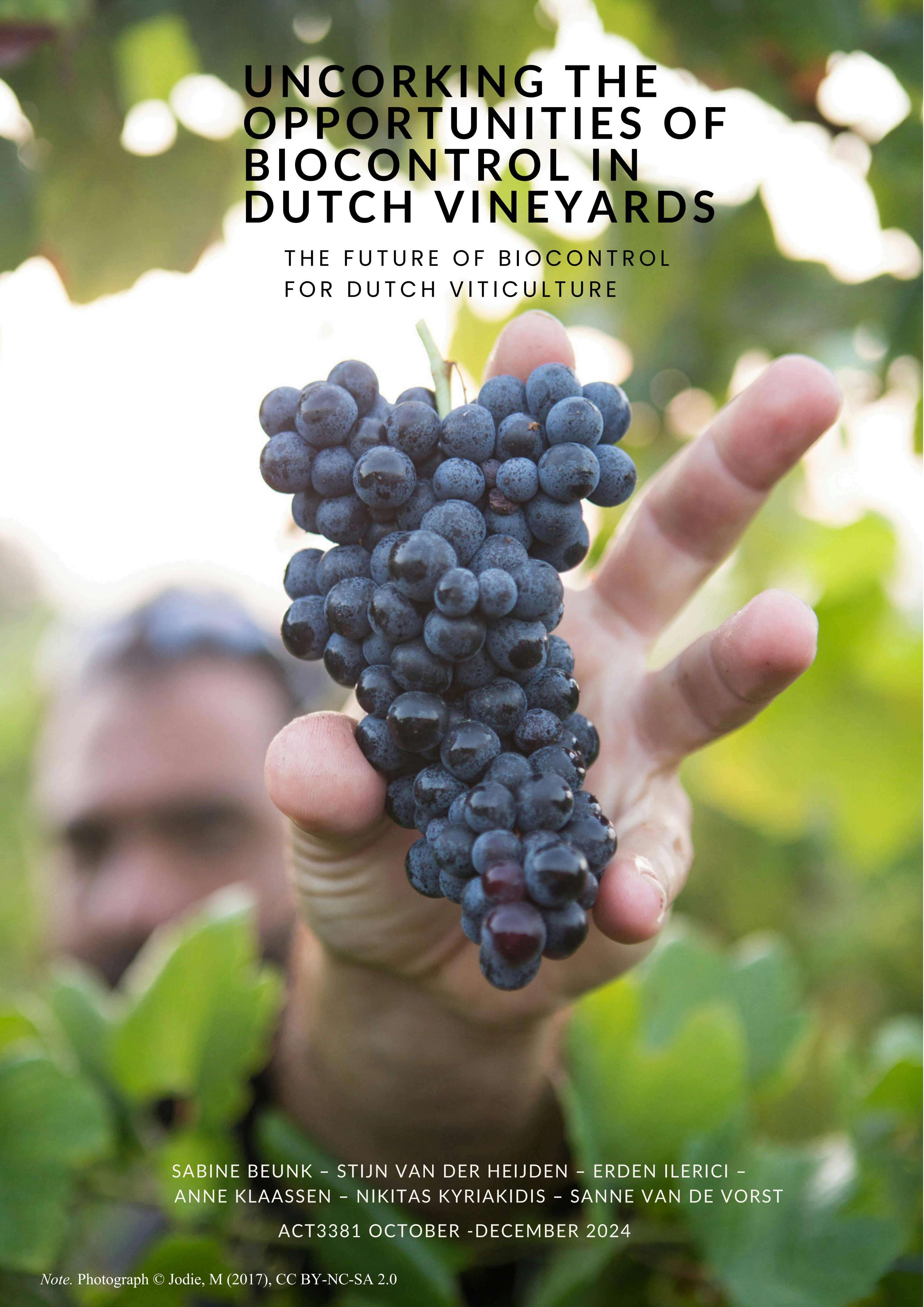

UNCORKING THE OPPORTUNITIES OF BIOCONTROL IN DUTCH VINEYARDS

THE FUTURE OF BIOCONTROL FOR DUTCH VITICULTURE

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The Future of Biocontrol for Dutch Viticulture

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1. Introduction

1.1 Introduction

Viticulture, the cultivation of grapevines, has a rich history and remains a vital agricultural activity in many European regions. As of 2020, there are approximately 3.2 million hectares of vineyards across the European Union, with Spain accounting for 28.5%, France for 24.5%, and Italy for 21.6% of the total vineyard area (Bukvić et al., 2020). Climate plays a crucial role in viticulture, influencing various factors such as microclimate, vine growth, yield, and ultimately the taste and quality of wine (Santos et al., 2020). However, recent decades have seen climate change profoundly impact viticulture, characterized by increased temperatures and unpredictable weather patterns. This shift in climate has made northern European regions, including the Netherlands, increasingly suitable for grape cultivation (Jones et al., 2021). Consequently, the number of grapevine growers in the Netherlands has been rising annually due to these warmer temperatures. In 2018, 136 commercial producers were registered and grown significantly over the past five years to 194 commercial producers (KvK, 2023)

Despite climatic conditions getting more favourable, Dutch grapevine growers face significant challenges from various plant diseases. The most detrimental pathogens affecting Dutch viticulture include grey mold, downy mildew, and powdery mildew (Rodríguez et al., 2020). In vineyards, fungicides are the primary practice to combat these fungal diseases. However, both Dutch and international regulations regarding pesticide usages becomes stricter and therefore limiting the approved registrations of pesticides as included in the EU Directive 2009/128/EC. This directive establishes a framework for the sustainable use of pesticides, and the EU Green Deal's 2019 "Farm to Fork" Strategy (European Commission, 2009; Leal and Gramaje, 2024). As part of these frameworks the European Commission has withdrawn pesticide use approvals or placed them on the list for substitution, set to expire by the end of 2025 (European Commission, 2018). This also includes the substitution of one of the most important antifungal compounds used in organic viticulture (Rantsiou et al., 2020). Such as commercially available copper-based compounds like copper hydroxide and copper oxychloride (EU Pesticides Database, 2024).

However, strict regulations and licensing requirements regarding the use of plant protection products in Dutch vineyards for amateur users, resulted in limited availability of most chemical products, affecting the Members of Wijnbouwers der Lage. The association of Wijnbouwers der Lage Landen comprises over 900 members, primarily hobbyists and pensioners managing small-scale vineyards. The association is dedicated to sharing knowledge through guidance, training, and resources aimed at optimizing wine growers. In response to these challenges, a sub-group within the association of Wijnbouwers der Lage Landen has emerged that is focusing on the most ecological and sustainable solutions possible. Additionally, this sub-group within the association is committed to sustainable farming practices that minimize reliance on active chemical compounds. While such compounds often are naturally derived elements, their application in large quantities can disrupt biological systems—sulphur being a prime example. Therefore, these members share a growing interest in potential biological control measures, focusing on biological antagonistic interactions as viable alternatives that can effectively reduce infection rates among amateur vineyard keepers.

The use of non-pathogenic microorganisms as biocontrol agents (BCAs), is representing an alternative to chemical products (Alabouvette et al., 2006; Fedele et al, 2020). While BCAs pose minimal risks to human health and environment, the BCAs are shown to be valuable in strategies to combat resistance through competition for nutrients and space, antibiosis, parasitism, and resistance induced in the host plant (Corkley et al., 2022). Among promising biological control agents, the use of antagonists belonging to *Trichoderma* species defend plants by producing biologically active compounds, including cell wall-degrading enzymes (Vinale et al., 2007). Another effective biological control measure is Serenade, based on *Bacillus subtillis*, inhibits plant pathogens by preventing spore germination (Highland & Timmer, 2004).

While biological control is recognized as an effective and sustainable approach in French viticulture for pest control (Charbonnier et al., 2021) and for fungal control in India (Sawant et al., 2017), there remains a critical knowledge gap regarding its practical application within Dutch viticulture. Simply implementing a biocontrol system from another region is unknown to yield success due to significant variations in environmental conditions—such as soil type, humidity, and temperature—that affect biocontrol activity (Leal et al., 2024; De Curtis et al., 2012). Given that climate and other environmental factors differ considerably between Dutch vineyards and traditional wine-producing areas, specific research into biocontrol activity tailored to Dutch vineyards is essential.

As the sub-group within the association of Wijnbouwers der Lage Landen already explored potential biocontrol opportunities independently, they encountered challenges stemming from diversity in both methods and timing among different vineyard keepers. This variability resulted in incomparable outcomes across vineyards and complicated efforts to draw research-based conclusions. Therefore, there is a pressing need for clear guidelines on suitable potential biological control approaches that can be utilized by Wageningen Research Open Teelten (OT) in collaboration with the vineyard keepers for future experiments.

1.2 Research questions

To support the internal goal of the subgroup within Wijnbouwers der Lage Landen of promoting ecological and sustainable viticulture, this research will focus on exploring potential biological control methods that Wageningen Research Open Teelten (OT) can evaluate in future experiments. The research will be guided by the following question:

"Which biological control measures are promising for disease control of powdery mildew, downy mildew and grey mold in Dutch vineyards, and could be evaluated on effectiveness in future experiments?"

In order to address this main question, the following sub-questions will guide the research:

- *"What are the lifecycles of the most prominent diseases - powdery mildew (Erysiphe necator), downy mildew (Plasmopara viticola) and grey mold (Botrytis cinerea) - in vineyards in the Dutch climate?"*
- *"What does the current Dutch viticulture look like in terms of grape varieties, cultivations systems, management practices, and diseases control measures?"*
- *"Which biological control measures can potentially be effective in Dutch vineyards, what are instructions for application, what are external effects of their application, are they commercially available, and what are the costs of application?"*
- *"What are the current disease management regulations for vineyards in the Netherlands?"*
- *"What are promising disease management practices, besides biological control measures, that could contribute to the efficient use of biocontrol measures?"*
- *"How can effective biological control measures be communicated to vineyard keepers in such a way they are willing to adopt new practices?"*

2. Methods

The research methodology for this project engaged a comprehensive approach to address multiple sub-research questions related to grape cultivation and disease management in Dutch vineyards. The methods utilized included interviews, literature reviews, multicriteria analysis, and regulatory document analysis.

2.1 interviews

Interviews were used as primary data collection method to get an impression of Dutch viticulture. Five in-person interviews were conducted with vineyard keepers of the Der Lage Landen from Gelderland, Flevoland and Limburg. Interview questions were composed based on the research questions and the literature review. The interviews questions can be found on the appendix (9.1).

The interviews were semi-structured. Semi-structured interviews are a qualitative data collection method that combines the systematic approach of structured interviews with the flexibility of unstructured formats (Adeoye-Olatunde & Olenik, 2021). This method is based on a predefined set of open-ended questions, ensuring consistency across participants while allowing the interviewer to investigate deeper into relevant responses.

Four vineyards (belonging to the interviewees) were visited, where observations were made regarding location, trellis system, training system, undergrowth, additional flora present, and overall vineyard health. With interviewee approval, photographic material was collected, and the interviews were recorded and transcribed using Sonix software (Sonix, Inc., 2024). The consent form can be found on appendix (9.2). The vineyard keepers stayed anonymous throughout the whole project.

Four of the interviews were conducted in Dutch, as the participants preferred to express themselves in their native language. The transcriptions were then reviewed, translated and coded to organize data into meaningful categories for in-depth analysis of the values and motives of the interviewed vineyard keepers.

