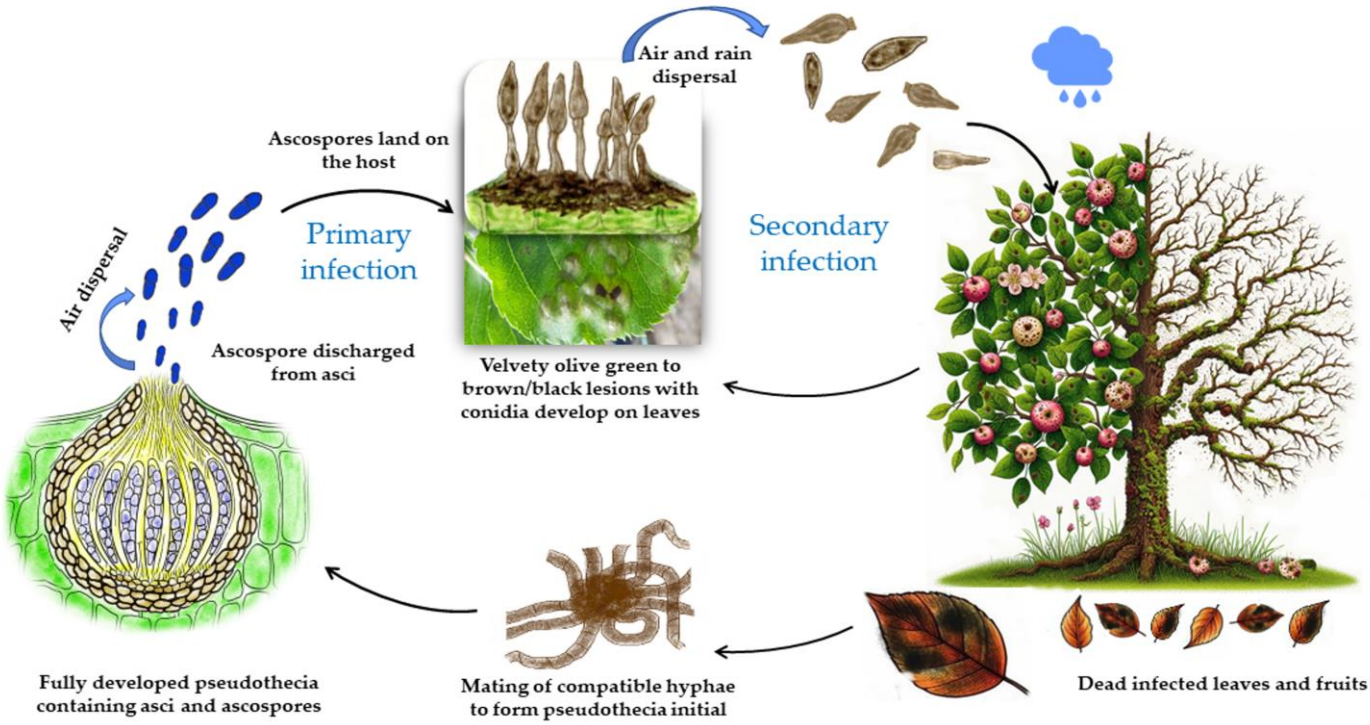

Figure 1. Life cycle of apple scab *V. inaequalis*.

The pathogen utilizes enzymatic hydrolysis of the cuticle, facilitated by enzymes like cutinase, to penetrate the host, forming nutrient-absorbing stromata which, in turn, lead to the development of characteristic lesions [9]. The single-celled, uninucleate conidia produced from these lesions on conidiophores emerge through the leaf cuticle, contributing to the velvety appearance of scab lesions [13]. The primary spread of the disease occurs within the canopy of the infected tree. Conidia are released by the pathogen and dispersed through water films, either locally or via splash-dispersion during rainfall events. This mode of dispersion allows the conidia to reach nearby susceptible host tissue, contributing to the spread and establishment of the disease within the orchard [2]. This leads to secondary infections during the growing season [13].

Susceptibility to *V. inaequalis* varies with the age of the host tissue. While young tissues of apple plants rely on a combination of quantitative trait loci (QTLs) and resistance genes (R genes), mature tissues exhibit ontogenic resistance [9]. This resistance develops as the leaves and fruit mature and is complete when leaves are fully expanded. Infections primarily occur on unfolding leaves and internodes between them, with ontogenic resistance developing faster on the ventral side of the leaves during expansion. The basis of this resistance is not yet understood, but it becomes inactive as leaves senesce in late seasons [2,9,14,15]. This phenomenon is observed in all apple cultivars and has never been overcome by the pathogen [9].

2. Management Strategies of Apple Scab

The introduction of synthetic fungicides in the late 1940s marked a turning point in the management of apple scab. Most apple cultivars are susceptible, and over time, fungicides became the predominant method for disease control, with limited exploration of alternative strategies on a commercial scale [7]. The heavy reliance on numerous fungicide applications results in substantial expenses for growers, coupled with undesirable environmental consequences [7,16]. To reduce dependence on fungicides, there is a huge concern to incorporate less-toxic control methods. This shift would not only mitigate costs for growers but also address environmental concerns associated with extensive fungicide use in apple cultivation [7,17]. Typically, management practices employed to control apple

scab focus on disrupting the disease cycle, specifically targeting its critical reproductive structures (e.g., spore germination).

2.1. Sanitation Practices

This approach involves minimizing overwintering inoculum through the mechanical removal of fallen leaves, ensuring meticulous orchard sanitation to control primary inoculum and, thereby, reduce the severity of scab infections [3]. Cultural control and sanitation practices play a pivotal role in mitigating scab infection, encompassing measures such as leaf shredding, pruning, burning, or burying leaves in the soil, along with the strategic application of a 5% urea solution to accelerate the decomposition process [6,8].

While cultural control proves indispensable in managing apple scab, it comes with inherent challenges. These include labour-intensive practices, limited efficacy, timing sensitivity, potential pathogen resurgence, weather dependency, long-term commitment requirements, and challenges related to varietal susceptibility [5–7]. Additionally, these inoculum reduction measures may be costly and impractical for certain operations [6].

MacHardy et al. [2] revealed that the introduction of horticultural practices in apple production has unintentionally facilitated the spread of *V. inaequalis*. Practices such as herbicide-treated strips, repeated mowing, and the use of semi dwarfing and dwarfing rootstocks, along with modern pruning techniques, create pathways for ascospores to reach the tree canopy more effectively [2]. These changes enhance ascospore dispersal efficiency, resulting in an increased number of successful infections. Initially, the impact of these modifications on scab epidemiology went unnoticed due to simultaneous advancements in fungicides, application strategies, and technology, which helped manage the challenges posed by these horticultural changes [2].

Additionally, overhead irrigation systems that promote prolonged leaf wetness have been linked to the increased disease severity of apple scab [18]. In contrast, drip irrigation systems that deliver water directly to the roots without wetting the foliage insure dry leaves which are less susceptible to infection and can help reduce disease development [5,18].

2.2. Mixed Cultivars

Implementing mixed-cultivar orchards, mimicking natural diversity, shows promise in reducing scab incidence [2]. Carisse and Bernier [1] emphasized the significant potential for reducing disease severity by taking advantage of cultivar susceptibility to *V. inaequalis* through a thoughtful orchard design. This was demonstrated in the work of Blaise and Gessler [19], where a careful consideration of cultivar selection and arrangement resulted in a substantial reduction in disease severity. By strategically mixing cultivars within or between rows, barriers to inoculum spread are created, offering a promising avenue for reducing susceptible tissue and enhancing disease control [19].

While often notable, the reduction in scab observed in mixed-cultivar orchards is typically insufficient for effective disease control in commercial production [8]. However, the most significant concern in implementing mixtures in commercial horticulture is the potential emergence and rapid build-up of a scab 'super race'. This combines virulence factors that can overcome most or all of the resistance genes in the host cultivars present in the mixture, rendering it ineffective as a mean for scab management [8,20].

2.3. Breeding for Resistance

Many commercially important apple cultivars are at risk of susceptibility to *V. inaequalis* [21,22]. Unfortunately, the slow and challenging transfer of disease resistance alleles from wild sources to apple cultivars with desirable fruit quality has led to a limited number of scab-resistant cultivars in commercial production [11]. The current strategy for cultivating scab-resistant cultivars involves incorporating *Rvi* resistance (R) genes into apple cultivars to enhance their defence mechanisms against *V. inaequalis* [20]. A total of 20 R genes against *V. inaequalis*, numbered from *Rvi1 to Rvi20*, have been documented, with the majority discovered in wild *Malus* accessions and landraces [20,23]. Several commercial

varieties show significant susceptibility to scab due to either a lack of known *Rvi* genes or a compromised effectiveness against specific races of scab [20]. The primary resistance gene used in breeding programs against apple scab has been *Rvi6*, which originated from the Japanese crab-apple *Malus floribunda* (Sieb.) sel. 821 [24]. Modern scab-resistant apple cultivars primarily rely on the *Rvi6* gene to enhance resistance against apple scab [25,26] (Table 1). Unfortunately, several *Rvi* genes, including the widely utilized *Rvi6* gene, have succumbed to new virulent strains of *V. inaequalis* [27]. The erosion of *Rvi6* has been observed in various varieties, including its origin, *M. floribunda* [20]. This breakdown of some of the *Rvi* genes was initially observed in Germany and later reported in various countries around the world [24,26–29]. Despite ongoing breeding efforts over the past decades, the majority of commercially grown scab-resistant apple varieties still rely on the *Rvi6* resistance [30]. Additionally, resistance gene pyramiding, involving the cultivation of cultivars with multiple resistance genes for durable resistance, has been explored [26]. While some combinations show positive results in field trials, their long-term effectiveness and commercial viability remain uncertain without direct empirical evidence [31].

Beyond resistance concerns, the widespread adoption of scab-resistant cultivars (such as Topaz, Prima, and Florina) in commercial orchards is limited due to worries about lower fruit quality and the uncertain durability of resistance [32]. Moreover, these challenges include the existence of multiple races of *V. inaequalis* [6] and the emergence of virulent races even in certain wild *Malus* species or genotypes like 'Golden Delicious' that remains highly susceptible despite carrying the *Rvi1* gene [6,20]. This situation raises concerns about fruit quality and poses obstacles for growers to replace existing trees due to cost and time considerations [3]. Hence, despite offering some protection against scab, resistant cultivars still face limitations such as less effectiveness against evolving pathogen strains, vulnerability to genetic uniformity, limitations in horticultural traits, longer development times, and an unclear long-term environmental impact [3]. Balancing resistant varieties with other tools is crucial in apple scab management programs.

Table 1. Commercially available apple cultivars and their reactions to apple scab.

Apple Cultivar	Reaction to Scab	*R*-Gene Type	References
Ahrista	R	*Rvi6*	[33]
Antonovka	R	*Rvi10*	[25]
Aychurok	S	-	[34]
Batul	HR	*Rvi4*	[23]
Cox Orange pippin	MS	-	[35]
Crimson Crisp	MR	*Rvi6*	[33]
Dayton	HR	*Rvi6*	[27]
Delicious	S	-	[27]
Discovery	HS	-	[35]
Elstar	S	-	[5]
Empire	S	-	[27]
Englischer Prinz	MR	*Rvi14*	[33]
Enterprise	HR	*Rvi6*	[6,36]
Florina	HR	*Rvi6*	[37,38]
Freedom	HR	*Rvi6*	[32]
Fuji	S	-	[6]
Gala	HS	-	[39,40]
Golab	S	-	[41]

Table 1. *Cont.*

Apple Cultivar	Reaction to Scab	*R*-Gene Type	References
Gold Rush	HR	*Rvi6, Rvi13*	[6,33]
Golden Delicious	HS	*Rvi1*	[27,37]
Granny Smith	S	-	[42]
Honeycrisp	R	*Rvi19, Rvi20*	[43]
Idared	S	-	[44]
Jonagold	S	-	[39]
Jonafree	R	Rvi6	[27]
Jonathan	S	-	[5]
Judeline	MS	*Rvi6*	[37]
Liberty	MR	*Rvi6*	[5,23]
Macfree	R	*Rvi6*	[5,24]
Makali	R	*Rvi6*	[32]
Malus floribunda 821	MR	*Rvi6, Rvi7*	[37]
Marshalll McIntosh	HS	None	[5]
Mutsu	HS	-	[44]
Nela	R	*Rvi6*	[33]
Nova Easygrow	HR	*Rvi6*	[6,23]
Pink lady	HS	-	[42]
Pioneer	R	*Rvi6*	[5]
Prima	HR	*Rvi1, Rvi6*	[37,44]
Primula	MR	*Rvi6*	[33]
Priscilla	HR	*Rvi6*	[37,45]
Realka	R	*Rvi2, Rvi4, Rvi6*	[33]
Redfree	R	*Rvi6*	[6,24]
Remo	R	*Rvi6*	[5]
Remura	R	*Rvi4*	[33]
Topaz	R	*Rvi6*	[33,38]
Vilmos renet	R	*Rvi2*	[23]
William's Pride	HR	*Rvi6*	[6,46]

R = Resistant; HR = Highly resistant; MR = Moderately resistant; S = Susceptible; MS = Moderately susceptible; HS = Highly susceptible; - = No resistance gene or unknown.

2.4. Scab Forecasting Models

Considering multiple factors like weather conditions, disease history, and the unique characteristics of different apple varieties, forecasting models play a crucial role in apple scab management [47,48]. Two prominent models come to the forefront. The Mills and LaPlante [49], a foundational framework, focuses on scab infection periods using a combination of leaf wetness duration and temperature to comprehend and predict scab onset. Several global studies were conducted to validate Mills' table and warning system, revealing some disagreements with the model [50–52]. Building upon this, the revised model by MacHardy and Gadoury [50], known as 'Mills/a - 3', addresses a three-hour discrepancy observed in Mills' studies. This curve, along with data from field weather stations, is used to assess infection risk. Additionally, the integration of sophisticated models, like the MacHardy/Gadoury ascospore maturity model, into forecasting tools,

along with the implementation of web-based Decision Support Systems (DSSs), has proven indispensable in offering real-time advice in horticultural extension services [47,53].