2.2 literature research

Literature research was conducted to gain insight into the disease cycles of powdery mildew, downy mildew and gray mold. The research focussed on determining:

- Responsible pathogen
- Disease symptoms
- Factors influencing susceptibility to the pathogen
- Life cycle of the pathogen in the Dutch climate
- Most susceptible period of the vines to the pathogen

Furthermore, literature research was conducted to support interview findings regarding the current state of Dutch viticulture. With biological control methods mentioned in the interviews taken as starting point, a literature review was conducted to find biological control agents that could be effective in Dutch viticulture. The findings were divided into two categories: microbial biological control agents, and non-microbial biological control agents. The review focussed on determining:

- Biological control agents used against powdery mildew, downy mildew or gray mold, tested in on grapes
- Biological control agents used against powdery mildew, downy mildew or gray mold, tested on other soft fruits
- Effectiveness of the biological control agent against powdery mildew, downy mildew, gray mold, measured in or converted to % disease control
- External effects of application on the environment, human health or grapes
- Application rate per growing season
- Required equipment for application
- Commercially available products containing those biological control agents
- Allowance of those commercial products in the Netherlands, allowed users and allowed application form on open fields (e.g. soil application or foliar application)
- Dosage per hectare
- Costs per hectare per growing season

Lastly, literature research was conducted to provide scientific base for communicating the findings in this report to the vineyard keepers in such a way that it would receive support and foster implementation. The review was focussed on determining:

- Communication differences between organic and conventional farmers
- Communication transmission model
- Practical implication, taking the theory of planned behaviour into mind

The literature review process was structured to ensure thorough coverage of each research question. For every research and sub-research question, a minimum of three scientific articles, journals, academic publications, websites, or books were selected, prioritizing recent publications and sources approved by the academic community. The team focused on keywords for each research question to increase the effectiveness of knowledge gathering. The context quality is ensured by conducted mutual assessing the literature and revising the information that each person of the team found and wrote.

2.3 Multi criteria analysis

A multicriteria analysis (MCA) was developed to evaluate the potential of different biological control solutions. MCA is a decision-making framework used to evaluate and prioritize options when multiple, often conflicting criteria must be considered (Dean, 2020). It systematically assesses alternatives by assigning weights to criteria based on their relative importance and scoring each option against these criteria. The criteria and scores were formulated based on literature, the views of the vineyard keepers, conversations with the commissioner, academic advisor and coach. The weights of the criteria were determined based on answers of vineyard keepers during interviews when asked to distribute 10 points across the four categories. The answers can be found in paragraph 4.6.4.

Twenty biological control methods were assessed by the MCA, using four main criteria:

- Effectiveness against powdery mildew, downy mildew or gray mold
  - Measured in % disease control
- External effects of application on environment, human health or grapes
  - Measured in number of categories (environment, human health, grapes) that the biological control agents have a negative effect on
- Easiness of application
  - Measured in the number of applications per growing season and the requirement of specialized equipment
- Costs
  - Measured in the euros per hectare per growing season

Biological control methods received between 0 and 4 points for each criterion, depending on the findings from interviews or in literature. The explanation of the scores is found in table 1.

Table 1: *Score explanations for each criterion*

category	weight	score = 0	score = 1	score = 2	score = 3	score = 4
Effectiveness	0.30	Effectiveness not proven	disease control 0-25%	disease control 25-50%	disease control 50-75%	disease control 75-100%
External effects	0.30	Negative influence on 3 out of 3 (environment, human health, wine quality)	Negative influence on 2 out of 3 (environment, human health, grapes)	Negative influence on1 out of 3 (environment, human health, grapes)	Negative influence on 1 out of 3 (environment, human health, grapes) + positive external effects	only positive external effects or no external effects
Easiness of application	0.25	-	> 10 applications per year + special equipment needed	6-9 applications per year + special equipment needed, or >10 applications per year + no special equipment needed	2-5 sprays per year + special equipment needed, or 6-9 application per year + no special equipment needed	2-5 applications per year + no special equipment needed
Costs	0.15	-	>$1000/ha per growing season	$500 - $1000/ha per growing season	$300 - $500/ha per growing season	$100 - $300/ha per growing season

Total scores of biological control methods were calculated by multiplying the scores for each criterion with their respective weights, dividing that total score by 4 and subsequently multiplying with 10 to end up with a score between 0 and 10.

To take into account the difference between the experimental conditions of the studies found in literature and the real application condition, deduction scores were introduced. These deduction scores would be translated into a lower score for the effectiveness criterion, as the found effectiveness could not be guaranteed if experimental conditions deviated from real application conditions. Three deviations criteria from real applications conditions were considered:

- Not tested on grapes
- Not tested in the Dutch/Belgium climate
- Not tested in long term field experiments

Biological control methods received between 0 and 4 points for each deviation criterion, depending on the experimental conditions of the studies used for determining the effectiveness. The explanation of the deduction scores is found in table 2. Deduction on effectiveness per each score can be found in table 3.

***Table 2:** Score explanations for each deviation criterion*

Category	**weight**	**score = 1**	**score = 2**	**score = 3**	**score = 4**
Tested on grapes	0.50	Tested on other	Tested on tomato's peppers, eggplants	Tested on other soft fruit	Tested on grapes
Tested in Dutch climate	0.20	Tested in other climates	Tested in cool mediterranean (Csb) **or** warm-summer continental (Dfb)	Tested in Temperate oceanic climate (Cfb): south/southeastern England **or** Northern germany **or** Denmark **or** Northern France	Tested in NL or Belgium (Cfb)
Tested in practice	0.30	Tested in lab setting	Tested in greenhouse conditions	Tested in controlled field experiments	Tested in long term (>3 years) field experiments

***Table 3:** Deduction on effectiveness per score*

Score	**1**	**2**	**3**	**4**
Deduction	75%	50%	25%	0%

Deduction on effectiveness was calculated by multiplying the deduction belonging to the score with the weight of the specific deviation category, and subsequently summing the three outcomes. For example, if the experimental conditions were similar to the real application conditions, 4 points were granted for each deviation criterion and no deduction on the effectiveness score was performed. If the biological control method scored 1 point on each deviation criterion, a deduction of 75% was applied on the score on effectiveness. If the biological control method scored 4 points on the first deviation criterion, 2 on the second deviation criterion, and 3 on the third criterion, a deduction of (0.50*0%) + (0.20*50%) + (0.30*25%) = 17% is applied to the score on effectiveness.

To cope with lacking information regarding the four criteria, range was introduced. If no information was available about a certain criteria, it did not receive a score. The maximum amount of points that could have been earned for that criteria was than added as a range later, to indicate to which extend the total score could increase when new information becomes available. For example, a certain method earned 4 points for the first three criteria (effectiveness, external effects, easiness of application), but did not receive a score for costs due to lacking information. The total score is then ((0.30*4)+(0.30*4)+(0.25*4))/4*10 = 8.5. However, a maximum of (0.15*4)/4*10 = 1.5 points could have been earned if information about costs was known. This means that the actual score is somewhere between 8.5 and 10.

The total scores (lower limit of range) for each biological control solution were then used to create a ranked list, with the highest-scoring solutions considered most suitable and ready for future experiments by the Wageningen Research OT. The team selected biological control solutions that are approved for use in grapevines in the Netherlands and achieved an MCA score higher than 5.5, which was the threshold set by the team.

2.4 Products

The final stage of the research process involved synthesizing data from the literature review, interviews and MCA into a comprehensive report. This report includes background knowledge on the three fungal diseases, key findings on current Dutch viticulture, a list with promising biological control methods ranked based on the MCA, and promising non-microbial diseases management strategies. The final advice of this research only includes biological control agents that are commercially available and are allowed to be used on grapes in the Netherlands, as they will be evaluated in experiments next year.

3. Results

3.1 Disease cycle of the three relevant diseases for grapevine health

Understanding the main diseases that threaten grapevine health is crucial for managing vineyard productivity and sustainability. This subchapter will therefore answer the sub question: *"What are the lifecycles of the most prominent diseases - powdery mildew, downy mildew and grey mold - in vineyards in the Dutch climate?"*. An in-depth overview of the most prevalent fungal diseases affecting Dutch viticulture is provided, focusing on their biology, life cycles, and the environmental factors that influence their severity.

3.1.1 Grey mold (Botrytis Cinerea)

Grey mold, or bunch rot is caused by *Botrytis cinerea* (Mundy et al., 2022). As a necrotrophic pathogen, *B. cinerea* can survive for long periods without a host, remaining viable in the soil and thriving on (dead) plant material and grape debris on the vineyard floor. This ability to persist in the vineyard environment makes controlling the disease more difficult (Pretorius and Høj, 2005). *Botrytis. cinerea* can often cause severe damage to grapevines to an extent that grapes are not suitable for wine production due to berry rotting as well as the reduced flavour, colour and storage stability from the fungal produced laccase enzymes (Pszczolkowski et al., 2001). Moreover, *B. cinerea* infection can open the way for increased susceptibility to other organisms. Leading to annual profit losses of US$ 34 million and US$ 22.4 million in respectively Australia and Chili (Esterio et al., 2009; Scholefield and Morison, 2010).

Botrytis cinerea is highly influenced by weather and microclimatic conditions. Prolonged periods of high humidity or rainfall, especially when coupled with mild temperatures, create sustained moisture on berry surfaces, providing ideal conditions for infection and disease progression (Steel et al., 2013). Excessive irrigation and rainfall can also lead to berry skin splitting, increasing the likelihood of *B. cinerea* infection. In addition to weather conditions, the cluster microclimate plays a significant role in determining susceptibility to the disease. Vines with dense, closed grape clusters trap moisture, providing an ideal environment for *B. cinerea* growth (Steel et al., 2013).

Botrytis cinerea survives the winter in two distinct forms: the fungus survives as mycelium in dead plant material, such as mummified berries, canes, and in small, hard resting structures called sclerotia (Elmer and Michailides 2004; Jacometti et al., 2010). These sclerotia are particularly resilient, therefore allowing the fungus to endure adverse weather conditions, including the cold winter months. When spring arrives, with warmer and wetter conditions, both the mycelium and sclerotia start producing conidia, the asexual spores that can be dispersed by wind, rain, and human activity, as shown in figure 1. These spores then settle on plant surfaces, where they most often remain dormant until conditions become favourable for infection (Jacometti et al., 2010).

The primary infection period occurs during the bloom stage, when the flower caps abscise and fall off, around the end of May and June. This process leaves small wounds at the sites where the caps have abscised, creating entry points for the fungal conidia. At this early stage, the fungus enters the plant tissues but remains latent (inactive and undeveloped), causing no visible symptoms until later in the growing season (Keller et al., 2002). These latent infections can remain dormant in the floral tissues until the grapes begin ripening, from August onwards. This natural resistance of the unripe berries is largely due to the presence of antifungal compounds such as resveratrol and other phenolic substances, which act as a biochemical defense barrier against the fungus (Steel et al., 2013). The ability of *B. cinerea* to remain dormant until just days before harvest makes it especially difficult to detect early infections and take effective management actions in the field.

The second and more critical period begins during véraison, the stage when the grape berries begin to soften and swell. During this period, significant physiological changes occur in the grape berries as sugar levels rise, acidity decreases, and the berries become more susceptible to fungal infections (Mundry and Beresford, 2007). The thinner skins and the increase in berry water content create an ideal environment for the fungus to develop. The infection during this period is not only influenced by environmental factors such as humidity and rainfall but also by mechanical and environmental damage (González-Domínguez et al., 2015). Vineyard practices, including the use of machines and wire lifting, as well as natural causes like hail, frost, berry splitting due to powdery mildew infection, and feeding by birds or insects, all create entry points for the fungus (Elmer and Michailides 2004; Keller et al., 2002). Once one berry is infected by the fungus, it can quickly spread to neighboring berries, particularly in compact grape clusters (Latorre et al. 2015). Therefore, increasing susceptibility varieties with thin skins and compactness of clusters, such as Pinot Noir, Chardonnay, Gewürztraminer, and Sauvignon Blanc (Paňitrur-De La Fuente et al, 2017).

Botrytis cinerea is a unique pathogen due to its ability to occasionally provide beneficial outcomes under specific conditions. Moist nights, foggy mornings, and dry, sunny afternoons create the ideal conditions for the gradual infection that leads to noble rot. In contrast, heavy rainfall and persistent high humidity encourage the rapid spread of the more aggressive grey mold (Negri et al., 2017; Steel et al., 2013). The process of noble rot concentrates sugars and produces glycerol rot. Noble rot is highly valued in the production of late-harvest dessert wines, such as Sauternes from France and Tokaji from Hungary (Steel et al., 2013). Despite this beneficial application, the overall impact of *B. cinerea* remains largely negative.

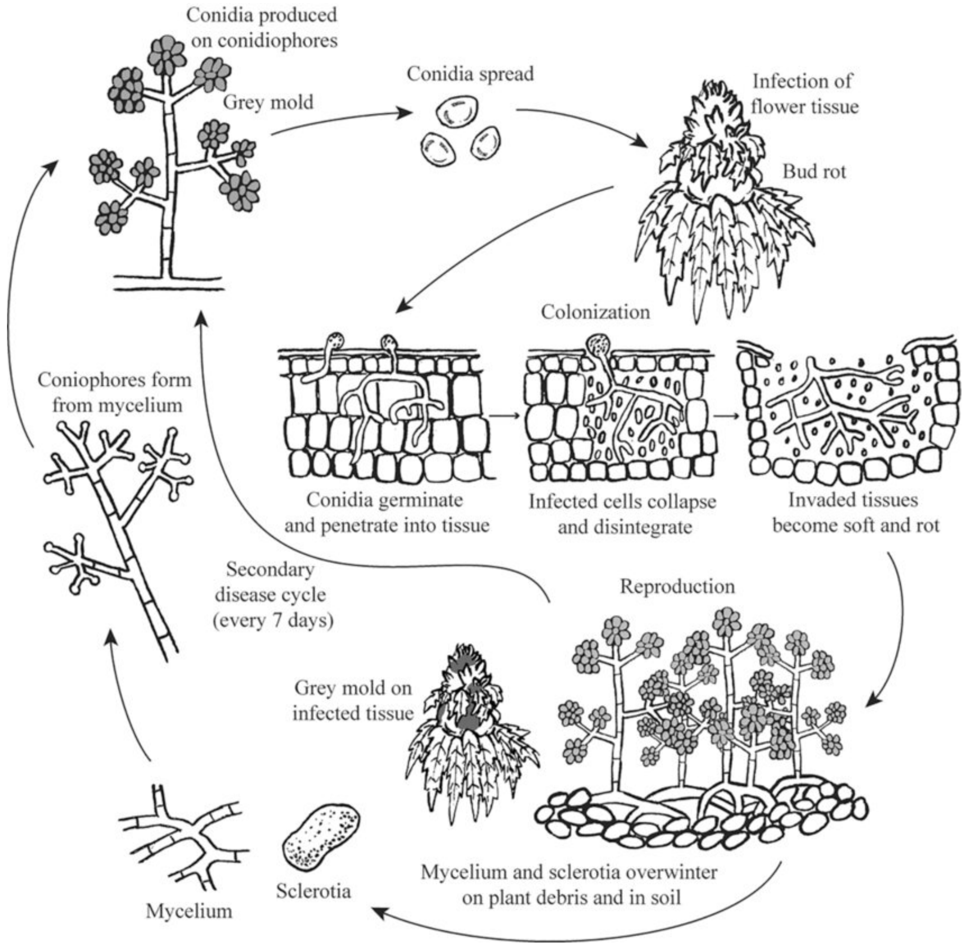


Figure 1: *Disease cycle of Botrytis cinerea, illustrating key stages from overwintering as sclerotia and mycelium to spore dispersal and infection to colonization, reproduction followed by secondary spread under favourable environmental conditions (Mahmoud et al., 2023).*

3.1.2 Downy Mildew (Plasmopara viticola)

Plasmopara viticola, which causes downey mildew, is an oomycete organism that can develop without the presence of a host (Gessler et al., 2011). The disease originated in North America and was introduced to Europe following the importation of American vine species to combat the insect pest phylloxera (*Dactylosphaera vitifoliae*). This introduction occurred in the late 19th century, around 1878, and had a profound impact on European viticulture, fundamentally changing grape cultivation practices in the viticultural region of Europe (Koledenkova et al., 2022).

While the economic damage caused by downy mildew has not been precisely quantified by any European country, studies in Western Australia have estimated the annual cost to be around AUD $7.3 million, which translates to approximately €4.5 million (Taylor and Cook, 2018). This gives an indication of the potential economic impact of the disease on viticulture.

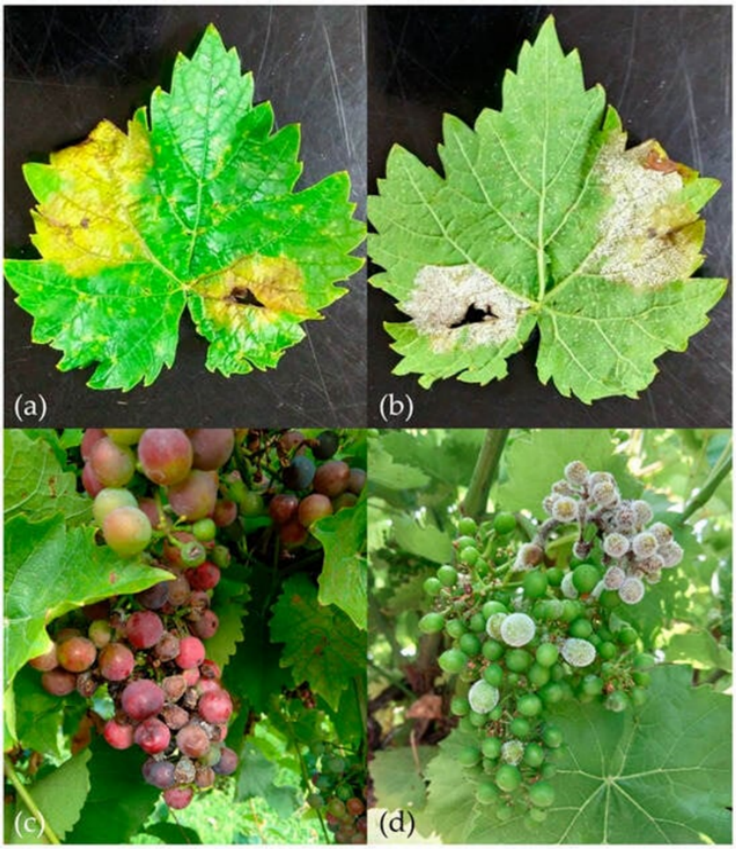


Figure 2: *Symptoms of downy mildew on a) the front side of a leaf, b) the back side of a leaf, c) on ripe grapes and on d) unripe grapes (Clippinger et al., 2024).*

The main symptoms of *P. viticola* infection include oil spots on leaves (Figure 2 a) and white downy growth on the underside of affected leaves (Figure 2 b), which impairs photosynthesis. Young berries turn brown, shrivel, and drop off (Figure 2 d), while older berries develop a hardened texture and show discoloration (Figure 2 c), reducing their quality (Clippinger et al., 2024). Severe infections can lead to premature defoliation, weakening the vine and significantly affecting the berry ripening process, ultimately resulting in a drop in yield quality (Moriondo et al., 2005). The symptoms of downy mildew are illustrated in figure 2.

The life cycle of *P. viticola* is complex and heavily influenced by environmental conditions. It can be divided into several key phases. The pathogen overwinters as oospores, which are produced under dry conditions or when leaves begin to senesce. These oospores are embedded in infected leaf tissue and mature over the winter months, surviving in fallen leaves and soil until suitable conditions for germination arise in the spring (Gessler et al., 2011).

In spring, typically around late April when soil temperatures are greater of 10°C, mature oospores germinate and release zoospores (Clippinger et al., 2024). These zoospores require a film of water to infect young grapevine tissues, which usually start budding in March (Gessler et al., 2011). This primary infection phase coincides with the grapevine's bud burst and initial leaf development stages, occurring from April to June, and can extend into July depending on environmental conditions.

Once primary infections are established, *P. viticola* undergoes asexual reproduction throughout the growing season (Clippinger et al., 2024). This involves the formation of sporangia on sporangiophores that emerge from infected grapevine tissues (Figure 3), typically under humid conditions (Gessler et al., 2011, Kennelly et al., 2007). These sporangia release motile zoospores that spread to other grape tissues, particularly during wet conditions, perpetuating secondary infections through multiple cycles as long as favorable conditions persist (Kennelly et al., 2007).

The secondary infection cycles are most active during the warmer, wetter summer months, primarily from July to September (Brischetto et al., 2021). During this period, secondary sporangia continue to spread the disease throughout the vine canopy, especially under humid conditions favourable for pathogen sporulation.

As the grapevine progresses through its development stages, the disease dynamics evolve. From April to June, during the bud burst and flowering stages, primary infections occur when oospores release zoospores that infect young leaves (Poeydebat et al., 2022). This initial infection phase is critical as it sets the stage for subsequent disease spread.

From June to July, as the weather warms and the grapevine enters the flowering and fruit set stages, secondary infections begin (Kennelly et al., 2007). This phase is characterized by repeated cycles of sporangia production and zoospore release (Figure 3), especially under humid conditions (Gouveia et al., 2024). This period is crucial because it coincides with flowering and early fruit formation, stages that are highly vulnerable to disease impact.

As grape berries grow from July to August, secondary infections continue (Brischetto et al., 2021). This phase is highly susceptible to mildew spread because dense leaf canopies increase humidity, aiding pathogen proliferation. The dense foliage creates an ideal environment for the pathogen to spread and infect more tissues.

Finally, with cooling temperatures in September, the grapevine reaches ripening and senescence. During this time, *P. viticola* produces oospores that settle in leaf litter (Figure 3), preparing to overwinter and restart the cycle in the spring (Rhouma et al., 2024, GESSLER et al., 2011). This completes the life cycle of *P. viticola*, ensuring its survival and the potential for future infections.