Despite notable advancements, challenges persist, particularly with discrepancies observed in the outcomes of forecasting tools [47,53]. While models utilizing weather data demonstrate accuracy in predicting infection periods, the ultimate effectiveness of control strategies depends on the availability of potent curative fungicides [54].

2.5. Chemical Fungicides

Over the past seven decades, apple scab management has heavily relied on weekly fungicide applications, with commercial plantations typically undergoing 10 to 30 treatments throughout the growing season [55]. *V. inaequalis* has been classified by the Fungicide Resistance Action Committee (FRAC) as a high-risk pathogen [56], signifying a substantial likelihood of fungicide resistance development in this pathogen [57]. Many different active ingredients with diverse modes of action have been deployed for apple scab management (Table 2). The primary objective is to effectively inhibit the initial ascospore-driven primary infections that occur during early spring [58].

Historically, several multisite fungicides such as Dodine, Captan, sulphur, copper, and Benzimidazole fungicides played a crucial role in apple scab control. However, fungicide resistance gradually emerged over time, restricting their effectiveness in managing apple scab [57,59]. Consequently, single-site fungicides targeting mitochondrial respiration or ergosterol biosynthesis, including quinone outside inhibitors (QoIs), sterol demethylation inhibiting fungicides (DMIs), and the newer generation II of Succinate dehydrogenase inhibitors (SDHIs), emerged as viable alternatives for the control of this pathogen [55,60]. Nevertheless, the specific modes of action of these single-site fungicides pose a significant risk for resistance development [60,61]. The mechanism underlying DMI fungicide resistance in *V. inaequalis* involves the overexpression of the *CYP51A1* gene, along with efflux mechanisms and point mutations [62,63]. Resistance to DMI fungicides has been reported globally [59,60,64–66]. Resistance to QoIs in *V. inaequalis* is primarily attributed to a target site mutation in the cytochrome b (*cyt b*) gene, known as *G143A* [57,67–69]. SDHI resistance in *V. inaequalis*, detected shortly after market introduction, was primarily observed in trial sites and associated with specific target site mutations (B-T258I, C-N85S, and C-H151R). Notably, resistance to SDHI fungicides in *V. inaequalis* was observed as individual cases and did not show an increase in frequency [57]. Nevertheless, it is important to note that the high intrinsic activity and target specificity of SDHI fungicides significantly raise the risk of resistance development, particularly as newer SDHI fungicides are increasingly relied upon, leading to a reduction in available alternatives for diversifying the chemical fungicide options [61].

2.6. Biological Control

Biological control is gaining increasing attention as an alternative means for plant disease management [76]. The availability of diverse products with different modes of action will play a crucial role in scab control and help mitigate the risk of over-reliance on individual products or synthetic fungicides. There is an urgent need to develop alternative products, specifically by exploring biocontrol options and by making multiple biocontrol products available that target distinct stages of the pathogen life cycle [8].

Table 2. Some common fungicides used to manage apple scab and their modes of action.

Fungicides Group	Chemical Name	Mode of Action	References
Anilinopyrimidine	Cyprodinil, Pyrimethanil	Inhibiting protein synthesis	[70]
Demethylation inhibitors (DMIs)	Imidazoles: triflumizole Triazoles: bitertanol, difenoconazole, fenbuconazole, flusilazole, hexaconazole, myclobutanil, propiconazole, tebuconazole	Inhibit sterol biosynthesis of fungal membranes by binding *Cyp51* gene	[6,71–73]
Dithiocarbamates	Mancozeb, maneb, metiram, propineb, ziram, zineb, maneb	Multi-site inhibitors inhibit fungal growth by disrupting metabolic processes through the release of ethylene-bis-isothiocyanate sulphide (EBIS)	[6,71,74]
Methyl benzimidazole carbamate (MBC)	Benomyl, carbendazim, thiophanate-methyl	Prevents mitosis and cell division in fungi via binding to the β-tubulin gene	[72]
Phenylpyrrole	Fludioxonil	Interferes with the osmolarity glycerol pathway	[70]
Multi-site contact activity: Chloronitriles Guanidines Phthalimides Quinones Inorganic	Chlorothalonil Dodine Captan Dithianon Copper salts and sulphur	Multi-site activity Cell membrane disruption Multi-site inhibitors with activity against thiol groups in proteins and peptides Active against thiol groups in proteins and peptides. Multi-site activity	[6,71] [70,71] [70,71] [70,71] [6,71]
SDHIs	Boscalid, fluopyram, fluxapyroxad, inpyrfluxam and pydiflumetofen	Inhibit fungal respiration by binding to the succinate dehydrogenase (SDH) complex in the mitochondrial electron transport chain	[70,75]
Quinone outside inhibitors (QoIs)	Methoxyacrylates: azoxystrobin Oximino acetates: trifloxystrobin, kresoxim-methyl Methoxycarbamates: pyraclostrobin	Inhibit mitochondrial respiration by binding to the quinone oxidizing site (QoI site) of the cytochrome bc1 enzyme complex	[71,72]

3. Insight into Integration of Biocontrol in Apple Scab Management

The biological control of plant pathogens involves inhibiting the diseases or their causative pathogens using living organisms or their specialized metabolites [76,77]. This encompasses both living and non-living agents, offering environmentally friendly and specific disease management [77]. Basically, biocontrol involves tipping the ecological balance in favour of the introduced agent to hinder pathogen development [3]. By utilizing natural antagonists like bacteria or fungi, biological control serves as an eco-friendly alternative to synthetic fungicides, potentially posing fewer environmental risks.

Integrating biological control agents (BCAs) and their by-products into scab disease management represents a transformative approach in sustainable agriculture [58]. Despite the potential benefits, there has been limited adoption of biocontrol methods for managing apple scab [78]. While BCAs and their products are often compared to fungicides, such fundamental comparisons are unjustified due to the living nature of BCAs and their gradual pathogen-suppressive effects [79]. Numerous studies have revealed that applying BCAs to a crop can achieve disease control levels comparable to or nearly equivalent to those achieved by fungicides [78,80]. Instead of replacing fungicides, it is more appropriate to consider BCAs as a central component of an integrated pest management program [79]. By BCAs-integration with other management approaches, their impact in agriculture can be significantly enhanced [48].

The first approach commonly employed for identifying novel BCAs against *V. inaequalis* involves screening several microbial libraries from the environment that might exhibit antagonistic potential against the pathogen [76]. Subsequently, the selection process may identify microorganisms capable of inhibiting *V. inaequalis* through in vitro studies. Once potential antagonists are identified, understanding the conditions influencing their inhibitory effects becomes crucial, ensuring their effectiveness across diverse environmental conditions [81].

In the initial screening for potential antagonists, in vitro studies are preferred over in vivo studies due to logistical and cost constraints [3]. The advantage of the in vitro approach lies in its ability to efficiently screen a large and diverse collection of strains for their antimicrobial or mycoparasitic activities [76]. This makes in vitro experiments a more manageable method for screening potential BCA candidates [3]. However, as noted by Collinge et al. [76], in vitro screening has limitations and can be misleading, with results not precisely indicating an efficacy on plant level. Notably, Fiss et al. [82] discovered significant antagonistic activity in a yeast strain (H25) against the apple scab pathogen in seedling assays, despite weak initial inhibitory effects in basic in vitro screenings. While the in vivo approach is a compromise, it offers a more realistic assessment under simulated field conditions, enabling a reasonable level of throughput and improved selection of promising BCA candidates [76]. In general, a comprehensive approach that combines both in vitro and in vivo methods might provide a more robust evaluation of BCAs against *V. inaequalis* [83]. This dual strategy leverages the advantages of high-throughput screening in vitro while ensuring selected candidates undergo realistic assessments in simulated field conditions [76,83].

Establishing a reliable and standardized method for assessing BCAs that considers specific stages in the pathogen's life cycle is essential for evaluating their impact on the scab pathogen. Most screening tests directed at traditional criteria like leaf rheology [84,85] and inhibition of mycelium and conidia production [78] have been considered unreliable or impractical [3]. Philion [3] argued that screening techniques targeted at the vegetative phase of *V. inaequalis* are useless because tests concentrated on mycelium inhibition may not conclusively demonstrate efficiency in inhibiting pseudothecia formation. The key criterion suggested for BCAs' selection is the in vitro inhibition of ascospore production [3]. By focusing on the in vitro inhibition of ascospore production, potential antagonistic agents that have the capability to suppress or reduce the formation and release of ascospores by the pathogen have been evaluated [3,16,86,87]. This ascospore inhibition is essential

for disrupting the pathogen's reproductive cycle and reducing its overall population size, ultimately leading to decreased disease severity [3].

Antagonists can alleviate pathogen development through multiple key mechanisms including niche or nutrient competition, parasitism, and antibiosis [58,77]. These mechanisms can act independently or synergistically [58]. While considerable efforts have been dedicated to elucidating the biological activity exhibited by beneficial microorganisms against plant pathogens, recent findings suggest that a synergistic interplay of various molecules, such as hydrolytic enzymes and antibiotics, contributes to their effectiveness [58,80,88–90]. Recognizing the crosstalk between potential antagonists and fungal pathogens highlights the potential for a more informed and rational application of these agents in agriculture [58].

BCAs demonstrate versatile effectiveness in managing plant diseases, both pre- and post-harvest [7]. Particularly, in apple scab control, BCAs strategically applied after harvest and before leaf fall influence the overwintering phase and subsequent pathogen development [91,92]. This tailored approach not only contributes to a comprehensive disease management paradigm throughout the orchard's life cycle but also highlights the remarkable adaptability of BCAs to various crop growth stages [91].

The history of apple scab biocontrol stretches back to several decades, with key contributions from pioneers like Cinq-Mars [93] and Ross [94] who explored microbial control strategies. Cinq-Mars focused on antagonists and culture filtrates, highlighting antibiosis and competition against *V. inaequalis*. Ross expanded these efforts, introducing microbial antagonism against pseudothecial development. Subsequent studies have highlighted the importance of using beneficial antagonists to reduce the overwintering pseudothecia in apple scab control strategies [86,95,96]. Heye [84] identified *Athelia bombacina* Pers. as an effective basidiomycete inhibiting pseudothecia formation. These approaches are preventive and can be seamlessly incorporated into existing management schemes. Nonetheless, integrating all or some of these methods is more intricate than the straightforward use of fungicides.

The effectiveness of BCAs is intricately tied to specific environmental conditions and the unique characteristics of the antagonist [97]. Different agents and target pathogens have unique preferences and dynamics, necessitating the consideration of specific conditions. For instance, Carisse and Bernier [1] indicate that for the optimal efficacy of *Microsphaeropsis* isolates as biocontrol agents, certain conditions such as temperatures between 20 and 25 °C, pH around 4, and a minimum of 8 h of light are favourable. In contrast, Ouimet et al.'s [81] study on *Ophiostoma* sp. demonstrated a high inhibitory effect on *V. inaequalis* (100%), and this antagonist remains effective regardless of temperature (5–25 °C) and pH variations (5–7), adapts to different carbon sources, and is not hindered by light. Therefore, it is essential to consider the specific characteristics of the BCAs and their favourable environmental conditions.

4. BCAs as Sustainable Alternatives

In recent years, there has been a significant increase in the interest in the biological control of plant pathogens [89]. This can be attributed, partially, to public concerns about the use of potentially hazardous chemical plant protection agents (PPAs) [98]. Additionally, biological control is increasingly seen as a viable approach for managing diseases that cannot be fully or adequately controlled by other means [76]. The primary objective of biocontrol is to offer supplementary tools for disease management, featuring modes of action distinct from conventional chemical PPAs [99]. Moreover, the likelihood of farmers adopting new products is higher when these products can be easily incorporated into existing production systems with minimal disruption [100]. By incorporating BCAs into an integrated pest management program, including sanitation, host resistance and reduced fungicide rates, the efficient utilization of available resources can be ensured. This approach not only results in safer production practices, but also maximizes the effectiveness of disease management [101].

Microbial-based biopesticides stand out as prime candidates to replace or complement synthetic PPAs, contributing to the promotion of sustainable agri-food production [89,102].

However, the availability of microbial biofungicides remains limited in comparison to conventional chemical synthetic fungicides [89]. A few biofungicides that have obtained official registration globally are primarily marketed for specific niches, notably high-value crops where there is a demand for PPA-free products [4]. These biofungicides typically comprise a single strain of an antagonist, limiting their effectiveness to a narrow range of diseases and limiting their broader application [4]. Considerable efforts have been made to manage apple scab using microbial biocontrol agents [103–105]. However, despite numerous reports in the literature, there are, so far, no commercially available BCAs specifically designed to control apple scab [17,20,106].

5. Biological Control Using Fungi, Yeasts, and Bacteria against *V. inaequalis*

A diverse range of fungal isolates, yeasts, and bacteria exhibit activity against both the biotrophic and saprophytic phases of *V. inaequalis*. Notably, *Athelia bombacina* [95], *Cladosporium* spp. [103], *Chaetomium globosum* [107], *Coniothyrium* sp. [86], *Ophiostoma* sp. [78], *Microsphaeropsis* sp. [108], *Aureobasidium pullulans* [109], *Trichoderma* and *Streptomyces* spp. [34], *Bacillus subtilis* [110], and *Pseudomonas syringae* [111] have been identified. In Table 3, we compile a summary of various BCAs and their application in scab management over the past two decades. Some of these agents, such as *P. syringae*, have demonstrated complete inhibition of *V. inaqualis* conidial germination in vitro. The observed effects were comparable to those obtained using Captan [111].