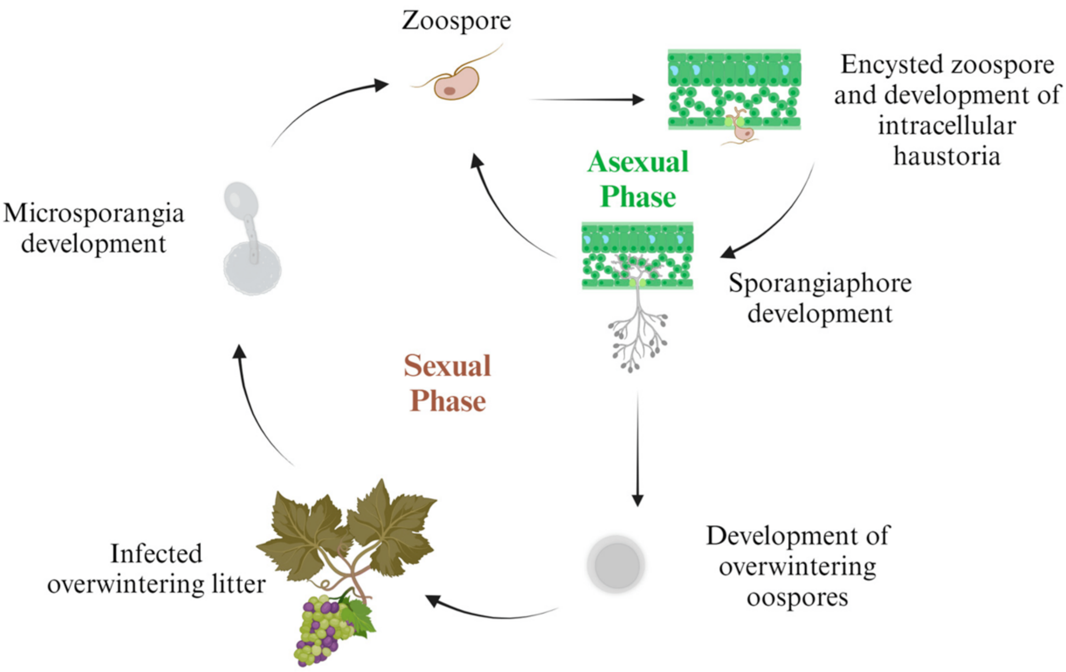


***Figure 3**: The life cycle of Plasmopara viticola (Clippinger et al., 2024).*

3.1.3 Powdery Mildew (Erysiphe necator)

Powdery mildew, is caused by the pathogen *Erysiphe necator*, previously known as *Uncinula necator*. This pathogen originates from America and can cause substantial economic losses as it can infect the green tissues of the plant and negatively impact the quality of the grapes and consequently the wine. (Miladinović et al., 2007).

Powdery mildew is a biotrophic fungal pathogen that is characterized by white powdery patches on the upper service of the leaves; in severe cases the infection will cause the leaves to curl and drop prematurely. Additionally, some black discoloured areas may appear on the infected areas after the powdery growth is removed or washed away (Thind et al., 2006).

Temperature plays a critical role in the development of powdery mildew. The pathogen thrives within its optimum temperature range, which is between 20-25°C, though it can still develop at temperatures between 6-32°C. While powdery mildew prefers dry conditions, it is still able to grow in in environments with high humidity (Thind et al., 2006). *E. necator* has two primary mechanisms to overwinter and survive until the following growing season:

The pathogen survives in dormant buds as mycelium, a network of fungal hyphae, or conidia within the buds of the grapevine. In spring, the mycelium will continue to spread when the buds of the grapevine get out of dormancy and vegetative growth resumes. The emerging grapevine shoots become overgrown with fungal mycelium, which produces conidia. These conidia release asexual spores, enabling the fungus to spread to uninfected plant tissues (Thind et al., 2006; Halleen & Holz, 2017).

The pathogen is also able to form cleistothecia, which are fungal fruiting structures, on infected tissues (Halleen & Holz, 2017). Furthermore, cleistothecia are able to overwinter and release ascospores in spring, which are spread due to rainfall. These spores are dispersed by wind and water, allowing the pathogen to infect new plant tissues. Cleistothecia formation begins around late July, with its abundance directly correlated to the severity of the disease rather than environmental factors or host characteristics. Higher disease severity results in greater cleistothecia production (Halleen & Holz, 2017).

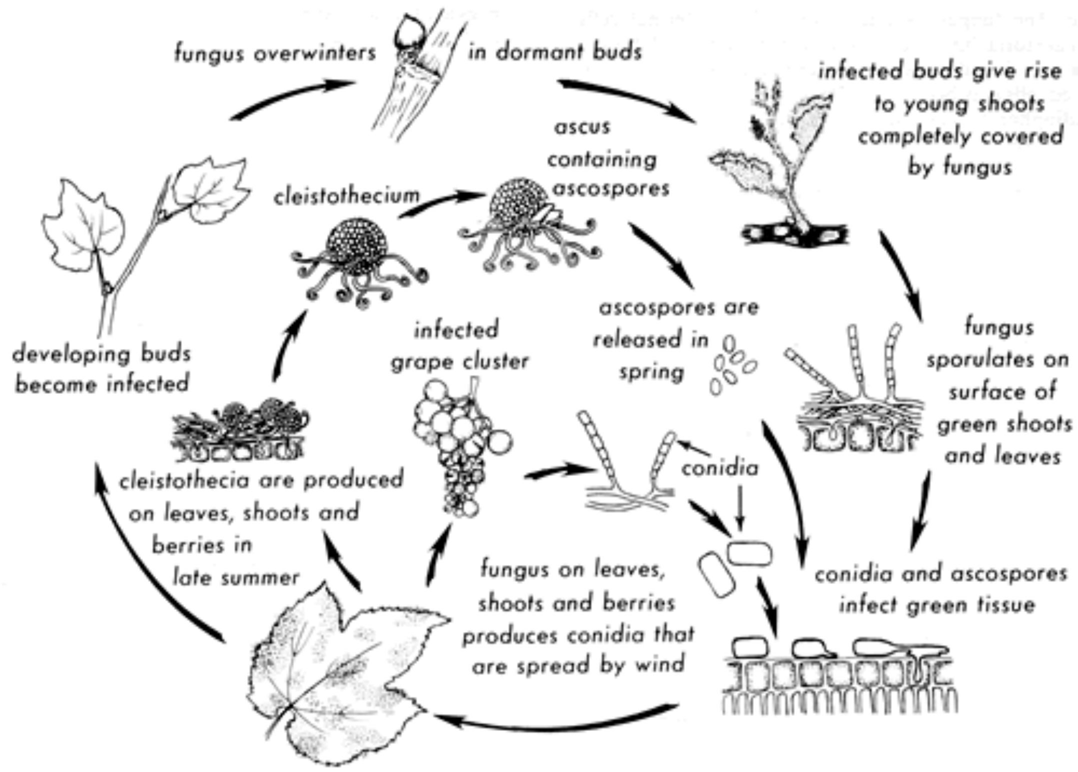


Figure 4: *Life cycle of powdery mildew (*Erysiphe necator*) has two primaty mechanism to survive winter. The first is by asexual spores that overwinters in dorment buds of the grapevine. Secondly, they survive by sexual spores produced by cleistothecia (Jackson, 2014).*

3.2 Current situation

Understanding the Dutch viticulture context is essential for proposing suitable solutions. This subchapter will therefore answer the sub question: *“What does the current Dutch viticulture looks like in terms of grape varieties, cultivations systems, management practices, and diseases control measures?”*. It gives a broad description of Dutch viticulture, based on data collected with interviews and supporting literature research. It discusses the influence of the Dutch climate, geography and hydrology on viticulture practices. In addition, special attention is paid to disease management in Dutch vineyards and frequently used measures/products.

3.2.1 Dutch climate

The Dutch climate, classified as Cfb under the Köppen-Geiger system, is a temperate oceanic climate (*World Bank Climate Change Knowledge Portal*, n.d.). This climate is characterized by mild winters and cool summers, with relatively moderate temperature fluctuations throughout the year. Precipitation is fairly evenly distributed across the seasons, with no distinct dry period, making the region susceptible to consistent rainfall and high humidity levels (Papacharalampous et al., 2023). These climatic conditions are a direct influence on viticulture practices in the Netherlands.

3.2.2 Grape varieties

Due to the moderate temperatures and high precipitation levels, grapevines in the Netherlands often experience challenges in ripening compared to warmer, drier wine regions (Keller, 2020; Cyr et al, 2010). The mild winters and cool summers limit the growth period, meaning that grape growers must select grape varieties that ripen in shorter growing seasons (Jones et al., 2010). One of the most common practices towards more sustainable Dutch viticulture is the planting of the so-called PIWI’s, short for pilzwiderstandsfähige Rebsorten. These grape varieties are specifically bred for fungal resistance to downy mildew and powdery mildew. Both fungal pathogens were introduced to Europe in the 1870s, likely through importation of American rootstocks, that had resistance to Phylloxera. These cultivars were used to graft with the highly susceptible European cultivars (Peressotti et al., 2010). With the introduction of the American rootstocks, downy and powdery mildew came along and has spread across Europe where they remain a major challenge in viticulture as of today. Because all *V. vinifera* cultivars are susceptible to both powdery and downy mildew, resistance must be found from other *V. vinifera* species (Peressotti et al., 2010, link). Through the classic crossing of American wild grapevines, like the *Muscadinia rotundifolia*, with natural resistance to the pathogens and European grapevines, which stand for high wine quality, it has been possible in recent years to breed new sustainable grape varieties in Germany that hardly require any plant protection. PIWI’s are seen as the most effective tool for increasing organic agriculture (Vršič and Vršič, 2021). In German viticulture, the PIWI varieties are gaining popularity over the last few years (Kiefer and Szolnoki, 2023). Moreover, among the interviewed Dutch vineyard keepers the majority is currently using

PIWI's, such as Johanniter, Solaris, Souvigner gris and Rondo (interviews, 2024). An overview of all grape varieties cultivated in the Netherlands can be found in appendix 9.3.

3.2.3 Site selection

Vineyards in the Netherlands are primarily situated in the provinces of Limburg and Gelderland due to their favourable microclimate and terrain, although smaller numbers of vineyards are found in other provinces (De Boer, 2024). Limburg and parts of Gelderland provide a slightly warmer and sunnier environment compared to the rest of the Netherlands, which helps to ripen the grapes more effectively (KNMI, 2023). Vineyards are commonly planted on slopes or in areas with well-draining soils to prevent excessive moisture accumulation, which can be harmful to vine health (Sanchez et al., 2024). Limburg's hilly landscape offers optimal drainage and increased sun exposure. Northern Limburg and parts of Gelderland receive relatively little precipitation compared to the rest of the Netherlands, reducing the risk of grapevine diseases (KNMI, 2023).

3.2.4 Soil properties

Vineyards located on silty-clay soils have a smaller rooting depth because of the moderately high-water table, but also faster growth due to absence of water stress, (interviews, 2024; Smart et al., 2006; White, 2003). Vineyards located on heavy clay soil could experience the opposite effect, where plant roots die because of prolonged lack of oxygen due to the high-water table (interviews, 2024; Manghwar et al., 2024). A vineyard located on sandy soil, with some clay ridges in the deeper soils layers, noticed decreased plant productivity on the location above those ridges (interviews, 2024). An explanation for this could be that the roots are not able to explore the deeper soil layers, due to physical limitations and lack of oxygen (Echeverría et al., 2017). Vineyards on loess soils (often classified as silty-loam) benefit from a lower water table than clay soils, but higher water holding capacity than sandy soils (interviews, 2024; University of California, Division of Agriculture and Natural Resources, n.d.). On sandy soils, irrigation for young plants is needed as they cannot reach the water table and the soil cannot retain much water (interviews, 2024; White, 2003). Multiple vineyards in the Netherlands are located on flood plains, where sediments deposited along rivers provide specific terroir to the wine. (interviews, 2024; Seguin, 1986).

3.2.5 Trellis system and training system

Due to cooler temperatures and high humidity, Dutch vineyards often use trellis systems that maximize sunlight exposure, like Vertical Shoot Positioning (VSP) or the Geneva Double Curtain system (GDC) (interviews, 2024).

In the VSP trellis system, vine shoots are trained vertically in a narrow upright structure, with the fruiting zone positioned below (as shown in figure 5). This design is widely used to control shading, which is essential in a cool climate (Danko et al., 2024). However, it is best used with low-vigour vines as the canopy could become too dense with high-vigour vines (Goldammer, 2018). The VSP trellis typically includes four to six tiers of wire, with the cordon or fruiting wire set approximately 90 cm above the ground. Moveable catch wires above the cordon guide the shoots to grow upward. The height of the top wire is usually between 150 and 180 cm. The shoots are often pruned at the top, creating a hedge-like row.

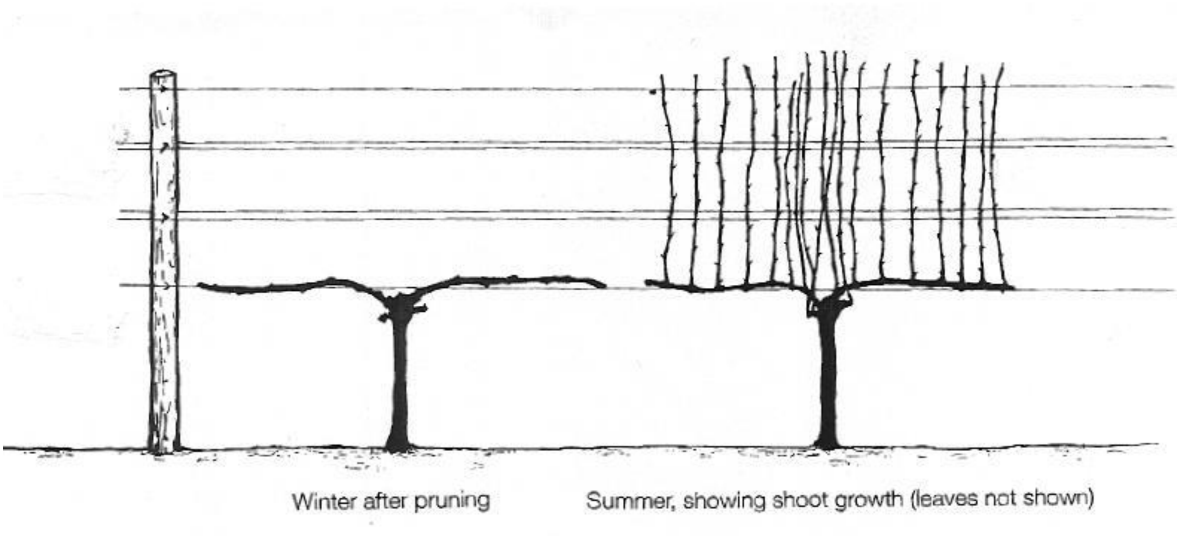


Figure 5: *Vertical Shoot Positioning trellis method (Trellis Systems | Best Practices Guide, n.d.)*

In the GDC trellis system, vine shoots are trained to hang down vertically from a high fruiting wire, creating a "curtain" of foliage and fruit (as shown in figure 6). The open hanging canopy improves sunlight penetration (Zoecklein, 2008). The fruiting wire is typically 150–180 cm above the ground. This system is particularly useful for high-vigour vines (Goldammer, 2018).

Figure 6: *Geneva Double Curtain trellis method (GmbH, 2019)*

Dutch vineyards often use the guyot method (interviews, 2024), which is the main training technique for VSP (Danko et al., 2024). In the Guyot system, each vine is typically trained with one (or two) main cane(s), and one or more short spurs (which carry the buds for next season's growth) left near the base of the vine (as shown in figure 7). These spurs can also be used to renew the cane each year, ensuring the vine's longevity. The susceptibility of cane-pruned vines to spring frost is usually less than that observed in spur-pruned fines (Poni et al., 2022). Another commonly used training method is the cordon system (interviews, 2024). In this system, the main trunk of the vine is trained horizontally along a wire, with fruiting spurs spaced evenly along the cordon (as shown in figure 7).

Vineyard's keepers use soft pruning in addition to guyot or cordon training techniques (interviews, 2024). This technique minimizes pruning wounds, thereby reducing the risk of trunk diseases such as *Esca* and *Eutypa* (Rosace et al., 2023). It involves smaller, strategically placed cuts close to younger wood, respecting the natural sap flow of the vine. By preserving the vine's vascular system and avoiding severe disruptions, soft pruning improves vine health and longevity (Simonit et al., 2023).

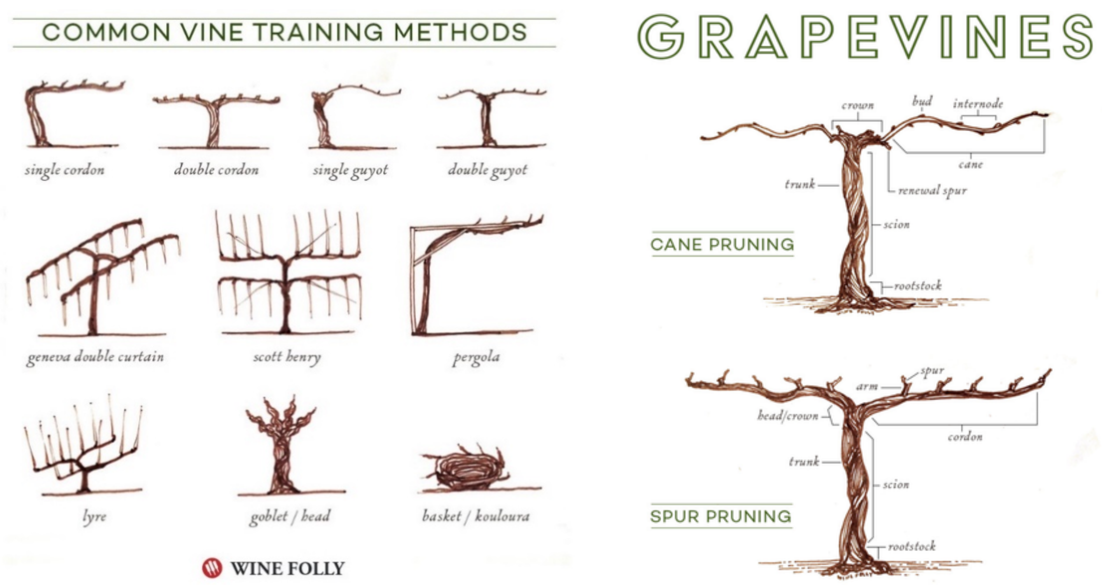


Figure 7: *Training and pruning methods (Illustrated Grape Vine Training Methods | Wine Folly, n.d.)*

3.2.6 Management practices

High precipitation levels and relatively high-water tables mean that drainage systems are critical to prevent waterlogging, as this could lead to damage and increase disease pressure (Sanchez et al., 2024). Dutch vineyards mostly rely upon natural precipitation for watering their vines, though supplemental irrigation may occasionally be used in drier periods or on establishing plants (interviews, 2024).

Given the typically high-water table and risk of soil compaction, cover crops like grasses and legumes are often used to prevent erosion and improve drainage in Dutch vineyards (interviews, 2024; Abad et al., 2021). The cover crops are cut once a year to avoid moisture build up around the plant, which could increase disease susceptibility (interviews, 2024). Furthermore, vineyard keepers indicated to use as little tillage as possible and avoid using heavy machinery in order to prevent soil compaction (interviews, 2024). Cover crops can also help to control weeds naturally and add soil organic matter, which is beneficial to plant growth (interviews 2024; Miglécz et al., 2015; Mcgourty et al., 2000). Some vineyards add natural fertilizer (e.g. plant residues, manure, mushroom residue, wood chips) to the soil to increase organic matter content and stimulate soil microbes and fungi (interviews, 2024; Zhang et al., 2012).

Due to high rainfall and humidity, canopy management is crucial for reducing mildew and botrytis in Dutch vineyards. Techniques like leaf thinning and shoot positioning are used to minimize shading and allow more airflow (interviews, 2024; Rombough, 2002). Some vineyard keepers indicated that they remove grapes to reduce the susceptibility to botrytis, especially when varieties produce very dense clusters of grapes, which potentially can lead to cluster rot (interviews, 2024; Steel et al., 2013; Hed et al., 2009). However, it is important to leave some bunches that offer competition to decrease the risk of either overly vigorous vegetative growth or excessive yield, which both lead to poorer fruit quality (interviews, 2024; Kliewer et al., 2005). Avoidance of chemical fertilizer was also mentioned as a way to prevent vigorous fruit growth (interviews, 2024).

Some Dutch vineyards utilize windbreaks, such as trees, to shield vines from strong winds while still promoting air circulation (interviews, 2024). Vineyard keepers might use frost protection techniques, such as over-vine sprinklers, to protect buds in early spring (interviews, 2024).

3.2.7 Yearly schedule

In winter, the vines are dormant, and pruning takes place while the branches are tied. In January and February, the vineyard owner prunes dry, woody branches to prevent uncontrolled growth of the vine. This pruning is crucial for grape cultivation as it helps determine the number of grapes that will grow on the vine (Previtali et al., 2022). Fewer branches and buds result in fewer, but higher-quality grapes. This process ensures better control over the vine's productivity and grape quality for the upcoming season. As spring begins, the plant awakens from its dormancy and gradually starts to bloom. The branches that were selected during winter pruning are bent and tied to wires. As the buds begin to grow, there's a risk of frost damage, which can kill the buds (Meier et al, 2018). On warm, sunny days, the vine grows quickly, and the vineyard keeper removes extra shoots to prevent the vine from becoming too vigorous. This ensures that nutrients and energy are directed to the right branches, where the grape clusters will eventually form (Palliotti et al., 2011). During summer, it's time to thin the grape clusters and remove leaves from the vine. From the flowers, small clusters of hard, green grapes begin to form. These continue to grow throughout the summer and gradually change colour. Meanwhile, the vine shoots also keep growing and must be tied up. Leaves surrounding the grape clusters may be removed to improve airflow and reduce the risk of fungal infections (Nicolosi et al, 2012). The vineyard keeper must remain vigilant as the vineyard faces threats from diseases, pests, or even sunburn. In august, the vines start to ripe. The sugar content in the grapes increases, the stems become thicker, and the colour of the seeds darkens. Grapes that are still unripe and not changing colour are cut off. This ensures only the best grapes remain to ripen for harvest, improving the quality of the yield. If there are too many leaves, the vineyard keeper may prune them to optimize the vine's growth and grape quality (Palliotti et al., 2011). In autumn, the harvest is the central focus. Depending on the grape variety, the vineyard keeper begins harvesting in September or October. Until then, there are many risks, such as fungal infections or damage from insects. Heavy rain or hail can also harm the crop. If the grapes survive and ripen properly, the harvest begins. Since Dutch vineyards are generally smaller and managed by hand, hand-harvesting is common (De Boer, 2024). This approach allows for selective picking, particularly important in unpredictable Dutch autumns where weather conditions can vary widely.