A holistic strategy for managing apple scab involves applying a BCA in autumn to disrupt the overwintering structures of the pathogen (i.e., pseudothecia formation) [7]. Several reports demonstrated that lower ascospore numbers were correlated with reduced disease severity in the subsequent spring [7]. This strategy has been comprehensively studied [7,16,93,94,108,112]. Additionally, certain fungal members, such as *A. bombacina* and *Coniothyrium* sp., contribute to leaf decomposition and, thus, interfere with pseudothecia development. These fungi accelerate the breakdown of senescent leaves while also exhibiting antifungal properties [3,112–114]. On the other hand, extensive research has been conducted on the disease management of summer epidemics, driven by conidia produced by the biotrophic mycelia that develop underneath the cuticle of apple leaves [38,82,103,105].

Recently, Caffier et al. [115] explored the potential of using hybridization and host adaptation to limit the impact of *V. inaequalis* on apple trees. *V. inaequalis* also causes scab disease in other Rosaceae hosts such as *Pyracantha* and *Loquat* [115,116]. These authors tested field isolates from different hosts and the progenies resulting from crosses between isolates from *Pyracantha* and apple for pathogenicity. The results revealed a strict host specificity between apple and *Pyracantha* isolates, with most causing disease on Loquat. Progeny resulting from crosses between *V. inaequalis* f.sp. *pyracantha* and *V. inaequalis* f.sp. *pomi* were unable to infect apple. The study suggests a potential biocontrol strategy, similar to sterile insect approaches, involving the introduction of *Pyracantha* isolates to reduce *V. inaequalis* populations in apple orchards without the use of chemicals. This strategy entails introducing *Pyracantha* isolates in autumn to produce hybrid ascospores incapable of causing apple disease in spring, with the aim of reducing the primary inoculum in apple-producing regions. However, experimental evaluation in orchards is necessary, along with an assessment of the risk posed by hybrids that may be pathogenic to apple or other Rosaceae hosts.

Table 3. Summary of notable antagonists and their usage in apple scab management.

BCA	Targeted Structure	Application Type	Assay	Mode of Action	Application Time	Efficacy	Reference
Athelia bombacina	Ascospore	Mycelial suspension	In vivo; applied on shredded scabbed leaves	-	Autumn	Reduced ascospore production up to 82%	[117]
Aureobasidium microstictum	Mycelim	-	In vitro—Cellophane membrane-based method	Antibiosis (VOC)	-	Suppressed *V. inaequalis* growth completely	[41]
Bacillus sp.	Conidia	Cell-free supernatant	Detached leaf assay in vitro; Agar plate test	Antibiosis	-	Growth inhibition up to 81%	[17]
Botrytis cinerea	Mycelia and conidia	Agar plugs and mycelial suspension	In vitro, agar plate Seedlings inoculation	-	-	Inhibited mycelial growth up to 86%; Reduced disease severity	[109]
Chaetomium globosum	Ascospore	Invert emulsion	In vivo; foliar spray on senescent leaves	-	Spring	Inhibited ascospore production up to 79%	[112]
C. globosum	Mycelia	Mycelial suspension	In vitro; cellophane membrane-based method In vivo; seedlings inoculation	Antibiosis	-	Completely inhibited *V. inaequalis* growth; Reduced disease severity	[41]
Cladosporium sp.	Conidia	Conidial suspension	In vivo; foliar spray, field trial	-	Spring and summer	Reduced disease severity up to 74%	[105]
Cladosporium sp. (I PK 14)	Mycelia	Agar plugs Spore suspension	In vitro; agar plate test, Seedlings inoculation	-		Inhibited mycelial growth up to 93%; Reduced scab severity	[109]
Coniochaeta endophytica	Conidia	Conidial suspension	In vivo; seedlings inoculation	-	-	Complete control of apple scab disease	[41]
Gliocladium sp.	Storage scab	Conidial suspension	In vivo; on orchards fruits	-	Late summer (harvest)	Controlled storage scab	[118]
Microsphaeropsis ochracea	Ascospore	Mycelial suspension	In planta; leaf discs and whole infected leaf In vivo; foliar leaf sprayed on tree canopy and ground	-	Autumn	Reduced ascospore production by 84%	[108]
Pantoea sp.	Mycelia	Cell suspension	In vivo, agar plate	-	-	Inhibited mycelial growth	[119]

Table 3. *Cont.*

BCA	Targeted Structure	Application Type	Assay	Mode of Action	Application Time	Efficacy	Reference
Pseudomonas spp.	Conidia	Cell-free supernatants	In vivo; agar plate test In planta; detached leaf assay	Antibiosis	-	Percentage growth inhibition up to 96%; Reduced disease incidence and severity	[17]
Sporidiobolus spp.	Mycelia	1.5×10^7 yeasts suspension per ml	In planta; seedling inoculation In vivo; foliar sprayed on field	Lysis	Spring	Complete suppression in planta; Scab reduction up to 81%	[82]
Trichoderma harzianum	Conidia	Conidial suspension	In vivo; foliar and soil application	-	-	Reduced scab disease incidence;	[120]
	Conidia	Conidial suspension	In vivo; on orchards fruits		Late summer (harvest)	Effective up to 30 days against storage scab	[118]
Trichodrma longibrachiatum	Ascospore	Invert emulsion	In vivo; foliar sprayed on senescent leaves	-	Spring	Inhibited ascospore production by 66%	[112]
Trichoderma viride	Conidia	Mycelial suspension	In planta; seedling inoculation In vivo; foliar spray	Mycopar-asitism	Spring	Prevented disease progression from 80–95%.	[34]

6. Modes of Action of BCAs against *V. inaequalis*

BCAs and plant pathogens can engage in various interactions, employing diverse mechanisms of action. These mechanisms can be broadly categorized into direct antagonism against the pathogen, including parasitism, antibiosis, and competition; and indirect biocontrol activities, such as the induction of systemic resistance and promotion of plant growth [77,89]. In many instances, the effective control of disease by BCAs is attributed to their utilization of multiple mechanisms. For instance, *T. harzianum* has been demonstrated to suppress various plant pathogens by employing a combination of mechanisms including antibiosis, cell wall degrading enzymes, and the induction of host resistance [77,121]. The viability of biological control for disease reduction relies on understanding its mechanisms. This knowledge is crucial for creating conditions supporting treatment efficacy. Identifying how biocontrol agents suppress a pathogen helps anticipate resistance and prevent interference from other ecosystem components [88].

6.1. Indirect Biocontrol Activities against V. inaequalis

A commonly observed mechanism associated with the protection of plants by BCAs involves the induction of host defence pathways [80]. Two main types of induced resistance exist: systemic acquired resistance (SAR), activated by pathogen infection and requiring salicylic acid, and induced systemic resistance (ISR), triggered by beneficial microorganisms [122]. This mechanism stems from BCAs releasing elicitors, such as proteins, antibiotics, and volatiles, ultimately leading to the activation of genes in the salicylic acid pathway or the jasmonic acid/ethylene pathway [80]. Bolar et al. [123] integrated genes encoding antifungal proteins, endochitinase, or exochitinase from the biocontrol fungus *T. atroviride* into 'Marshall McIntosh' apple plants. The resulting plants were evaluated for resistance to *V. inaequalis*. The study found that disease resistance correlated with the expression level of each enzyme when expressed individually, with endochitinase proving more effective than exochitinase. The persistent expression of fungal chitinases in apple lines was associated with enhanced resistance against *V. inaequalis*. Particularly, a significant positive correlation was observed between the level of endochitinase expression and scab resistance.

On the other hand, various other compounds have been investigated as defence elicitors to enhance disease management and resistance against apple scab (Table 4) [38] including chitosan [124] and fructans [38]. Plant defence inducers, including harpins, and salicylic acid derivatives demonstrated efficacy against *V. inequalis* under field conditions [125,126]. While these inducers showed a reduction in leaf and fruit scab when applied at different growth stages, their efficacy was markedly enhanced when used in combination with fungicides (Boscalid + Pyraclostrobin). In addition, Rusevski et al. [54] explored the biofungicidal potential of Vacciplant, a biofungicide containing laminarin as its active ingredient, for the biological control of apple scab. Laminarin, derived from brown seaweed, is a polysaccharide recognized for its antifungal and defence-inducing properties [126]. The study revealed comparable efficacy to the standard fungicide Captan, showing promising results in decreasing infection levels on the leaves and fruits of two apple varieties.

6.2. Direct Biocontrol Activities against V. inaequalis

6.2.1. Competition

Competition in the context of biological control refers to niche overlap, where two or more microbial populations simultaneously demand the same resources [127]. Competition is a widely utilized mechanism in biocontrol. It manifests in various ways, including reducing inoculum potential through nutrient competition, increasing saprotrophic competition during substrate colonization, and decreasing the actual pathogen density during survival or growth phases [127].

Table 4. Commercially available biopesticides and their actions against apple scab.

Biopesticide	Commercial Name	Group/a.i. *	Efficacy	Defence Inducer	Targeted Propagule	Application Type	Country	Ref.
Chitosan	ARMOUR-Zen 15	Polysaccharide	Low efficacy	-	Ascospore	Foliar spray	Botry-Zen Ltd. (Dunedin, New Zealand)	[124]
Chitosan	-	Polysaccharide	Active on leaf scab	Inducer	Conidia	Foliar spray	Viresco Ltd., (Thirsk, UK)	[125]
Fructans (Levans)	-	Fructose-based oligo- and polysaccharide	Active	Inducer	Mycelial growth	Foliar spray	-	[38]
Harpin protein	Messenger®	Harpin αβ protein	Active on leaf and fruit scab	Inducer	Conidia	Foliar spray	Plant Health Care, (Manchester, England	[125]
Laminarin	Vacciplant®	oligosaccharide	Active	Inducer	Conidia	Foliar spray	-	[54]
Salicylic acid	Rigel-G	Salicylic acid	Active on leaf and fruit scab	Inducer	Conidia	Foliar spray	Orion, Future Tech., (Colchester, England)	[125]
Serenade ASO	-	*B. subtilis* QST 713	Active on scab	-	Conidia and ascospore	Foliar spray	Bayer AG (Leverkusen, Germany)	[124]
SERENADE Garden		*B. subtilis* QST 713	Active on scab	-	Scab	Foliar spray	AgraQuest, Inc., (British Columbia, Canada)	[6]

* a.i. = Active ingredient.

BCAs utilize strategies like sequestering iron through the production of iron-binding siderophores, aiming to reduce the availability of iron to other organisms [80]. This is particularly significant in the context of the effectiveness of biological control against pathogenic fungi, as these fungi heavily rely on iron (Fe^{3+}) for their growth and virulence [128]. In their study, Miliute et al. [119] found that sterile culture filtrates from *Pantoea* species inhibited the growth of *V. inaequalis* and produced siderophores. They attributed the inhibition of *V. inaequalis* mycelial growth to these biochemical traits. Padder et al. [17] also attributed the mechanism of induced iron starvation as one of the possible reasons for the antifungal behaviour of *Bacillus* and *Pseudomonas* sp. against *V. inaequalis*. They also observed that iron chelation through this mechanism does not impact plant development, as plants can grow at significantly lower iron levels than the invading pathogenic microflora. While competition for nutrients and space plays a major role in biocontrol, appropriate methods are lacking to separate these two mechanisms of action [127].

6.2.2. Mycoparasitism

Mycoparasitism involves a series of steps in microbial interaction, including close contact with the pathogen, mutual recognition, the release of lytic enzymes, penetration, and active development inside the host [128]. Through mechanisms like haustoria formation or invading the pathogen's mycelium, mycoparasites absorb nutrients from the host for their survival and growth [106]. While mycoparasites weaken the host gradually, they do not completely eradicate it. Applying mycoparasites when the pathogen is first detected is crucial, as these biocontrol agents depend on a host for their survival [106].

Compared to synthetic chemical fungicides, mycoparasites exhibit a slower onset of action and require sufficient time to colonize and eliminate the pathogen. Consequently, when rapid action is imperative, biofungicides employing direct antibiosis should be prioritized [106].

Benyagoub et al. [58] offer valuable insights into the cellular mechanisms of mycoparasitism, specifically focusing on the interactions between the antagonistic fungus *Microsphaeropsis* sp. and the apple pathogen *V. inaequalis*. Through ultrastructural and cytochemical analyses, they showed a sequence of events in the mycoparasitic process, including attachment, penetration, fungal host structural response, and active multiplication of the mycoparasite. The antagonist, *Microsphaeropsis* sp., penetrated *V. inaequalis* hyphae by disrupting the osmiophilic coating layer. This disruption likely caused significant metabolic changes, exposing the underlying wall layers to mechanical pressure and potential enzymatic attack.

6.2.3. Antibiosis

Antibiosis stands out as the most extensively studied mechanism, as it provides a direct means for evaluating candidates based on their inhibitory activity [78]. Antibiosis is specifically characterized by the secretion of volatile and/or non-volatile products that inhibit or restrict the growth of the target pathogen [81]. It constitutes a chemically diverse group of organic, low-molecular-weight compounds synthesized by microorganisms [90]. Over the past decades, a multitude of studies has unequivocally shown that various metabolites, including antibiotics, enzymes, proteins, and volatiles produced by antagonistic bacteria and fungi, play crucial roles in controlling a wide array of plant pathogens [90]. The identification of specific secondary metabolites considered potential biological control agents against *V. inaequalis* has roots dating back several decades.

Early research led by Cinq-Mars [93] involved isolating microorganisms from apple leaves. Among these, *Penicillium* species, especially, demonstrated the production of antibiotics inhibiting *V. inaequalis* mycelial growth. In 1953, Ross [94] isolated 13 saprophytic fungi from apple leaf litter. These fungi were found to produce antibiotic substances in liquid culture, leading to complete or partial inhibition of *V. inaequalis* mycelial growth. Notably, only *Oospora lactis* and *Penicillium* species exhibited the secretion of toxic metabolites with activity against the pathogen. However, their fungitoxic effects were found to

be lower than that of the organo-mercurial fungicide ('Tag') used for comparison. Later, Simard et al. [129] evaluated some fungal isolates for their antibiotic properties against *V. inaequalis*. Among the organisms isolated from apple leaf litter, 34 were identified to impede the germination of conidia, as determined by the sprayed-plate test. Remarkably, twelve of these isolates belonged to the genus *Penicillium*, consistent with the findings previously reported by Cinq-Mars [93]. In 1984, Cullen and Andrews [130] investigated the potential of *Chaetomium globosum* and *Athelia bombacina* for apple scab control, revealing that *C. globosum* produced essential antibiotics. Antibiotic production varied among *C. globosum* strains, with higher levels positively correlating with increased antagonism against *V. inaequalis* on seedlings. Chetomin, tentatively identified in culture extracts, was observed using thin-layer chromatography and ultraviolet absorbance. In 1997, Ouimet et al. [78] identified *Aureobasidium* sp., *Phoma* sp., and *Ophiostoma* sp. as strong inhibitors, suppressing over 80% of *V. inaequalis* mycelial growth. Meanwhile, *Ophiostoma* sp. exhibited a prolonged inhibitory effect lasting beyond 58 days. However, the impact of metabolites released by the fungi results in an inhibitory effect of less than 5%. Recently, Padder et al. [17] isolated and characterized bacterial endophytes with antifungal properties from apple germplasm. They reported a 100% suppression of scab disease using bacterial cell-free supernatants. The observed impact on disease severity and incidence at all concentrations was linked to the presence of antimicrobial compounds, including but not limited to phenazines, ammonia, pyrrolnitrin, hydrocyanic acid, and pyoluterorin. In their study, Ebrahimi et al. [41]. investigated the antifungal properties of various endophytic fungi against *V. inaequalis* in vitro. The study aimed to determine the release of metabolites from *Aureobasidium* sp. and *C. globosum*. Notably, the volatile organic compounds produced by specific isolates, including *A. microstictum* 7F2 and *C. globosum* 2S1, along with others, were found to completely prevent the mycelial growth of *V. inaequalis*.

Desmyttere et al. [131] conducted a comprehensive investigation into the antifungal properties of *B. subtilis* lipopeptides against *V. inaequalis*. Fengycin displayed potent antifungal activity comparable to the chemical fungicide tebuconazole, particularly against sensitive strains. Surfactin and mycosubtilin, either alone or in combinations, displayed varying effectiveness. In a similar study, Leconte et al. [110] explored the efficacy of *B. subtilis* lipopeptides in combating apple scab. Their in vitro tests on three lipopeptide families and their mixtures revealed varying levels of antagonistic activity against *V. inaequalis*. Notably, Fengycin exhibited significant inhibition among the three. Orchards trials revealed a reduction in scab incidence by 70%, with the mycosubtilin/surfactin mixture demonstrating better reproducibility. Combining these potent antifungal molecules in orchard treatments against *V. inaequalis* is likely to result in a synergistic effect against the pathogen [110].

Considering chitin as a main component of fungal cell wall, several studies have particularly focused on identifying antagonistic BCAs with chitinolytic properties [123,132–135]. This interest has led to the exploration of diverse chitinolytic microorganisms as potential biological control agents effective against various fungal plant pathogens [90,119,123]. Taking a genetic approach, Bolar et al. [132] sought to enhance apple resistance against apple scab by introducing genes from *Trichoderma harzianum* encoding endochitinase and exochitinase. The simultaneous expression of these enzymes resulted in a synergistic reduction in disease severity, with minimal effects on plant vigour. Later, Miliute et al. [119] extended the exploration by identifying chitinase-producing endophytes from apple plants, specifically *Pantoea* species and the *Pseudomonas fluorescens* group. These endophytes exhibited the ability to inhibit the mycelial growth of *V. inaequalis.* Three isolates of *Pantoea* spp. demonstrated inhibitory effects, with one exhibiting additional trait, including siderophore production and hydrogen cyanide generation.

Chitosan, a deacetylated derivative of chitin, has emerged as a particularly promising compound due to its well-documented antifungal activity [124,125,136,137]. The potential synergies resulting from combining chitosan with BCAs was investigated by DeGenring et al. [124]. They found that chitosan, when applied pre-harvest, reduced the incidence and

severity of apple scab, sooty blotch, flyspeck, and rust. The combination of chitosan with a biopesticide was even more effective in disease suppression.

In line with the ongoing efforts to advance scab management strategies, Hossain et al. [138] explored the inhibitory potentials of proteins from *P. fluorescens* Bk3. Their research revealed substantial inhibition in bacterial cell suspension (73%) and extracellular proteins (100%). Key proteins, such as solute-binding protein, alkaline metalloprotease, and peptidoglycan-associated lipoprotein, exhibited inhibitory effects ranging from 20% to 42%.

7. Biological Control of Apple Scab Using Botanicals

Plant extracts offer promising alternatives for pest and pathogen control [139,140]. They have long been utilized as natural pesticides due to their cost-effectiveness, ease of preparation, and eco-friendly properties with no resistance risks [141]. Plant extracts contain low-molecular-weight secondary metabolites, produced in response to stress, that serve as potential alternatives for controlling apple scab without harm to non-target organisms [139,142]. Various promising plant extracts have demonstrated effective control against apple scab (Table 5). Bengtsson et al. [140] investigated the impact of *Yucca schidigera* extract in controlling *V. inaequalis*, comparing it with the chemical resistance inducer acibenzolar-S-methyl (ASM). Yucca extract and ASM significantly reduced apple scab symptoms and sporulation in seedling assays, exhibiting similar control efficacy to sulphur. Yucca extract and sulphur inhibited conidial germination in vitro, while ASM did not. Yucca extract inhibited conidial germination by 98% to 100%, sulphur exhibited inhibition ranging from 72% to 97%, and ASM showed inhibition of 5% to 25%. Yucca extract primarily acted fungitoxically, inhibiting pre-penetration and penetration, while ASM hindered the subsequent infection stages. Gene expression studies on apple seedlings suggested that Yucca extract may influence plant defence, with the upregulation of *PR1* and *PR8* genes resembling levels observed after ASM treatment. Additionally, essential oils like those from *Thymus vulgaris* and other extracts are well-suited for addressing plant pathogen control, including apple scab, owing to their antimicrobial and antifungal properties [143,144]. However, the effectiveness of extracts and essential oils may vary under different circumstances, especially in field conditions. Therefore, a well-developed formulation is crucial to maintaining consistency and ensuring reliable performance during field trials [144,145].

Table 5. Some botanicals and their actions against apple scab.

Plant Extracts	Source	Solvent	Targeted Propagule	Assay Type	Efficacy	Active Components	Ref.
Artemisia, Mentha and Thyme extracts	*Artemisia annua* *Mentha piperita* *Thymus vulgaris*	Hexane	Ascospore	In vitro; ascospore infected floral buds in petri dishes	Reduced the ascosporic inocula between 85 and 90% at 6% conc.	Artemisinin Menthol Thymol	[146]
Essential oils of Thyme and Cinnamon	*T. vulgaris* *Cinnamonum verum*	-	Conidia	In vivo; field trial	Reduced disease severity	Thymol -	[143]
Hop cone and leaf extract	Hop plant	Hydro- ethanolic dichloromethane	Conidia	In vitro; laboratory study, liquid medium	Significant activity against two strains with IC_{50} of 1.6 and 5.1 mg L^{-1}	Xanthohumol	[147]
Juncus effusus extract	Medulla of *J. effusus*	Ethyl acetate	Conidia	In planta; greenhouse and growth chambers	95% disease control at 500 µg mL^{-1}	Dihydrophena- nthrene dehydroeffuso	[145]
Magnolia officinalis bark extract	*M. officinalis*	Ethyl acetate	Conidia	Seedling assay	97% efficacy at 1 mg extract mL^{-1}	Honokiol and Magnolol	[148]
Morinda royoc crude extract	*Morinda royoc* roots	Ethanol	Conidia	In vitro; Agar plate test	Complete inhibition of conidial growth at 4.8 to 0.3 mg mL^{-1}	-	[149]
Morus root bark	*Morus* sp.	Methanol	Conidia	In vitro; glass slide and microscopy detached leaf assay	100% germination inhibition at 300 µg/mL. Antifungal efficacy of 98% at 10.0 mg/mL	Diels-Alder adducts	[142]
Populin	Black popular buds	Hexane	Conidia	In vitro; agar plate test In vivo; foliar spray	Slow down conidia growth Reduced scab severity	Populin	[139]
Saponin	Fruit pericarp of *Sapindus mukorosis*	Aqueous extract and chloroform-methanol	Conidia	In vitro; greenhouse and field trials	Reduced sporulation by 43% seedlings symptom reduction up to 99%	Sapindoside B	[150]
Yucca schidigera extract	*Y. schidigera* (Norponin® BS Liquid)	-	Conidia	In vitro; glass slide and microscopy In planta; seedling assay	No conidia germinated significantly reduced apple scab	Saponin	[140]

8. Risks and Challenges of Utilizing BCAs in Apple Scab Management

Introducing new microorganisms to leaf and fruit surfaces, while common, presents potential risks and both positive and negative outcomes [97]. The complex interactions between these introduced microorganisms and the existing balance in the plant environment need careful consideration due to the potential for unexpected effects [151,152].

Concerns arise from the potential misidentification of microorganisms during screening processes, leading to the inadvertent selection of harmful strains, including those that may pose threats to both plants and humans [77]. In the context of commercialization, screening for BCAs presents a substantial challenge, as it involves identifying organisms suitable for commercial development while ensuring the exclusion of potential pathogens [3]. Instances of initially identified BCAs turning out to be possible human pathogens highlight the need for accurate identification to mitigate risks [77]. *Trichothecium roseum* Link. have demonstrated pathogenic traits under certain conditions, affecting both apple orchards and other plant species [3]. Effectively navigating this challenge is crucial for ensuring that selected BCAs not only prove effective in pest management but also pose minimal risks to agriculture, the environment, and human health when deployed on a commercial scale [76,77]. The integration of genomic sequencing has emerged as a possible solution [77], enhancing the identification process and thereby minimizing the potential hazards associated with introducing new microorganisms into diverse ecosystems.

Another associated risk with BCAs is the production of harmful metabolites or mycotoxins [3,76]. A case in point is the ascomycete *C. globosum*, which initially showed promise in effectively controlling *V. inaequalis* within the phyllosphere. However, despite its efficacy, the production of toxins including chaetomin led to its eventual abandonment as a commercially viable BCA [76,130,153]. This case highlights the delicate balance required in selecting BCAs, emphasizing the necessity of a thorough evaluation not only for their efficacy in pest control but also for their potential to produce harmful substances that could have adverse consequences on both the target species and the surrounding environment [76]. This risk calls for a comprehensive understanding of the metabolic pathways and secondary metabolite profiles of potential BCAs to ensure their safety and effectiveness in practical applications.

Furthermore, establishing a reliable method for assessing BCAs, particularly in the context of apple scab, is complicated by difficulties in the in vitro production of fungal asexual and sexual structures (e.g., conidia or pseudothecia) [3]. The difficulty in replicating these structures outside their natural environment hampers the precision of BCA evaluations. In response to this challenge, alternative parameters, with mycelium being one such focus, have been explored [3]. However, relying on mycelium alone in evaluating the efficacy may potentially lead to misleading conclusions [3]. This emphasizes the continual need to refine and diversify evaluation methods for a comprehensive understanding of BCAs' efficacy and potential against *V. inaequalis*.

The effectiveness of specific BCAs against plant pathogens can be influenced by various factors, including environmental conditions, the timing of treatment, the season of application, the nature or method of treatment, and the frequency of application [1,81,154]. BCAs in orchards encounter challenges in colonizing apple leaves under cold conditions, adapting to industrial production and employing multiple modes of action against *V. inaequalis* [1,2]. The efficacy of BCAs during colder periods, particularly in the autumn, represents a substantial hurdle, demanding the development of strategies to enhance their effectiveness under these challenging conditions [3,7]. In colder climates characterized by harsh winter conditions, the conidia and mycelia of *V. inaequalis* cannot withstand the low temperatures and freezing [155–157]. Consequently, *V. inaequalis* overwinters within scabbed leaves as pseudothecia [155]. The ability of BCAs to establish a robust presence on apple leaves in cold climates is crucial for their success in scab management, necessitating innovative approaches to address these specific environmental challenges [158].

On the other hand, biological products encounter challenges in achieving widespread adoption due to inconsistencies in their performance observed in both in vitro and in vivo

assays [159]. Ouimet et al. [78] explored the effect of fungal antagonists on the in vitro inhibition of mycelial growth of *V. inaequalis* and found that *Ophiostoma* sp. emerged as a potent inhibitor and completely prevented mycelial growth across various environmental conditions, including temperature, pH, and light variations. The same BCA reduced ascospores production up to 88.7% in a single trial [86]. However, subsequent trials conducted by Carisse et al. [108] presented a contrasting outcome. Despite the recorded successes of the inhibitory potential of this antagonist, the *Ophiostoma* sp. isolate showed a notable failure to inhibit ascospore production. Across all tests, the average ascospore inhibition was merely 8.2%. Additionally, the slow commercial adoption of BCAs for plant disease management also stems from issues related to host specificity [154] as exemplified by the study on *Epicoccum purpurascens* and *T. roseum* [3]. Despite their reported antagonistic activities in other plant-pathogen systems, these fungi showed no antagonistic ability against apple scab in the specific conditions of the study. This emphasizes the importance of understanding the context-specific interactions between BCAs and their target pathogens to ensure their effectiveness in practical applications.