3.2.8 Pest and disease management

Due to high humidity, Dutch vineyards are prone to fungal diseases like downy and powdery mildew, as well as Botrytis (Danko et al., 2024). Disease management is essential and for commercial growers, this often involves using chemical pesticides. The target group of this project, amateur vineyard keepers or small biological vineyards, is restricted by the Dutch regulations and cannot use most commercially available pesticides (interviews, 2024). Therefore, they mainly focus on organic treatments like sulphur and biological control when necessary (interviews, 2024).

Sulphur is effective against powdery mildew, applied preventatively and often in combination with other treatments (Savocchia et al., 2010; Williams, 2004). Copper compounds are used as foliar application to control downy mildew but are not allowed in Netherlands from 2025 onwards (ctgb, 2024). As an alternative to copper, potassium phosphonate can be used as curative treatment against downy mildew (*AANLEG EN BEHEER VAN DE WIJNGAARD. – Domein Wolder*, n.d.; Speiser et al., 2000). However, the application of potassium phosphonate inevitably leads to residues in the wine (Speiser et al., 2000). Potassium phosphite works as preventive and curative treatment (Pinto et al., 2012). It is applied as elicitor that activates the plant's own defence mechanisms, but also inhibits growth of the pathogen (Jackson et al., 2000).

Products based on *Trichoderma spp.* have received much attention lately as promising biological control method in viticulture for managing fungal diseases such as powdery mildew and downy mildew (Sawant et al., 2017; Küpper et al., 2023). These beneficial fungi work through multiple mechanisms, including competition for nutrients and space, production of antifungal compounds, and mycoparasitism, where fungi of the genus *Trichoderma* directly attack and degrade the cell walls of pathogenic fungi (Tyśkiewicz et al., 2022). Additionally, Saravanakumar et al. (2015) showed that *T. asperellum* CCTCC-

RW0014 enhances plant resistance by releasing elicitors, which primes the vine's immune system for future pathogen attack. Trichoderma is mainly applied as foliar spray for curative treatment, but also soil spraying occurs to prevent the pathogenic fungi from surviving through winter (interviews, 2024). Commonly used strains are *T. harzanium* T9 for foliar application, *T. harzanium* T22 for soil application, and *T. atroviride* SC1 (product Vintec®) to combat trunk disease Esca (interviews, 2024). Several vineyard keepers indicated to spray compost tea on the leaves four days after the application of Trichoderma (interviews, 2024). Composition and manufactures of the compost tea's differed (interviews, 2024). Ketterer (1990) found that spray application of horse manure compost tea was able to reduce botrytis on grapes, while Tränkner (1992) found that horse manure compost tea was able to reduce downy mildew and powdery mildew. Evans et al. (2012) found that aerated compost tea, prepared from immature compost, was able to supress botrytis and powdery mildew on grapes. However, no scientific literature is available about the combined effect of Trichoderma and compost tea. Furthermore, it is difficult to produce the exact same compost tea composition twice. It is believed that the vines are temporarily more susceptible to diseases after Trichoderma application, because also the beneficial microbes are removed by Trichoderma (interviews, 2024). By adding compost tea, the beneficial microbe community on the leaves is believed to re-establish quicker (interviews, 2024). These statements need to be evaluated in future research. A vineyard keeper used the aerobe sap from horsetail (*Equisetum Telmateia)* to make plant extract spray (interviews, 2024). Plant extracts from *E. Telmateia* are known to have antibacterial and-fungal effects (Radojevic et al., 2012). The spray was applied to the vines together with the compost tea, and seemed to visually benefit plant health (interviews, 2024). Other products used for re-establishment of the microbiome are soft cheese or milk (interviews, 2024), but these contain anaerobe bacteria while compost tea consists of aerobe bacteria. Aerobic produced compost teas are known to contain a larger microbial diversity and abundance than anaerobic produced compost tea's (Scheuerellet al., 2002). Further research is needed to investigate the influence of this on the re-establishment process.

A relatively new product used by vineyards keepers is Oenosan® (interviews, 2024). Oenosan® is produced by Oenosan (Westkerke, Belgium). It is microfine calcite which is applied to leaves and is said to act on the stomata. There, it induces plant defence reactions by activating calcium-dependent signalling pathways. This defence reaction would prime the plants for future danger, such as mildew infection (interviews, 2024). Although scientific studies on the effect of Oenosan® are not conducted yet, calcium carbonate treatments have proved to influence stomatal behaviour and defence priming by activating reactive oxygen species production and triggering immune responses (Negi et al., 2023).

Another product that was mentioned was VitiSan (interviews, 2024). VitiSan is produced by Andermatt (Leersum, Netherlands). It is a contact fungicide used to control powdery mildew, with potassium bicarbonate as the active ingredient. It is effective both preventively and curatively. The fungicide works through a combination of mechanisms (*VitiSan*, n.d.). It first hardens the plant surface, making the plant more resistant to fungal infections. It also increases the pH, which slows the growth of mycelium, the fungal filaments. Additionally, it alters osmotic pressure, causing the mycelia to burst open and eventually dry out. VitiSan is often applied in combination with other treatments (e.g. copper, sulphur) in scientific studies (Döring et al., 2015; Žežlina et al., 2010), so the effectiveness of VitiSan is difficult to determine. A study where VitiSan was used solitary did not find reliable effects on disease incidence (Lukas et al., 2016).

In some vineyards, roses were planted for early detection of mildews and botrytis because they tend to get infected earlier than grapes, but this was generally perceived as non-effective (interviews, 2024). This could partly be because visible symptoms appear much later than the infection itself. However, the temperature of the rose leaves changes after infection, and infrared and RGB imaging system could potentially be used to detect early disease infection before visible symptoms appear (Vagelas et al., 2021). In another vineyard, Weinberg perzik was used for early detection (interviews, 2024). Vineyard keepers had the impression that trees around or within the vineyards benefitted plants health (interviews, 2024). Studies in agroforestry systems suggest that trees improve soil quality and offer protection from environmental stresses, creating a favourable microenvironment for vines. For instance, research on grapevine agroforestry systems indicates that while vines near trees may have slightly reduced yields, the use of trees in vineyards results in increased water infiltration and water-holding capacity, greater nutrient availability, better soil quality, and more efficient vine rooting patterns (Favor et al., 2021).

3.3 Interview insights

Understanding the values and motives of vineyard keepers helps in selecting solutions that will find support, increasing the chance of implementation. This subchapter examines the practices and perspectives of five vineyard keepers that were interviewed across the Netherlands (N=5) more in depth. To make the interview data more quantitative, it is indicated with N=x by how many vineyard keepers the subjects discussed below were mentioned. Basic attributes of the vineyards that were visited are presented in table 4.

Table 4: Basic attributes of the visited vineyards

Vineyard	**Location**	**Soil**	**Varieties**
1	Gelderland	Heavy clay	PIWI's
2	Flevoland	Silty-clay	PIWI's
3	Flevoland	Silty-clay	PIWI's
4	Gelderland	Sand	PIWI's
5	Limburg	Loess (silty-loam)	*V. vinifera*

3.3.1 Viticultural Practices

The majority of the vineyard keepers were amateurs and produced solely for own consumption (N=3). Two vineyards (part of the same organization) were certified organic and sold part of their wine commercially (N=2). Most of the vineyards processed their grapes into wine themselves (N=4), while one sends the grapes to the Moezel to be processed into wine (N=1). Volunteers were involved in three vineyards (N=3), where one also featured an adoption program of vine ranks (N=1).

Two vineyards were located in Flevoland (N=2, same organization), two in Gelderland (N=2), and one in Limburg (N=1). The vineyards are situated on contrasting soils: One is located on heavy river clay (N=1), one is located on loess (silty-loam) soil (N=1), one is located on sandy soil (N=1), while the other two have silty-clay soils (N=2). They all mentioned advantages and drawback of their specific soil type, but two vineyard keepers indicated to take regular soil samples (N=2). Regarding these soil samples, one vineyard keeper indicated to use the Kinsey-Albrecht method for soil surveys (N=1). Most of the vineyard keepers showed investment in supporting soil life and creating long-term soil health (N=4).

All the vineyards used a vertical trellis system (N=5), where some vineyard keepers experimented with other trellis systems (N=2). One vineyard keeper tried the Double Curtain system (N=1) but found the maintenance of such a curtain system too labour intensive. Another vineyard keeper experimented with several other trellis systems (N=1). Pruning techniques varied across the vineyards, reflecting adaptability to specific needs. Guyot pruning is employed in all vineyards (N=5), but the Cordon method was mentioned as well (N=1. Vineyard 4 had also a little vineyard at home, where he used the

Cordon method. Additionally, soft pruning, aimed at minimizing vine stress, is practiced in three vineyards (N=3). One vineyard keeper indicated to adapt his pruning technique depending on the physical state of the vine rank (N=1).

The choice of grape varieties showcases a commitment to cultivating PIWI's. Almost all vineyard keepers were enthusiastic about the fungus-resistant grapes (N=4). This can be illustrated with the following quote:

Vineyard keeper 4: *"I believe PIWIs are essential because of the weather here. Our Belgian colleagues focus more on traditional vines, but I think starting with those isn't ideal. With PIWIs, you have a certain level of resistance, which is exactly what's needed in this country."*

Regarding white grapes, Solaris and Johanniter are grown in three vineyards (N=3), while Muscaris is found two vineyards (N=2). Regarding red varieties, Regent and Rondo are common across two vineyards (N=2). Marechal Foch, Muscat Blue and Cabernet Cortis are found in only vineyard 1 (N=1, same vineyard). Monarch and Sauvignier gris are also cultivated exclusively in one vineyard (N=1, different vineyards). Different vineyard keepers mentioned that early ripening varieties like the Solaris experience less disease pressure, as the most susceptible period occur after their ripening (N=2).

One vineyard 5 keeper was committed to using classical varieties that are part of *V. vinifera* (N=1). He mentioned that Limburg is more oriented on Luxembourg and France viticulture, while Gelderland is more oriented towards Germany. He also mentioned that preference for classical varieties or PIWI's it is matter of taste. This vineyard cultivated Pinot grigio permanently, but also conducted experiments with Sauvignac and another Pinot variety with less compact bunches. Several other vineyard keepers indicated to prefer varieties with less compact bunches, because they are less susceptible to diseases (N=3).

The vineyard keeper with Pinot grigio indicated that the sugar content of the grapes had increased over the years due to climate change (N=1). He also mentioned that warmer temperatures due to climate change enabled cultivation of varieties that were not suitable for this climate before. The other vineyard keepers did not mention a direct effect of climate change on their cultivation practices or grapes.

All vineyards-maintained grass as ground cover (N=5), where two vineyards actively managed a herb rich grass mix because they believed this benefitted the vines (N=2, same organization). Most vineyards keepers had a positive attitude towards a planting trees in the vineyard (N=4), but only one planted them in between wine ranks (N=1). Several vineyards planted roses for early detection, but they all perceived this as ineffective (N=3). One vineyard keeper used Weinberg perzik for early detection because he believed that roses nowadays are disease resistant (N=1). One vineyard keeper indicated to possess a frost protection system (N=1), while two others (N=2, same organization) would like to have such a system but financial constraint inhibited this.

3.3.2 Disease Management

Despite differences, all growers (N=5) face powdery mildew, downy mildew, and botrytis. However, not all vineyards experienced the same disease pressure from each disease. For example, the severity of botrytis is described as moderate in two vineyards (N=2) and low in another vineyard (N=1). One vineyard only experienced severe disease pressure from downy mildew and not from powdery mildew, while another had mostly downy mildew (N=1). All vineyard keepers found the diseases still manageable (N=5), but one mentioned that he observed the vines very often to be able to act immediately (N=1). Two vineyard keepers mentioned to experience severe damage caused by animals (N=2).