To fully harness the potential of biocontrol, a shift towards comprehensive, field-oriented research and the optimization of commercial formulations is essential [77]. Regrettably, the transition from laboratory efficacy to real-world field success remains a challenge for many biological agents [128,154]. The discrepancy in effectiveness is often attributed to the limitations imposed by physiological and ecological factors inherent in field conditions [128]. Genetic engineering and molecular methods offer new ways to improve biocontrol agent selection and evaluation [128]. Manipulating the genetic makeup of these agents optimizes their performance, ensuring a more seamless transition from controlled laboratory conditions to the complex and dynamic environments found in actual fields [128,160]. These molecular approaches provide a valuable toolset to finetune the characteristics of biocontrol agents, potentially overcoming the hurdles posed by physiological and ecological factors that hinder their success in real-world applications.

Another significant challenge with BCAs is ensuring consistent efficacy and addressing limited shelf life, attributed to the variability of BCAs and external environmental factors [77]. Köhl et al. [103] identified *Cladosporium cladosporioides* (H39) as a promising biocontrol agent, demonstrating significant reduction of *V. inaequalis* sporulation in orchard conditions. However, challenges in maintaining efficacy and shelf life hindered commercial success [104]. To address these challenges, there is a critical need to advance the development of new formulations of BCAs that boast a higher degree of stability, efficiency, and survival [89,128,161,162]. This necessitates the integration of novel biotechnological practices to enhance the overall performance and reliability of BCAs in practical agricultural applications [154]. Fenta et al. [128] suggested the development of both dry and liquid formulations to enhance the efficacy of biocontrol and extending its shelf life. These formulations offer practical solutions for the challenges faced in commercial applications, ensuring that biocontrol agents remain effective and viable under diverse packaging conditions. By leveraging cutting-edge biotechnological approaches, working towards creating formulations that are more resilient, adaptable, and effective across a broader range of environmental conditions can be achieved [89,154,161]. This proactive approach is crucial for unlocking the full potential of biological control in plant disease management, ensuring that BCAs can provide consistent and reliable results in various agricultural settings [154,162]. As the field of biotechnology continues to evolve, it partly holds the key to overcome the challenges hindering the widespread commercial use of BCAs and establishing them as integral components of sustainable agriculture [89,128,162].

The commercial viability of microbial BCAs hinges on their efficacy at defined doses, adherence to industrial production standards, and practical benefits for growers [7]. The upscaling of a specific BCA to the commercialization stage is an expensive and multi-step process [154]. Initial steps include the isolation of the microorganism in pure culture or its enrichment, followed by accurate identification and characterization processes. Subsequent stages involve the development of a suitable formulation, mass production, efficacy testing

of the product, inspection of storage stability, registration of the product, and subsequent marketing efforts to make the BCA available to end-users [154]. The complex nature of these processes underlines the significant challenges and resource investments associated with bringing a BCA from research and development stages to practical, commercially viable application [76]. For instance, *Microsphaeropsis ochracea* demonstrated a robust 80–90% efficacy in reducing ascospore production in the field, making it a promising biofungicide [163]. However, challenges related to commercialization, including significant time and financial investments, coupled with grower acceptance, have impeded its progress into widespread use [164]. Despite extensive research and numerous reports on the biological control of plant pathogens, the global availability of registered biofungicides remains rather limited [4,89,128,165]. The majority of these biofungicides are commercialized for specific niches, particularly in high-value crops where there is a demand for pesticide-free products [4]. This targeted commercialization reflects the challenges associated with scaling up and registering biofungicides for broader agricultural applications.

9. Future Trends and Conclusions

In the forthcoming years, the outlook for biological control in apple scab management is highly promising. Ongoing advancements in research tools, such as next-generation sequencing and functional genomics, are providing detailed insights into the specialized metabolic pathways of potential fungal and bacterial antagonists. This deeper understanding opens new avenues for the development of more potent BCAs against *V. inaequalis*. The evolution of the omics approach holds great potential for integrating biocontrol into apple scab disease management. A comprehensive understanding of the molecular processes underlying the biocontrol activity of BCAs against plant pathogens lays the groundwork for subsequent experiments using functional genetics approaches.

Generally, addressing challenges related to adoption, susceptibility to environmental factors, and enhancing the efficacy and persistence of microbial biocontrol agents is imperative for sustainable disease management. Overcoming these challenges requires united efforts to develop innovative strategies and bridge existing gaps in our understanding. Genetic engineering and genomic sequencing offer avenues to enhance the specificity and effectiveness of BCAs. By identifying and modifying key genes or traits, BCAs can be customized to more effectively counter specific pathogens, withstand adverse environmental conditions, and improve their overall performance. Moreover, a deeper understanding of the ecological dynamics within orchard ecosystems can guide the development of strategies to optimize the application and persistence of BCAs under varying conditions. Integrating this knowledge with innovative biotechnological approaches holds promise for creating more adaptable BCAs that can survive in adverse orchard environments.

In conclusion, despite extensive research on microbial antagonists, the formulation of BCAs for managing apple scab epidemics remains elusive. Additional research and innovative strategies are essential to fill these gaps and enhance our understanding, ultimately paving the way for the successful management of scab outbreaks. The primary goal of biocontrol research is to provide supplementary tools for disease management and the successful integration of BCAs into existing production systems. Moving forward, continued research and collaborative efforts will be key to realizing the full potential of biocontrol in apple scab management.

Funding: Chisom. A. Okoro was supported by Deutscher Akademischer Austauschdienst (DAAD), under funding number 57552340.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

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

References

1. Carisse, O.; Bernier, J. Effect of Environmental Factors on Growth, Pycnidial Production and Spore Germination of *Microsphaeropsis* Isolates with Biocontrol Potential against Apple Scab. *Mycol. Res.* **2002**, *106*, 1455–1462. [CrossRef]
2. MacHardy, W.E.; Gadoury, D.M.; Gessler, C. Parasitic and Biological Fitness of *Venturia inaequalis*: Relationship to Disease Management Strategies. *Plant Dis.* **2001**, *85*, 1036–1051. [CrossRef] [PubMed]
3. Philion, V. *The Screening of Potential Fungal Antagonists of Pseudothecial Formation by the Apple Scab Pathogen Venturia inaequalis*; National Library of Canada = Bibliothèque Nationale du Canada: Ottawa, ON, Canada, 1996; ISBN 978-0-612-05615-2.
4. Majeed, M.; Bhat, N.A.; Badri, Z.A.; Yousuf, V.; Wani, T.A.; Hassan, M.; Saleem, M.; Dorjey, S.; Paswal, S. Non-Chemical Management of Apple Scab-A Global Perspective. *Int. J. Environ. Agric. Biotechnol.* **2017**, *2*, 912–921. [CrossRef]
5. Nu, A.; Rani, R.; Sharma, J.R. Studies on Biology and Management of Apple Scab Incited by *Venturia inaequalis*. *Int. J. Curr. Microbiol. Appl. Sci.* **2019**, *8*, 162–182. [CrossRef]
6. Belete, T.; Boyraz, N. Critical Review on Apple Scab (*Venturia inaequalis*) Biology, Epidemiology, Economic Importance, Management and Defense Mechanisms to the Causal Agent. *J. Plant Physiol. Pathol.* **2017**, *5*, 2. [CrossRef]
7. Carisse, O.; Dewdney, M. A Review of Non-Fungicidal Approaches for the Control of Apple Scab. *Phytoprotection* **2005**, *83*, 1–29. [CrossRef]
8. Xu, X.; Harvey, N.; Roberts, A.; Barbara, D. Population Variation of Apple Scab (*Venturia inaequalis*) within Mixed Orchards in the UK. *Eur. J. Plant Pathol.* **2013**, *135*, 97–104. [CrossRef]
9. Bowen, J.K.; Mesarich, C.H.; Bus, V.G.M.; Beresford, R.M.; Plummer, K.M.; Templeton, M.D. *Venturia inaequalis*: The Causal Agent of Apple Scab. *Mol. Plant Pathol.* **2011**, *12*, 105–122. [CrossRef]
10. Shiller, J.; Van De Wouw, A.P.; Taranto, A.P.; Bowen, J.K.; Dubois, D.; Robinson, A.; Deng, C.H.; Plummer, K.M. A Large Family of AvrLm6-like Genes in the Apple and Pear Scab Pathogens, *Venturia inaequalis* and *Venturia pirina*. *Front. Plant Sci.* **2015**, *6*, 165188. [CrossRef] [PubMed]
11. Khajuria, Y.P.; Akhoon, B.A.; Kaul, S.; Dhar, M.K. Avirulence (Avr) Genes in Fungal Pathogen *Venturia inaequalis*, a Causal Agent of Scab Disease on Apple Trees. *Physiol. Mol. Plant Pathol.* **2023**, *127*, 102101. [CrossRef]
12. Thakur, K.; Chawla, V.; Bhatti, S.; Swarnkar, M.K.; Kaur, J.; Shankar, R.; Jha, G. De Novo Transcriptome Sequencing and Analysis for *Venturia inaequalis*, the Devastating Apple Scab Pathogen. *PLoS ONE* **2013**, *8*, e53937. [CrossRef]
13. Gauthier, N. Apple Scab. In *The Plant Health Instructor*; University of Kentucky: Lexington, UK, 2018. [CrossRef]
14. Kollar, A. Evidence for Loss of Ontogenetic Resistance of Apple Leaves against *Venturia inaequalis*. *Eur. J. Plant Pathol.* **1996**, *102*, 773–778. [CrossRef]
15. Zajicova, I.; Tihlarikova, E.; Cifrova, P.; Kyjakova, P.; Nedela, V.; Sechet, J.; Havelkova, L.; Kloutvorova, J.; Schwarzerova, K. Analysis of Apple Epidermis in Respect to Ontogenic Resistance against *Venturia inaequalis*. *Biol. Plant.* **2019**, *63*, 662–670. [CrossRef]
16. Porsche, F.M.; Pfeiffer, B.; Kollar, A. A New Phytosanitary Method to Reduce the Ascospore Potential of *Venturia inaequalis*. *Plant Dis.* **2017**, *101*, 414–420. [CrossRef] [PubMed]
17. Padder, S.A.; Mansoor, S.; Bhat, S.A.; Baba, T.R.; Rather, R.A.; Wani, S.M.; Popescu, S.M.; Sofi, S.; Aziz, M.A.; Hefft, D.I.; et al. Bacterial Endophyte Community Dynamics in Apple (*Malus domestica*) Germplasm and Their Evaluation for Scab Management Strategies. *J. Fungi* **2021**, *7*, 923. [CrossRef] [PubMed]
18. Schwabe, W.F.S. Wetting and Temperature Requirements for Infection of Mature Apples by *Venturia inaequalis* in South Africa. *Ann. Appl. Biol.* **1982**, *100*, 415–423. [CrossRef]
19. Blaise, P.; Gessler, C. Cultivar Mixtures in Apple Orchards as a Means to Control Apple Scab? *Nor. J. Agric. Sci.* **1994**, *17*, 105–122.
20. Stewart, K.; Passey, T.; Verheecke-Vaessen, C.; Kevei, Z.; Xu, X. Is It Feasible to Use Mixed Orchards to Manage Apple Scab? *Fruit Res.* **2023**, *3*, 28. [CrossRef]
21. Chizzali, C.; Gusberti, M.; Schouten, H.J.; Gessler, C.; Broggini, G.A.L. Cisgenic *Rvi6* Scab-Resistant Apple Lines Show No Differences in *Rvi6* Transcription When Compared with Conventionally Bred Cultivars. *Planta* **2016**, *243*, 635–644. [CrossRef]
22. Kaymak, S.; Kaçal, E.; Öztürk, Y. Screening Breeding Apple Progenies with vf Apple Scab (*Venturia inaequalis* (Cke.) Wint.) Disease Resistance Gene Specific Molecular Markers. *Integr. Prot. Fruit Crops IOBC-WPRS Bull.* **2013**, *91*, 361–365.
23. Papp, D.; Király, I.; Tóth, M. Suitability of Old Apple Varieties in Organic Farming, Based on Their Resistance against Apple Scab and Powdery mildew. *Org. Agric.* **2016**, *6*, 183–189. [CrossRef]
24. Papp, D.; Singh, J.; Gadoury, D.; Khan, A. New North American Isolates of *Venturia inaequalis* Can Overcome Apple Scab Resistance of *Malus floribunda*. 821. *Plant Dis.* **2020**, *104*, 649–655. [CrossRef]
25. Jha, G.; Thakur, K.; Thakur, P. The *Venturia* Apple Pathosystem: Pathogenicity Mechanisms and Plant Defense Responses. *J. Biomed. Biotechnol.* **2009**, *2009*, 680160. [CrossRef] [PubMed]
26. Sokolova, O.; Moročko-Bičevska, I. Evaluation of Apple Scab and Occurrence of *Venturia inaequalis* Races on Differential *Malus* Genotypes in Latvia. *Proc. Latv. Acad. Sci. Sect. B Nat. Exact Appl. Sci.* **2022**, *76*, 488–494. [CrossRef]
27. Papp, D.; Gao, L.; Thapa, R.; Olmstead, D.; Khan, A. Field Apple Scab Susceptibility of a Diverse *Malus* Germplasm Collection Identifies Potential Sources of Resistance for Apple Breeding. *CABI Agric. Biosci.* **2020**, *1*, 16. [CrossRef]
28. Patocchi, A.; Wehrli, A.; Dubuis, P.-H.; Auwerkerken, A.; Leida, C.; Cipriani, G.; Passey, T.; Staples, M.; Didelot, F.; Philion, V.; et al. Ten Years of VINQUEST: First Insight for Breeding New Apple Cultivars With Durable Apple Scab Resistance. *Plant Dis.* **2020**, *104*, 2074–2081. [CrossRef]