Compost tea, used by most vineyards (N=4), is considered essential for maintaining balance in the ecosystem, while Trichoderma applications (N=5) have seen mixed success. This can be illustrated by the following quote:

> Vineyard keeper 1: "*Some people firmly believe in using Trichoderma to combat fungal problems, based on positive experiences. The idea is that when a fungus is present, you can apply Trichoderma to suppress it. It is thought to block, encapsulate, or neutralize the fungus. However, this belief is not universally accepted, and doubts remain about its actual effectiveness.*"

Some vineyards (N=2) reported positive effects from Trichoderma, one vineyard keeper (N=1) was not sure about its effectiveness, while the other vineyard keepers (N=2) remained unconvinced of its efficacy. From the latter, one indicated that downy mildew infection increased after application of Trichoderma (N=1). One vineyard keeper that did see effect mentioned that the skin of the grapes showed discoloration, but the taste was not affected (N=1). It was difficult for some vineyard keepers to say whether they had actually seen an effect, or whether it was simply due to one of the other circumstances (N=2). Furthermore, all vineyard keepers said that it was difficult for them to say what the effects were exactly (N=5). One vineyard keeper mentioned that the white powder from downy mildew should disappear, but that the Trichoderma spores were also white, making it difficult to see the effect (N=1). Preventative use of Trichoderma is practiced in two vineyards (N=2), while the other three (N=3) apply it only when fungal issues are detected. The Trichoderma species used for foliar application was *Trichoderma harzanium* T9 (N=5), which is not commercially available. The vineyard keepers bought it from the Wijnbouwers Der Lage Landen association, who imported it from Czech Republic. The exact composition and origin were unclear. Oenosan® was used by three vineyard keepers (N=3), of which two were convinced of its effect (N=2). The other vineyard keeper mentioned that they had sprayed all the vines with Oenosan®, so had no comparison material (N=1). A scheme developed by one of the vineyard keepers for the application of Trichoderma, compost tea, and Oenosan® is added in appendix (9.4). Three vineyard keepers were interested in the influence the pump and spray mouth of spraying devices had on application effectiveness (N=3). One tested different pressures and mouth shapes but did not find a significant difference in effect (N=1).

3.3.3 Attitudes Toward Sustainability

All vineyard keepers exhibited a proactive attitude, reading scientific literature and actively searching for possible management improvements (N=5). Some of them mentioned that they had participated in courses (N=3). All vineyard keepers were part of multiple associations, where knowledge was shared (N=5). They were open to experimenting with either disease control strategies (N=5), cultivation system (N=5) or varieties (N=5). Four vineyard keepers mentioned explicitly that it was important for them to produce natural wines (N=4). This was important for them, as well as being biological and organic. This could be illustrated by a clear quote:

Vineyard keeper 1: *"I have always farmed organically, and I don't have a spray license. Honestly, I don't really want one either"*

None of the vineyard keepers used chemical pesticides, but some used or had used sulphur (N=2). Trichoderma is used by all vineyards but with caution, as its short survival time and potential to disrupt microbial balance are recognized drawbacks (N=5). Compost tea is valued by the majority (N=4), especially for restoring balance after Trichoderma applications or during the summer growing season. Their propensity to biological control methods and natural bio stimulants underscores their preference for sustainable practices (N=5). One vineyard keeper mentioned to take precaution measures like protective glasses, gloves and face mask (N=1), while another vineyard keeper said that didn't take precaution measure except making sure he didn't breathe in the Trichoderma (N=1).

3.3.4 Weights MCA

To determine the weight of the different categories in the multi criteria analysis, the vineyard keepers were asked to divide ten points among the categories based on what aspects of a biological control methods they valued most. The results are presented in table 5. As shown, weight distribution differed among the vineyard keepers. Weight scoring for vineyard 3 is missing, as only an answer for vineyard 2 was received from the organization. The average (rounded to nearest 0.5) was used to define the final weights.

Table 5: weight distribution categories MCA as valued by vineyard keepers

Vineyard	Effectiveness	External effects	Easiness of application	Costs
1	1	5	3	1
2	4	3	2	1
3	-	-	-	-
4	5	0	3	2
5	3	3	2	2
Mean	***3*** *(0.30)*	***3*** *(0.30)*	***2.5*** *(0.25)*	***1.5*** *(0.15)*

3.3.5 Conclusion

The interviews revealed a shared commitment to sustainability and experimentation, with most favoring PIWI grape varieties for their climate resistance and prioritizing soil health and natural wine production while avoiding chemical pesticides. Despite varied soils and practices, common trends included the use of Trichoderma for disease management, although with mixed views on its effectiveness. There was also a universal interest in improving practices through knowledge-sharing and innovation, highlighting the need for tailored organic solutions.

3.4 Promising biological control methods allowed in NL

Understanding the characteristics of biological control methods in terms of effectiveness, external effects, easiness of application, commercial availability, and costs will enable ranking of the methods from most promising to least promising. The following two subchapters will therefore answer the sub question: *"Which biocontrol measures can potentially be effective in Dutch vineyards, what are instructions for application, what are external effects of their application, are they commercially available, and what are the costs of application?"*. Furthermore, is important to check if commercial products containing biological control agents are allowed to be used on grapes in the Netherlands, and if they are allowed to be used by amateurs. This subchapter will therefore also answer the following sub question: *"What are the current disease management regulations for vineyards in the Netherlands?"*. This subchapter will discuss characteristics of seven biological control methods that are allowed in the Netherlands, ranked on points they scored in the multi criteria analysis. The next subchapter will discuss ten biological control methods that are not allowed in the Netherlands, ranked on points they scored in the multi criteria analysis. An overview of the scores that were allocated to the different methods is presented in table 6, also the chapter were the method is fully explain will be indicated in this table. An explanation of the score is given in the text under each method.

Table 6: All the biological control methods and their respective MCA scores for each category.

Biological control method	Approved in nl	Approved on grapes	Target diseases*	Effectiveness						External effects	Easiness of application	Cost	Overall score	
				Initial effectiveness	Tested in Dutch Climate	Tasted on grapes	Tasted in practice	Deduction	Final effectiveness					
B. subtilis (3.4.1)	YES	YES	G	3	3	4	4	5%	2.85	4	3	3	8.1	Allowed Biological control methods
A. Quisqualis (3.4.2)	YES	NO	P	3	3	4	4	5%	2.85	4	3	3	8.1	
L. digitata (3.4.3)	YES	YES	G, D, P	4	2	4	3	18%	3.3	3	3	4	7.7	
***T. atroviride* SC-1** (3.4.4)	YES	YES	G	4	1	3	2	43%	2.3	4	3	1	7.0	
***T. harzanium* strain T-22** (3.4.5)	YES	YES	G	3	2	4	4	10%	2.7	3	4	-	6.8-8.3	
T. asperellum* strain *T-34 (3.4.6)	YES	YES	P	3	1	4	2	30%	2.1	4	-	4	6.1-8.6	
Compost tea (3.4.7)	YES	YES	G, P	-	1	4	3	-	-	3	3	4	5.6-8.6	
Potassium bicarbonate (3.6.1)	YES	YES	B, D	4	2	4	3	18%	3.3	3	3	4	8.1	Biological compounds
Calcium carbonate (3.6.2)	YES	YES	-	-	-	-	-	-	-	4	-	4	-**	

5-chlorosalicylic acid (5CSA) (3.6.3)	NO	NO	G	3	3	4	3	13%	2.6	2	1	-	4.1-5.6
A. Pullalans (3.5.1)	NO	NO		4	1	4	4	15%	3.4	4	4	-	7.8-9.3
Myco-Sin (3.5.2)	NO	NO	D,P	4	3	4	4	5%	3.8	1	3	4	7
***T. harzanium* T-39** (3.5.3)	NO	NO	D,P	3	2	4	4	10%	2.7	4	3	-	6.9-8.4
***G.candidum* JYC1146** (3.5.4)	NO	NO	G	4	4	3	1	35%	2.6	4	3	-	6.8-8.3
***C. rosea* – Prepstop** (3.5.5)	NO	NO	G	3	-	4	2	15%	2.6	4	3	-	6.8-8.3
U. oudemansii (3.5.6)	NO	NO	B	3	3	4	3	13%	2.6	4	1	2	6.4
B. licheniformis (3.5.7)	NO	NO	G	4	3	3	1	48%	2.1	3	4	-	5.7-7.2
P.polymyxa (3.5.8)	NO	NO	B	3	1	1	2	68 %	1	4	3	-	4.9-6.4
P. oxalicum (3.5.9)	NO	NO	B	3	1	1	3	60 %	1.2	4	-	-	3.9
***T. harzianum* strain T-9** (3.5.10)	NO	NO	-	2	1	1	1	75%	0.5	-	-	-	- **

Non-allowed biological controlcontrol methods

*Abbreviations of diseases are D; downy mildew P; powdry mildew G; grey mold

**No final score was given as there was no scientific-based evidence available

3.4.1 Bacillus subtilis **– Serenade Max** ***(score = 8.1)***

<u>Allowance in the Netherlands</u>

Bacillus subtilis is the active biological control agent in the commercialized product Serenade Max. This product has been evaluated and it is allowed to be used in the Netherland by professionals to be used on several crops including wine grapes by means of spraying (ctgb, 2024).

<u>Mode of action</u>

Serenade Max is a biological control agent (bio-fungicide) containing a strain of *B. subtilis* (QST 713). Serenade is able to combat diseases such as powdery mildew and gray mold (Thomidis et al., 2016). Its primary mode of action involves the production of lipopeptides, which are able to disrupt the cell membranes of the plant pathogens. This kills the cells of the pathogen and thereby suppressing infections. This is a unique mode of action for fungicides and Serenade Max can therefore be synergistically used with other fungicides. This helps against resistance from the pathogen against the *Bacillus* strain (Thomidis et al., 2016).

In addition, these lipopeptides stimulate systemic acquired resistance (SAR) and other plant defense responses, which means it can also be used as a preventative measure (Thomidis et al., 2016). When the biological control agent is applied to the soil, *Bacillus subtilis* rapidly colonizes the plant roots and grows alongside them while feeding on root exudates. This process strengthens the root system, protects against soil-borne fungi like *Pythium*, *Rhizoctonia*, and silver scurf, and enhances nutrient uptake and stress tolerance. Colonization of the *B. subtilis* goes swiftly and will continues as the roots grow. Also, the bacterium adheres strongly to roots and is can therefore not be washed away, regardless of soil or moisture conditions (Bayer Agro, n.d.).

<u>Effectiveness against downy mildew, powdery mildew and grey mold</u>

Serenade Max has been proven effective in controlling grey mold on grapes. In the field experiments that were conducted in Greece over two consecutive years they applied the product two times and an 75% reduction in infections of grey mold was recorded (Thomidis et al., 2016).

Studies in Crimea's South-Western and South Coastal viticulture zones (Serbia) also demonstrated positive outcomes with the biological control agent *B. subtilis*. In this study they applied the spray five times at seven-day intervals (Aleinikova et al., 2023).

<u>Products, Application, and Costs</u>

Serenade Max is applied as a foliar spray, and a backpack sprayer is needed to be able to apply the solution to the crops (Thomidis et al., 2016). For effective application, the spray solution of Serenade Max must be mixed thoroughly with water to ensure a uniform coverage on the crops. A maximum of 8 litters of the Serenade Max mixed in 500 litters of water can be used. The solutions should not be left standing for prolonged periods, as this can degrade the active compounds and therefore lessen the effectiveness of the solution (Bayer Agro, n.d.).