29. Soriano, J.M.; Madduri, M.; Schaart, J.G.; Van Der Burgh, A.; Van Kaauwen, M.P.W.; Tomic, L.; Groenwold, R.; Velasco, R.; Van De Weg, E.; Schouten, H.J. Fine Mapping of the Gene *Rvi18*(V25) for Broad-Spectrum Resistance to Apple Scab, and Development of a Linked SSR Marker Suitable for Marker-Assisted Breeding. *Mol. Breed.* **2014**, *34*, 2021–2032. [CrossRef]
30. Kellerhals, M.; Baumgartner, I.O.; Schütz, S.; Lussi, L.; Andreoli, R.; Gassmann, J.; Patocchi, A. Approaches in Breeding High-Quality Apples with Durable Disease Resistance. In Proceedings of the Ecofruit, 17th International Conference on Organic-Fruit Growing, Hohenheim, Germany, 15–17 February 2016; pp. 15–17.
31. Papp, D.; Gangadharappa Harigondra, S.; Paredes, C.; Karacs-Végh, A.; Penksza, K.; T.-Járdi, I.; Papp, V. Strong Genetic Differentiation between Generalist Populations of *Venturia inaequalis* and Populations from Partially Resistant Apple Cultivars Carrying *Rvi3* or *Rvi5*. *Diversity* **2022**, *14*, 1050. [CrossRef]
32. Švara, A.; Ilnikar, K.; Carpentier, S.; De Storme, N.; De Coninck, B.; Keulemans, W. Polyploidy Affects the Development of *Venturia inaequalis* in Scab-Resistant and -Susceptible Apple Cultivars. *Sci. Hortic.* **2021**, *290*, 110436. [CrossRef]
33. Höfer, M.; Flachowsky, H.; Schröpfer, S.; Peil, A. Evaluation of Scab and Mildew Resistance in the Gene Bank Collection of Apples in Dresden-Pillnitz. *Plants* **2021**, *10*, 1227. [CrossRef]
34. Doolotkeldieva, T.; Bobusheva, S. Scab Disease Caused by *Venturia inaequalis*; on Apple Trees in Kyrgyzstan and Biological Agents to Control This Disease. *Adv. Microbiol.* **2017**, *7*, 450–466. [CrossRef]
35. Wenneker, M.; Goedhart, P.W.; Van Der Steeg, P.; Van De Weg, W.E.; Schouten, H.J. Methods for the Quantification of Resistance of Apple Genotypes to European Fruit Tree Canker Caused by *Neonectria ditissima*. *Plant Dis.* **2017**, *101*, 2012–2019. [CrossRef] [PubMed]
36. Crosby, J.A.; Janick, J.; Pecknold, P.C.; Goffreda, J.C.; Korban, S.S. 'Enterprise' Apple. *HortScience* **1994**, *29*, 825–826. [CrossRef]
37. Caffier, V.; Patocchi, A.; Expert, P.; Bellanger, M.-N.; Durel, C.-E.; Hilber-Bodmer, M.; Broggini, G.A.L.; Groenwold, R.; Bus, V.G.M. Virulence Characterization of *Venturia inaequalis* Reference Isolates on the Differential Set of *Malus* Hosts. *Plant Dis.* **2015**, *99*, 370–375. [CrossRef] [PubMed]
38. Svara, A.; Tarkowski, Ł.P.; van Rensburg, H.C.J.; Deleye, E.; Vaerten, J.; De Storme, N.; Keulemans, W.; Van den Ende, W. Sweet Immunity: The Effect of Exogenous Fructans on the Susceptibility of Apple (*Malus x domestica* Borkh.) to *Venturia inaequalis*. *Int. J. Mol. Sci.* **2020**, *21*, 5885. [CrossRef] [PubMed]
39. Petkovsek, M.M.; Stampar, F.; Veberic, R. Parameters of Inner Quality of the Apple Scab Resistant and Susceptible Apple Cultivars (*Malus domestica* Borkh.). *Sci. Hortic.* **2007**, *114*, 37–44. [CrossRef]
40. Bus, V.G.M.; Rikkerink, E.H.A.; Caffier, V.; Durel, C.-E.; Plummer, K.M. Revision of the Nomenclature of the Differential Host-Pathogen Interactions of *Venturia inaequalis* and *Malus*. *Annu. Rev. Phytopathol.* **2011**, *49*, 391–413. [CrossRef]
41. Ebrahimi, L.; Hatami Rad, S.; Etebarian, H.R. Apple Endophytic Fungi and Their Antagonism against Apple Scab Disease. *Front. Microbiol.* **2022**, *13*, 1024001. [CrossRef]
42. Marolleau, B.; Gaucher, M.; Heintz, C.; Degrave, A.; Warneys, R.; Orain, G.; Lemarquand, A.; Brisset, M.-N. When a Plant Resistance Inducer Leaves the Lab for the Field: Integrating ASM into Routine Apple Protection Practices. *Front. Plant Sci.* **2017**, *8*, 1938. [CrossRef]
43. McLaughlin, M.S.; Roy, M.; Abbasi, P.A.; Carisse, O.; Yurgel, S.N.; Ali, S. Why Do We Need Alternative Methods for Fungal Disease Management in Plants? *Plants* **2023**, *12*, 3822. [CrossRef]
44. Holb, I.J. Apple Scab Management in Organic Fruit Orchards: Epidemiology, Forecasting and Disease Control Strategies. *Acta Hortic.* **2013**, *1001*, 223–234. [CrossRef]
45. Bénaouf, G.; Parisi, L. Genetics of Host-Pathogen Relationships Between *Venturia inaequalis* Races 6 and 7 and *Malus* Species. *Phytopathology* **2000**, *90*, 236–242. [CrossRef] [PubMed]
46. Sandskär, B.; Gustafsson, M. Classification of Apple Scab Resistance in Two Assortment Orchards. *Genet. Resour. Crop Evol.* **2004**, *51*, 197–203. [CrossRef]
47. Garofalo, E.W.; Tuttle, A.F.; Clements, J.M.; Cooley, D.R. Discrepancies Between Direct Observation of Apple Scab Ascospore Maturation and Disease Model Forecasts in the 2014 and 2015 Growing Seasons. *Fruit Notes* **2016**, *8*, 17–21.
48. Shuttleworth, L.A. Alternative Disease Management Strategies for Organic Apple Production in the United Kingdom. *CABI Agric. Biosci.* **2021**, *2*, 34. [CrossRef]
49. Mills, W.D.; LaPlante, A.A. Diseases and Insects in the Orchard. *Cornell Univ. Ext. Bull.* **1951**, *711*, 21–27.
50. MacHardy, W.E.; Gadoury, D.M. A Revision of Mill's Criteria for Predicting Apple Scab Infection Periods. *Phytopathology* **1989**, *79*, 304–310. [CrossRef]
51. Schwabe, W.F.S. Wetting and Temperature Requirements for Infection by *Venturia inaequalis* in South Africa. *Phytophylactica* **1980**, *12*, 69–80.
52. Sys, S.; Seonen, A. Investigation on the Infection Criteria of Scab (*Venturia inaequalis*) on Apples with Respect to Mills &Laplante. *Agric. Louvain* **1970**, *18*, 3–8.
53. Gadoury, D.M.; Seem, R.C.; MacHardy, W.E.; Wilcox, W.F.; Rosenberger, D.A.; Stensvand, A. A Comparison of Methods Used to Estimate the Maturity and Release of Ascospores of *Venturia inaequalis*. *Plant Dis.* **2004**, *88*, 869–874. [CrossRef] [PubMed]
54. Rusevski, R.; Kuzmanovska, B.; Petkovski, E.; Bandzo, K. Biological Control of *Venturia inaequalis*—The Cause of Apple Scab in Apple. *J. Agric. Food Environ. Sci.* **2018**, *72*, 16–18. [CrossRef]
55. Rusevski, R.; Kuzmanovska, B.; Petkovski, E.; Bandzo Oreskovic, K. New Opportunities for Chemical Control of *Venturia inaequalis* and *Podosphaera leucotricha* in Apple Orchards in Macedonia. *J. Agric. Food Environ. Sci.* **2018**, *72*, 12–15. [CrossRef]

56. FRAC Pathogen at Risk 2019. Available online: https://www.frac.info/docs/default-source/publications/pathogen-risk/frac-pathogen-list-2019.pdf (accessed on 12 December 2023).
57. Hoffmeister, M.; Scheu, P.; Glaab, A.; Zito, R.; Stammler, G. Sensitivity Evolution in *Venturia inaequalis* towards SDHIs in Comparison to Other Modes of Action. *Eur. J. Plant Pathol.* **2023**. [CrossRef]
58. Benyagoub, M.; Benhamou, N.; Carisse, O. Cytochemical Investigation of the Antagonistic Interaction between a *Microsphaeropsis* Sp. (Isolate P130A) and *Venturia inaequalis*. *Phytopathology* **1998**, *88*, 605–613. [CrossRef] [PubMed]
59. Chapman, K.S.; Sundin, G.W.; Beckerman, J.L. Identification of Resistance to Multiple Fungicides in Field Populations of *Venturia inaequalis*. *Plant Dis.* **2011**, *95*, 921–926. [CrossRef] [PubMed]
60. Mondino, P.; Casanova, L.; Celio, A.; Bentancur, O.; Leoni, C.; Alaniz, S. Sensitivity of *Venturia inaequalis* to Trifloxystrobin and Difenoconazole in Uruguay. *J. Phytopathol.* **2015**, *163*, 1–10. [CrossRef]
61. Ayer, K.M.; Villani, S.M.; Choi, M.-W.; Cox, K.D. Characterization of the *VisdhC* and *VisdhD* Genes in *Venturia inaequalis*, and Sensitivity to Fluxapyroxad, Pydiflumetofen, Inpyrfluxam, and Benzovindiflupyr. *Plant Dis.* **2019**, *103*, 1092–1100. [CrossRef]
62. Jobin, T.; Carisse, O. Incidence of Myclobutanil- and Kresoxim-Methyl-Insensitive Isolates of *Venturia inaequalis* in Quebec Orchards. *Plant Dis.* **2007**, *91*, 1351–1358. [CrossRef]
63. Pfeufer, E.E.; Ngugi, H.K. Orchard Factors Associated with Resistance and Cross Resistance to Sterol Demethylation Inhibitor Fungicides in Populations of *Venturia inaequalis* from Pennsylvania. *Phytopathology* **2012**, *102*, 272–282. [CrossRef]
64. Cordero-Limon, L.; Shaw, M.W.; Passey, T.A.; Robinson, J.D.; Xu, X. Cross-resistance between Myclobutanil and Tebuconazole and the Genetic Basis of Tebuconazole Resistance in *Venturia inaequalis*. *Pest Manag. Sci.* **2021**, *77*, 844–850. [CrossRef]
65. Villani, S.M.; Biggs, A.R.; Cooley, D.R.; Raes, J.J.; Cox, K.D. Prevalence of Myclobutanil Resistance and Difenoconazole Insensitivity in Populations of *Venturia inaequalis*. *Plant Dis.* **2015**, *99*, 1526–1536. [CrossRef] [PubMed]
66. Villani, S.M.; Hulvey, J.; Hily, J.-M.; Cox, K.D. Overexpression of the *CYP51A1* Gene and Repeated Elements Are Associated with Differential Sensitivity to DMI Fungicides in *Venturia inaequalis*. *Phytopathology* **2016**, *106*, 562–571. [CrossRef] [PubMed]
67. Fontaine, S.; Remuson, F.; Fraissinet-Tachet, L.; Micoud, A.; Marmeisse, R.; Melayah, D. Monitoring of *Venturia inaequalis* harbouring the QoI Resistance G143A Mutation in French Orchards as Revealed by PCR Assays. *Pest Manag. Sci.* **2009**, *65*, 74–81. [CrossRef] [PubMed]
68. Frederick, Z.A.; Villani, S.M.; Cooley, D.R.; Biggs, A.R.; Raes, J.J.; Cox, K.D. Prevalence and Stability of Qualitative QoI Resistance in Populations of *Venturia inaequalis* in the Northeastern United States. *Plant Dis.* **2014**, *98*, 1122–1130. [CrossRef] [PubMed]
69. Lesniak, K.E.; Proffer, T.J.; Beckerman, J.L.; Sundin, G.W. Occurrence of QoI Resistance and Detection of the G143A Mutation in Michigan Populations of *Venturia inaequalis*. *Plant Dis.* **2011**, *95*, 927–934. [CrossRef] [PubMed]
70. Chatzidimopoulos, M.; Zambounis, A.; Lioliopoulou, F.; Vellios, E. Detection of *Venturia inaequalis* Isolates with Multiple Resistance in Greece. *Microorganisms* **2022**, *10*, 2354. [CrossRef]
71. FRAC Code List 1: Fungicides Sorted by FRAC Code 2005. Available online: https://ipm.ifas.ufl.edu/resources/success_stories/t&pguide/pdfs/appendices/appendix6-frac.pdf (accessed on 12 December 2023).
72. Polat, Z.; Bayraktar, H. Resistance of *Venturia inaequalis* to Multiple Fungicides in Turkish Apple Orchards. *J. Phytopathol.* **2021**, *169*, 360–368. [CrossRef]
73. Xu, X.-M.; Gao, L.-Q.; Yang, J.-R. Are Insensitivities of *Venturia inaequalis* to Myclobutanil and Fenbuconazole Correlated? *Crop Prot.* **2010**, *29*, 183–189. [CrossRef]
74. Thind, T.S.; Hollomon, D.W. Thiocarbamate Fungicides: Reliable Tools in Resistance Management and Future Outlook. *Pest Manag. Sci.* **2018**, *74*, 1547–1551. [CrossRef]
75. Hirayama, K. Curative Effects of Fungicides against *Venturia inaequalis* Causing Apple Scab. *J. Gen. Plant Pathol.* **2022**, *88*, 264–269. [CrossRef]
76. Collinge, D.B.; Jensen, D.F.; Rabiey, M.; Sarrocco, S.; Shaw, M.W.; Shaw, R.H. Biological Control of Plant Diseases—What Has Been Achieved and What Is the Direction? *Plant Pathol.* **2022**, *71*, 1024–1047. [CrossRef]
77. Lahlali, R.; Ezrari, S.; Radouane, N.; Kenfaoui, J.; Esmaeel, Q.; El Hamss, H.; Belabess, Z.; Barka, E.A. Biological Control of Plant Pathogens: A Global Perspective. *Microorganisms* **2022**, *10*, 596. [CrossRef]
78. Ouimet, A.; Carisse, O.; Neumann, P. Evaluation of Fungal Isolates for the Inhibition of Vegetative Growth of *Venturia inaequalis*. *Can. J. Bot.* **1997**, *75*, 626–631. [CrossRef]
79. NIAB EMR, UK. *Integrated Management of Diseases and Insect Pests of Tree Fruit*; Xu, X., Fountain, M., Eds.; Burleigh Dodds Series in Agricultural Science; Burleigh Dodds Science Publishing: Cambridge, UK, 2019; ISBN 978-1-78676-256-6.
80. O'Brien, P.A. Biological Control of Plant Diseases. *Australas. Plant Pathol.* **2017**, *46*, 293–304. [CrossRef]
81. Ouimet, A.; Carisse, O.; Neumann, P. Environmental and Nutritional Factors Affecting the in Vitro Inhibition of the Vegetative Growth of *Venturia inaequalis* by Five Antagonistic Fungi. *Can. J. Bot.* **1997**, *75*, 632–639. [CrossRef]
82. Fiss, M.; Barckhausen, O.; Gherbawy, Y.; Kollar, A.; Hamamoto, M.; Auling, G. Characterization of Epiphytic Yeasts of Apple as Potential Biocontrol Agents against Apple Scab (*Venturia inaequalis*). *Z. Pflanzenkrankh. Pflanzenschutz* **2003**, *110*, 513–523.
83. Fiaccadori, R. In Vitro, in Vivo and in Field Sensitivity of *Venturia inaequalis* to Anilinopyrimidine Fungicides with Different Types of Scab Management and Degree of Control. *OALib* **2018**, *5*, 1–13. [CrossRef]
84. Heye, C.C. Biological Control of the Perfect Stage of the Apple Scab Pathogen, *Venturia inaequalis* (Cke.) Wint. Ph.D. Thesis, University of Wisconsin, Madison, WI, USA, 1982.

85. Ross, R.G.; Burchill, R.T. Experiments Using Sterilized Apple-Leaf Discs to Study the Mode of Action of Urea in Suppressing Perithecia of *Venturia inaequalis* (Cke.) Wint. *Ann. Appl. Biol.* **1968**, *62*, 289–296. [CrossRef]
86. Philion, V.; Carisse, O.; Paulitz, T. In Vitro Evaluation of Fungal Isolates for Their Ability to Influence Leaf Rheology, Production of Pseudothecia, and Ascospores of *Venturia inaequalis*. *Eur. J. Plant Pathol.* **1997**, *103*, 441–452. [CrossRef]
87. Thakur, V.S.; Sharma, R.D. Effect of Urea on Microbial Degradation of Apple Leaf Litter and Its Relationship to the Inhibition of Pseudothecial Development of *Venturia inaequalis*. *Indian J. Agric. Sci.* **1999**, *69*, 147–151.
88. Arseneault, T.; Filion, M. Biocontrol through Antibiosis: Exploring the Role Played by Subinhibitory Concentrations of Antibiotics in Soil and Their Impact on Plant Pathogens. *Can. J. Plant Pathol.* **2017**, *39*, 267–274. [CrossRef]
89. Palmieri, D.; Ianiri, G.; Del Grosso, C.; Barone, G.; De Curtis, F.; Castoria, R.; Lima, G. Advances and Perspectives in the Use of Biocontrol Agents against Fungal Plant Diseases. *Horticulturae* **2022**, *8*, 577. [CrossRef]
90. Raaijmakers, J.M.; Vlami, M.; de Souza, J.T. Antibiotic Production by Bacterial Biocontrol Agents. *Antonie Leeuwenhoek* **2002**, *81*, 537–547. [CrossRef] [PubMed]
91. Bosshard, E.; Schüepp, H.; Siegfried, W. Concepts and Methods in Biological Control of Diseases in Apple Orchards. *EPPO Bull.* **1987**, *17*, 655–663. [CrossRef]
92. Burchill, R.T.; Hutton, K.E.; Crosse, J.E.; Garrett, C.M.E. Inhibition of the Perfect Stage of *Venturia inaequalis* (Cooke) Wint., by Urea [37]. *Nature* **1965**, *205*, 520–521. [CrossRef]
93. Cinq-Mars, L. Interactions between *Venturia inaequalis* (Cke) Wint. And Saprophytic Fungi and Bacteria Inhabiting Apple Leaves. Master's Thesis, McGill University, Montréal, QC, Canada, 1949.
94. Ross, R.G. The Microflora of Apple Leaves and ilS Relationship to *Venturia inaequalis*(Cke.) Wint. Master's Thesis, McGiIl University, Montréal, QC, Canada, 1953.
95. Heye, C.C.; Andrews:, J.H. Antagonism of *Athelia bombacina* and *Chaetomium globosum* to the Apple Scab Pathogen *Venturia inaequalis*. *Phytopathology* **1983**, *73*, 650–665. [CrossRef]
96. Burchill, R.T.; Cook, R.T.A. *The Interaction of Urea and Micro-Organisms in Suppressing the Development of Perithecia of Venturia inaequalis (Cke.) Wint*; Ecology of Leaf Surface Micro-Organisms; Preece, T.F., Dickinson, C.H., Eds.; Academic Press: New York, NY, USA, 1970; pp. 471–483.
97. Alaphilippe, A.; Elad, Y.; David, D.R.; Derridj, S.; Gessler, C. Effects of a Biocontrol Agent of Apple Powdery Mildew (*Podosphaera leucotricha*) on the Host Plant and on Non-Target Organisms: An Insect Pest (*Cydia pomonella*) and a Pathogen (*Venturia inaequalis*). *Biocontrol. Sci. Technol.* **2008**, *18*, 121–138. [CrossRef]
98. Raymaekers, K.; Ponet, L.; Holtappels, D.; Berckmans, B.; Cammue, B.P.A. Screening for Novel Biocontrol Agents Applicable in Plant Disease Management—A Review. *Biol. Control* **2020**, *144*, 104240. [CrossRef]
99. Fravel, D.R. Commercialization and Implementation of Biocontrol. *Annu. Rev. Phytopathol.* **2005**, *43*, 337–359. [CrossRef]
100. Rosenberger, D.A. Factors Limiting IPM-Compatibility of New Disease Control Tactics for Apples in Eastern United States. *Plant Health Prog.* **2003**, *4*, 22. [CrossRef]
101. Poleatewich, A.M.; Ngugi, H.K.; Backman, P.A. Assessment of Application Timing of *Bacillus* Spp. to Suppress Pre- and Postharvest Diseases of Apple. *Plant Dis.* **2012**, *96*, 211–220. [CrossRef]
102. Heydari, A.; Pessarakli, M. A Review on Biological Control of Fungal Plant Pathogens Using Microbial Antagonists. *J. Biol. Sci.* **2010**, *10*, 273–290. [CrossRef]
103. Köhl, J.J.; Molhoek, W.W.M.L.; Groenenboom-De Haas, B.B.H.; Goossen-Van De Geijn, H.H.M. Selection and Orchard Testing of Antagonists Suppressing Conidial Production by the Apple Scab Pathogen *Venturia inaequalis*. *Eur. J. Plant Pathol.* **2009**, *123*, 401–414. [CrossRef]
104. Köhl, J. Screening of Biocontrol Agents for Control of Foliar Diseases. In *Recent Developments in Management of Plant Diseases*; Gisi, U., Chet, I., Gullino, M.L., Eds.; Springer: Dordrecht, The Netherlands, 2010; pp. 107–119. ISBN 978-1-4020-8803-2.
105. Köhl, J.; Scheer, C.; Holb, I.J.; Masny, S.; Molhoek, W. Toward an Integrated Use of Biological Control by *Cladosporium cladosporioides* H39 in Apple Scab *Venturia inaequalis* Management. *Plant Dis.* **2015**, *99*, 535–543. [CrossRef] [PubMed]
106. Pertot, I.; Puopolo, G.; Giovannini, O.; Angeli, D.; Sicher, C. Advantages and Limitations Involved in the Use of Microbial Biofungicides for the Control of Root and Foliar Phytopathogens of Fruit Crops. *Italus Hortus* **2016**, *23*, 3–12.
107. Andrews, J.H.; Berbee, F.M.; Nordheim, E.V. Microbial Antagonism to the Imperfect Stage of the Apple Scab Pathogen *Venturia inaequalis*. *Phytopathology* **1983**, *73*, 228. [CrossRef]
108. Carisse, O.; Philion, V.; Rolland, D.; Bernier, J. Effect of Fall Application of Fungal Antagonists on Spring Ascospore Production of Apple Scab Pathogen, *Venturia inaequalis*. *Phytopathology* **2000**, *90*, 31–37. [CrossRef] [PubMed]
109. Fiss, M.; Kucheryava, N.; Schönherr, J.; Kollar, A.; Arnold, G.; Auling, G. Isolation and Characterization of Epiphytic Fungi from the Phyllosphere of Apple as Potential Biocontrol Agents against Apple Scab *Venturia inaequalis*. *Z. Pflanzenkrankh. Pflanzenschutz* **2000**, *107*, 1–11.
110. Leconte, A.; Tournant, L.; Muchembled, J.; Paucellier, J.; Héquet, A.; Deracinois, B.; Deweer, C.; Krier, F.; Deleu, M.; Oste, S.; et al. Assessment of Lipopeptide Mixtures Produced by *Bacillus subtilis* as Biocontrol Products against Apple Scab *Venturia inaequalis*. *Microorganisms* **2022**, *10*, 1810. [CrossRef] [PubMed]
111. Burr, T.J.; Matteson, M.C.; Smith, C.A.; Corral-Garcia, M.R.; Huang, T.-C. Effectiveness of Bacteria and Yeasts from Apple Orchards as Biological Control Agents of Apple Scab. *Biol. Control* **1996**, *6*, 151–157. [CrossRef]

112. Bensaci, O.A.; Aliat, T.; Berdja, R.; Popkova, A.V.; Kucher, D.E.; Gurina, R.R.; Rebouh, N.Y. The Use of Mycoendophyte-Based Bioformulations to Control Apple Diseases: Toward an Organic Apple Production System in the Aurès (Algeria). *Plants* **2022**, *11*, 3405. [CrossRef]
113. Miedtke, U.; Kennel, W. Athelia Bombacina and *Chaetomium globosum* as Antagonists of the Perfect Stage of the Apple Scab Pathogen (*Venturia inaequalis*) under Field Conditions. *Z. Pflanzenkrankh. Pflanzenschutz* **1990**, *97*, 24–32.
114. Young, C.S.; Andrews, J.H. Inhibition of Pseudothecial Development of *Venturia inaequalis* by the Basidiomycete *Athelia bombacina* in Apple Leaf Litter. *Phytopathology* **1990**, *80*, 536–542. [CrossRef]
115. Caffier, V.; Shiller, J.; Bellanger, M.-N.; Collemare, J.; Expert, P.; Gladieux, P.; Pascouau, C.; Sannier, M.; Le Cam, B. Hybridizations Between Formae Speciales of *Venturia inaequalis* Pave the Way for a New Biocontrol Strategy to Manage Fungal Plant Pathogens. *Phytopathology* **2022**, *112*, 1401–1405. [CrossRef] [PubMed]
116. Gladieux, P.; Caffier, V.; Devaux, M.; Le Cam, B. Host-Specific Differentiation among Populations of *Venturia inaequalis* Causing Scab on Apple, *Pyracantha* and *Loquat*. *Fungal Genet. Biol.* **2010**, *47*, 511–521. [CrossRef] [PubMed]
117. Vincent, C.; Rancourt, B.; Carisse, O. Apple Leaf Shredding as a Non-Chemical Tool to Manage Apple Scab and Spotted Tentiform Leafminer. *Agric. Ecosyst. Environ.* **2004**, *104*, 595–604. [CrossRef]
118. Singh, K.P.; Singh, A.; Prasad, R.K.; Kumar, J. Postharvest Applicaton of Fungicides, Antagonists and Plant Products for Controlling Storage Scab and Rots of Apple Fruits. *Indian Phytopathol.* **2017**, *70*, 315–321.
119. Miliute, I.; Buzaite, O.; Gelvonauskiene, D.; Sasnauskas, A.; Stanys, V.; Baniulis, D. Plant Growth Promoting and Antagonistic Properties of Endophytic Bacteria Isolated from Domestic Apple. *Zemdirbyste* **2016**, *103*, 77–82. [CrossRef]
120. Çaltili, O.; Arici, Ş.E. The Determination of the Efficacy of Some Microbial Preparations against Apple Scab Disease (*Venturia inaequalis* (CKE) Wint.) in Isparta. *Black Sea J. Agric.* **2018**, *1*, 6–10.
121. Elad, Y. Biological Control of Foliar Pathogens by Means of *Trichoderma harzianum* and Potential Modes of Action. *Crop Prot.* **2000**, *19*, 709–714. [CrossRef]
122. Ons, L.; Bylemans, D.; Thevissen, K.; Cammue, B.P.A. Combining Biocontrol Agents with Chemical Fungicides for Integrated Plant Fungal Disease Control. *Microorganisms* **2020**, *8*, 1930. [CrossRef] [PubMed]
123. Bolar, J.P.; Norelli, J.L.; Wong, K.-W.; Hayes, C.K.; Harman, G.E.; Aldwinckle, H.S. Expression of Endochitinase from *Trichoderma harzianum* in Transgenic Apple Increases Resistance to Apple Scab and Reduces Vigor. *Phytopathology* **2000**, *90*, 72–77. [CrossRef] [PubMed]
124. DeGenring, L.; Peter, K.; Poleatewich, A. Integration of Chitosan and Biopesticides to Suppress Pre-Harvest Diseases of Apple. *Horticulturae* **2023**, *9*, 707. [CrossRef]
125. Percival, G.; Graham, S. Evaluation of Inducing Agents and Synthetic Fungicide Combinations for Management of Foliar Pathogens of Urban Trees. *Arboric. Urban For.* **2021**, *47*, 85–95. [CrossRef]
126. Reglinski, T.; Havis, N.; Rees, H.J.; De Jong, H. The Practical Role of Induced Resistance for Crop Protection. *Phytopathology* **2023**, *113*, 719–731. [CrossRef] [PubMed]
127. Jamalizadeh, M.; Etebarian, H.R.; Aminian, H.; Alizadeh, A. A Review of Mechanisms of Action of Biological Control Organisms against Post-harvest Fruit Spoilage. *EPPO Bull.* **2011**, *41*, 65–71. [CrossRef]
128. Fenta, L.; Mekonnen, H.; Kabtimer, N. The Exploitation of Microbial Antagonists against Postharvest Plant Pathogens. *Microorganisms* **2023**, *11*, 1044. [CrossRef]
129. Simard, J.; Pelletier, R.I.; Coulson, J.G. Screening Ofmicroorganisms Inhubiting Apple Leaf for Their Antibiotic Propenies against *Venturia inaequalis*(Cke.) Wint. *Annu. Rep. Qué. Soc. Prot. Plants* **1957**, *39*, 59–67.
130. Cullen, D.; Andrews, J.H. Evidence for the Role of Antibiosis in the Antagonism of *Chaetomium globosum* to the Apple Scab Pathogen, *Venturia inaequalis*. *Can. J. Bot.* **1984**, *62*, 1819–1823. [CrossRef]
131. Desmyttere, H.; Deweer, C.; Muchembled, J.; Sahmer, K.; Jacquin, J.; Coutte, F.; Jacques, P. Antifungal Activities of *Bacillus subtilis* Lipopeptides to Two *Venturia inaequalis* Strains Possessing Different Tebuconazole Sensitivity. *Front. Microbiol.* **2019**, *10*, 487665. [CrossRef]
132. Bolar, J.P.; Norelli, J.L.; Harman, G.E.; Brown, S.K.; Aldwinckle, H.S. Synergistic Activity of Endochitinase and Exochitinase from *Trichoderma atroviride* (*T. harzianum*) against the Pathogenic Fungus (*Venturia inaequalis*) in Transgenic Apple Plants. *Transgenic Res.* **2001**, *10*, 533–543. [CrossRef]
133. Schäfer, T.; Buscot, F.; Kaldorf, M.; Flachowsky, H.; Hanke, M.-V.; König, S. First Results on the Effect of Increased Chitinase Expression in Transgenic Apple Trees on Mycorrhization with *Glomus intraradices* and *G. mosseae*. *Acta Hortic.* **2009**, *839*, 719–724. [CrossRef]
134. Kobayashi, D.Y.; Reedy, R.M.; Bick, J.; Oudemans, P.V. Characterization of a Chitinase Gene from *Stenotrophomonas maltophilia* Strain 34S1 and Its Involvement in Biological Control. *Appl. Environ. Microbiol.* **2002**, *68*, 1047–1054. [CrossRef]
135. Pan, H.; Wei, Y.; Xin, F.; Zhou, M.; Zhang, S. Characterization and Biocontrol Ability of Fusion Chitinase in *Escherichia coli* Carrying Chitinase cDNA from *Trichothecium roseum*. *Z. Naturforschung-Sect. C J. Biosci.* **2006**, *61*, 397–404. [CrossRef]
136. Bautista-Baños, S.; Hernández-Lauzardo, A.N.; Velázquez-del Valle, M.G.; Hernández-López, M.; Ait Barka, E.; Bosquez-Molina, E.; Wilson, C.L. Chitosan as a Potential Natural Compound to Control Pre and Postharvest Diseases of Horticultural Commodities. *Crop Prot.* **2006**, *25*, 108–118. [CrossRef]

137. Yu, T.; Yu, C.; Chen, F.; Sheng, K.; Zhou, T.; Zunun, M.; Abudu, O.; Yang, S.; Zheng, X. Integrated Control of Blue Mold in Pear Fruit by Combined Application of Chitosan, a Biocontrol Yeast and Calcium Chloride. *Postharvest Biol. Technol.* **2012**, *69*, 49–53. [CrossRef]
138. Hossain, M.B.; Piotrowski, M.; Lensing, J.; Gau, A.E. Inhibition of Conidial Growth of *Venturia inaequalis* by the Extracellular Protein Fraction from the Antagonistic Bacterium *Pseudomonas fluorescens* Bk3. *Biol. Control* **2009**, *48*, 133–139. [CrossRef]
139. Bálint, J.; Nagy, S.; Thiesz, R.; Nyárádi, I.-I.; Balog, A. Using Plant Extracts to Reduce Asexual Reproduction of Apple Scab (*Venturia inaequalis*). *Turk. J. Agric. For.* **2014**, *38*, 91–98. [CrossRef]
140. Bengtsson, M.; Wulff, E.; Lyngs Jørgensen, H.J.; Pham, A.; Lübeck, M.; Hockenhull, J. Comparative Studies on the Effects of a Yucca Extract and Acibenzolar-S-Methyl (ASM) on Inhibition of *Venturia inaequalis* in Apple Leaves. *Eur. J. Plant Pathol.* **2009**, *124*, 187–198. [CrossRef]
141. Ganchev, D. In Vitro Antifungal Activity of Ethanol Plant Extracts against Conidiospores of Apple Scab (*Venturia inaequalis*). *Moroc. J. Agric. Sci.* **2023**, *4*, 61–67.
142. Rollinger, J.M.; Spitaler, R.; Menz, M.; Marschall, K.; Zelger, R.; Ellmerer, E.P.; Schneider, P.; Stuppner, H. *Venturia inaequalis* -Inhibiting Diels−Alder Adducts from Morus Root Bark. *J. Agric. Food Chem.* **2006**, *54*, 8432–8436. [CrossRef] [PubMed]
143. Hochbaum, T.; Petróczy, M.; Ladányi, M.; Nagy, G. The Efficacy of Essential Oils against *Venturia inaequalis*(Cooke) G. Winter and *Podosphaera leucotricha* (Ellis & Everh.) E. S. Salmon in Vivo. *Acta Univ. Sapientiae Agric. Environ.* **2018**, *10*, 5–19. [CrossRef]
144. Nagy, G.; Hochbaum, T.; Sárosi, S.; Ladányi, M. In Vitro and in Planta Activity of Some Essential Oils against *Venturia inaequalis* (Cooke) G. Winter. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2014**, *42*, 109–114. [CrossRef]
145. Thuerig, B.; Ramseyer, J.; Hamburger, M.; Oberhänsli, T.; Potterat, O.; Schärer, H.; Tamm, L. Efficacy of a *Juncus effusus* Extract on Grapevine and Apple Plants against *Plasmopara viticola* and *Venturia inaequalis*, and Identification of the Major Active Constituent. *Pest Manag. Sci.* **2016**, *72*, 1718–1726. [CrossRef]
146. Thiesz, R.; Balog, A.; Ferencz, L.; Albert, J. The Effects of Plant Extracts on Apple Scab (*Venturia inaequalis* Cooke) under Laboratory Conditions. *Rom. Biotechnol. Lett.* **2007**, *12*, 3295–3302.
147. Moureu, S.; Jacquin, J.; Samaillie, J.; Deweer, C.; Rivière, C.; Muchembled, J. Antifungal Activity of Hop Leaf Extracts and Xanthohumol on Two Strains of *Venturia inaequalis* with Different Sensitivities to Triazoles. *Microorganisms* **2023**, *11*, 1605. [CrossRef]
148. Thuerig, B.; Ramseyer, J.; Hamburger, M.; Ludwig, M.; Oberhänsli, T.; Potterat, O.; Schärer, H.-J.; Tamm, L. Efficacy of a *Magnolia officinalis* Bark Extract against Grapevine Downy Mildew and Apple Scab under Controlled and Field Conditions. *Crop Prot.* **2018**, *114*, 97–105. [CrossRef]
149. Linares, C.; Cid, G.A.; Capdesuner, Y.; Buchelle, M.; Martinez-Montero, M.E.; Scheer, C.; Quiñones-Galvez, J. Optimization of the Process for Obtaining *Morinda royoc* Crude Extract Bioactive against Phythopathogens. *Vegetos* 2023. [CrossRef]
150. Porsche, F.M.; Molitor, D.; Beyer, M.; Charton, S.; André, C.; Kollar, A. Antifungal Activity of Saponins from the Fruit Pericarp of *Sapindus mukorossi* against *Venturia inaequalis* and *Botrytis cinerea*. *Plant Dis.* **2018**, *102*, 991–1000. [CrossRef]
151. He, D.-C.; He, M.-H.; Amalin, D.M.; Liu, W.; Alvindia, D.G.; Zhan, J. Biological Control of Plant Diseases: An Evolutionary and Eco-Economic Consideration. *Pathogens* **2021**, *10*, 1311. [CrossRef]
152. Teem, J.L.; Alphey, L.; Descamps, S.; Edgington, M.P.; Edwards, O.; Gemmell, N.; Harvey-Samuel, T.; Melnick, R.L.; Oh, K.P.; Piaggio, A.J.; et al. Genetic Biocontrol for Invasive Species. *Front. Bioeng. Biotechnol.* **2020**, *8*, 452. [CrossRef]
153. Boudreau, M.A.; Andrews, J.H. Factors Influencing Antagonism of *Chaetomium globosum* to *Venturia inaequalis*: A Case Study in Failed Biocontrol. *Phytopathol.* **1987**, *77*, 1470–1475. [CrossRef]
154. Thambugala, K.M.; Daranagama, D.A.; Phillips, A.J.L.; Kannangara, S.D.; Promputtha, I. Fungi vs. Fungi in Biocontrol: An Overview of Fungal Antagonists Applied Against Fungal Plant Pathogens. *Front. Cell. Infect. Microbiol.* **2020**, *10*, 604923. [CrossRef] [PubMed]
155. Carisse, O.; Rolland, D. Effect of Timing of Application of the Biological Control Agent *Microsphaeropsis ochracea* on the Production and Ejection Pattern of Ascospores by *Venturia inaequalis*. *Phytopathology* **2004**, *94*, 1305–1314. [CrossRef]
156. Odile, C.; David-Mathieu, T. Disease Decision Support Systems: Their Impact on Disease Management and Durability of Fungicide Effectiveness. In *Fungicides*; Carisse, O., Ed.; InTech: London, UK, 2010; ISBN 978-953-307-266-1.
157. Passey, T.A.J.; Robinson, J.D.; Shaw, M.W.; Xu, X.-M. The Relative Importance of Conidia and Ascospores as Primary Inoculum of *Venturia inaequalis* in a Southeast England Orchard. *Plant Pathol.* **2017**, *66*, 1445–1451. [CrossRef]
158. Köhl, J.; Molhoek, W.; Haas, L.G.; de Geijn, H.G. New Approaches in Biological Control of Apple Scab. In Proceedings of the 13th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit-Growing, Weinsberg, Germany, 24 July 2008.
159. Rancāne, R.; Valiuškaitė, A.; Zagorska, V.; Komašilovs, V.; Rasiukevičiūtė, N. The Overall Environmental Load and Resistance Risk Caused by Long-Term Fungicide Use to Control *Venturia inaequalis* in Apple Orchards in Latvia. *Plants* **2023**, *12*, 450. [CrossRef] [PubMed]
160. Mahyar Mirmajlessi, S.; Ahari Mostafavi, H.; Loit, E.; Najdabbasi, N.; Mänd, M. Application of Radiation and Genetic Engineering Techniques to Improve Biocontrol Agent Performance: A Short Review. In *Use of Gamma Radiation Techniques in Peaceful Applications*; Almayah, B.A., Ed.; IntechOpen: London, UK, 2019; ISBN 978-1-83962-259-5.
161. Martinez, Y.; Ribera, J.; Schwarze, F.W.M.R.; De France, K. Biotechnological Development of *Trichoderma*-Based Formulations for Biological Control. *Appl. Microbiol. Biotechnol.* **2023**, *107*, 5595–5612. [CrossRef] [PubMed]

162. Ayaz, M.; Li, C.-H.; Ali, Q.; Zhao, W.; Chi, Y.-K.; Shafiq, M.; Ali, F.; Yu, X.-Y.; Yu, Q.; Zhao, J.-T.; et al. Bacterial and Fungal Biocontrol Agents for Plant Disease Protection: Journey from Lab to Field, Current Status, Challenges, and Global Perspectives. *Molecules* **2023**, *28*, 6735. [CrossRef]
163. Carisse, O.; Holloway, G.; Leggett, M. Potential and Limitations of *M. ochraceaan* Agent for Biosanitation of Apple Scab. In *Biological Control: A Global Perspective: Case Studies from around the World*; Vincent, C., Goettel, M.S., Lazaovits, G., Eds.; CABI Publishing: Wallingford, UK, 2007; pp. 234–240.
164. Vincent, C.; Goettel, M.S.; Lazarovits, G. (Eds.) *Biological Control: A Global Perspective: Case Studies from around the World*; CABI Publishing: Wallingford, UK, 2007; 440p.
165. Maciag, T.; Kozieł, E.; Rusin, P.; Otulak-Kozieł, K.; Jafra, S.; Czajkowski, R. Microbial Consortia for Plant Protection against Diseases: More than the Sum of Its Parts. *Int. J. Mol. Sci.* **2023**, *24*, 12227. [CrossRef]