The recommended dosage ranges from 5 to 8 litres per hectare, depending on the severity of the disease. Costs are estimated between €10 and €20 per litre, with a maximum expense of approximately €340 per hectare (Bayer Agro, n.d.).

External Effects

Serenade Max has no harmful effects on beneficial insects or pollinators, making it an environmentally friendly option. As a fungicide with a unique mode of action in comparison to other used fungicides, it is not prone to resistance development, which enhances its use in integrated pest management (IPM) strategies (Bayer Agro, n.d.).

Scores

- For effectiveness a score of **3** was given, because its ability to control grey mold was around 75% and lower (Thomidis et al., 2016).
- A score of **4** was given for application on grapes, and its use in practice, so there was no deduction. This score was given because this product has been thoroughly tested on grapes and in practical settings (Thomidis et al., 2016; Aleinikova et al., 2023). A deduction score of **3** was given for not testing under Dutch climate conditions, because it was tested in Serbia.
- External effects got a score of **4**, as Serenade Max does not negatively impact beneficial insects or pollinators and supports resistance management due to its multi-site action (Bayer Agro, n.d.).
- The product's ease of application received a **3**, because its need for a backpack sprayer to spray it evenly on the crop (Thomidis et al., 2016)
- Finally, the costs were given a **3**, because it was in the range of €300-€500, indicating good affordability relative to its dosage and benefits (Bayer Agro, n.d.).
- The total score of *B. subtilis* was an **8.1**.

3.4.2 Ampelomyces quisqualis - AQ10 (score = 8.1)

Allowance in the Netherlands

Ampelomyces quisqualis is the biological control agent in the commercialized product AQ10. This product is currently approved for use in the Netherlands by professionals on crops such as cucumber, tomato, pepper and strawberry. Unfortunately, this product is not permitted to be used on wine grapes (ctgb, 2024).

Mode of action

Ampelomyces quisqualis is a parasitic fungus that can parasitise on multiple other fungus species, among which is powdery mildew. This parasitic fungus is commonly used as a biological control agent, marketed as AQ10, as it is able to parasitize on both the sexual and asexual structures of powdery mildew (Manjunatha et al., 2020).

When sprayed plant surface of the host the spores of *A. quisqualis* will germinate, and its hyphae will find and penetrate the hyphae of the powdery mildew by excreting lytic enzymes that are able to degrade the cell wall of the pathogen. After penetrating the cell wall the parasitic fungus will grow further into the pathogen and produce intracellular pycnidia in the mycelium of the pathogen. This will lead to the collapse and eventually death of the pathogen. Once the pathogen dies, *A. quisqualis* will realise spores from the intracellular pycnidia (CBC (Europe) S.r.l., 2020). Additionally, *A. quisqualis* is also able to survive on plant tissues alone and can therefore compete powdery mildew for nutrients and space and causes the death of this pathogen due to starvation (Manjunatha et al., 2020).

For optimal growth and germination, *A. quisqualis* requires a high relative humidity of around 60%. What often is done to be able to use this biological control agent is adding paraffin, mineral oils or additives, which lowers the relative humidity that is needed to be able to germinate and grow. These substances help prevent the biological control agent from drying out in lower relative humidity conditions will lead to a higher diseases control of the powdery mildew (Pertot, 2008).

Effectiveness against downy mildew, powdery mildew and grey mold

Ampelomyces quisqualis has been well studied in field conditions in different host plants. It was found, in field studies in New York, that *A. quisqualis* is a successful biological control agent in cucumber, apple seedlings and on grapevines. In the field conditions of the grapevine a 50%-60% reduction in the infection was seen (Falk, 1995).

Another study conducted in the Catholic University of Piacenza in Italy found similar results to the study done in New York. They found that the biological control agent AQ10 caused a 50%-70% reduction in infection in the grapevines (AgriBio Shop, n.d.).

Lastly a study three-year study done in northern Italy, found that when applying the AQ10 product late in season it reduced the infection by 40% after applying it one time and a reduction of 64% when it was applied for a second time (Legler et al., 2011).

Products, application and costs

AQ10 is commercially available as a water-soluble powder. It needs to be applied two times, right before harvest and right after harvest (late September – mid-October). This is the time when there is a higher amount of rainfall which provides good conditions for *A. quisqualis*, because it needs high relative humidity (60%) to be able to grow and germinate properly (Schweigkofler, 2006).

To apply the AQ10 it the powder needs to be dissolved into a liquid solution. Then paraffin, mineral oils or additives are added to the solution to help *A. quisqualis* stay hydrated longer to be able to grow and germinate, which will elongate the effect of AQ10. Alternatively, when Helioterpen Film or summer oils like AddQ and Biolid are added, AQ10's effectiveness increases by slowing evaporation and protecting it from UV degradation, enabling better control of powdery mildew in hot and dry conditions (Schweigkofler, 2006). The solution can then be applied to the vines with a foliar spray (Hortipro, n.d).

It is recommended to use 35-70 g/ha for wine grapes. This needs to be sprayed 2 times per season which means a max of 140 grams is needed. At Fargo AQ10 costs around €85,- for 35 grams, so a maximum amount of €340,-/ha/year (Fargro, n.d.). At AgriBio Shop it is sold for around €70,- for 30 grams, so it will cost maximum of around €327,-/ha /year (AgriBio Shop, n.d.).

External effects

It has been found that the use of AQ10 two times per year will reduce the occurrence of powdery mildew in the following year, without it having a negative effect on the quality of the wine. Furthermore, according to EFSA (2017) it has not been found that AQ10 has a negative effect on the human and animal health. Also, resistance of the pathogen against this biological control agent has not yet been recorded in literature (Arena et al., 2017).

AQ10 is also approved for organic farming and is an excellent addition to integrated pest management (IPM) strategies due to its unique mode of action and lack of phytotoxicity (Hofstein & Chapple, 2003).

Scores

- The effectiveness was given a score of **3**, because there were three studies that found on average between 50%-60% reduction in disease infection (Falk, 1995 ; AgriBio Shop, n.d. ;Legler et al., 2011).
- A deduction score of **3** was given to tested in Dutch climate, because it was not tested in the Netherlands or a country with the same climate, but it was tested in northern Italy which has similar climate (Legler et al., 2011). Tested on grapes and tested in practices was both given a score of **4**, because it was both done on grapes and longer field studies were done (Legler et al., 2011.
- Then external effects were given a score of **4**, because it there were no negative effects found on both the environment and on human/animal health (Arena et al., 2017).
- A score of **3** was given to easiness of application due to the need for a sprayer to be able to apply the solution, but besides that it was not labour intensive as it only is sprayed two times a year (Hortipro, n.d.).
- And lastly the cost was given a score of **3**, because it was between €300,-and €500,-.
- The total score for *A. Quisqualis* was an **8.1**

3.4.3 Laminaria digitata - Vacciplant® (score = 7.7)

Allowance in the Netherlands

The Vacciplant® based on brown algae *Laminaria digitata* extract of laminarin, is currently registered in the Netherlands as elicitor (Pugliese et al., 2018; ctgb, 2024). Elicitors are substances that induce the defence mechanisms of plants (Labarre and Orieux, 2010).

Working Mechanism

Laminarin is a β-1,3-glucan polysaccharide derived from the brown algae *L. digitata (Aziz et al., 2003).* Specifically, this substance acts as an elicitor of plant defence responses, which are natural or naturally derived molecules capable of mimic the presence of a pathogen or the signalling molecules that plants produce in response to a pathogen attack (Thakur and Sohal, 2013). This recognition initiates signalling for the plant to including a rapid influx of calcium ions (Ca^{2+}) into the cytoplasm (Aziz et al., 2003). The calcium influx acts as secondary messengers to transmit the defence signals to downstream pathways (Aziz et al., 2003).

Laminarin also triggers the production of reactive oxygen species (ROS), a process known as an oxidative burst (Aziz et al., 2003). ROS, such as hydrogen peroxide (H_2O_2), serve dual roles: they have direct antimicrobial effects and act as signalling molecules that activate other defence mechanisms (Baker & Orlandi, 1995). Concurrently, laminarin induces extracellular alkalinization, which is associated with the strengthening of cell walls and enhanced resistance to pathogen penetration (Aziz et al., 2003).

The signal transduction continues with the activation of mitogen-activated protein kinases (MAPKs). These proteins relay the defence signals, which are mobilizing the transcription of defence-related genes. Specifically, Laminarin upregulates genes involved in the production of pathogenesis-related (PR) proteins, such as chitinases and β-1,3-glucanases, which degrade fungal cell walls, thereby directly inhibiting their growth (Aziz et al., 2003). Additionally, it activates the phenylpropanoid pathway, leading to the synthesis of phytoalexins like resveratrol and ε-viniferin, compounds with strong antimicrobial properties (Langcake, 1981). In addition to these localized effects, laminarin primes plants for systemic acquired resistance (SAR), a state in which the entire plant becomes more resistant to subsequent infections (Aziz et al., 2003).

Effectiveness against downy mildew, powdery mildew and grey mold

The laminarin tested in-vitro by Aziz et al., 2003 against *B. cinerea* and *P. viticola* under control conditions on cells of grapevines (V. vinifera cv. Gamay and V. vinifera cv. Chardonnay 75) in a laboratory. The results of the experiment reported 55% and 75% reduction of infection from the *B. cinerea* and *P. viticola*, respectively. However, in 2016 a two-year experiment contacted by Pugliese et al. (2018) in vineyards of *V. vinifera cultivar Moscato* in Piedmont, Italy to study the effects of laminarin against powdery mildew. The first year the weather conditions were rainier with a lower infections pressure, where the laminarin reduced the diseases severity on the leaves 77.1% compared to the untreated control. In the second year warmer and frier weather conditions occurred, with increased presence of the disease. Whereas laminarin reduced the disease severity on the leaves by 94.5% compared to the untreated control. Moreover, on a field experiment that conducted for two years in 6 years old commercial vineyards in the central-eastern Italy to observe the control pressure of Laminarin alone or with other product against downy mildew (Gianfranco Romanazzi et al., 2016). The results showed that laminarin together with *Streptomyces sp.* has an increase of reducing the severity of downy mildew compared to laminarin individually.

External Effects

No specific external effects have been identified literature research for negative effects on environment, human health and grapes. However, the product label of Vacciplant® is mentioning that for the application you have to use personal protective equipment, and that is harmful if swallowed, absorbed through skin, or inhaled (*UPL | NL*, 2024).

Products, Application, and Costs

In the market exist one product based on laminarin, which is Vacciplant® by UPL Limited. In the study by Pugliese et al. (2018) in total nine applications took place in a period of time of three months, 7-9 gap between treatments. The manufacture company is suggesting for against powdery mildew and *B. cinerea* 20 applications per season with gap between treatment 10 days (*UPL | NL*, 2024). The recommended dosage is 1.0 litter of Vacciplant® per 1 hectare of land. Also, the company suggest starting applications of Vacciplant® preventatively or when conditions for disease become favourable (*UPL | NL*, 2024). Moreover, the Vacciplant® in the Netherlands can be sold only to farmers that possess permission for professional use. However, in an online store in Greece one litter of Vacciplant® has been sold at the price of €39.55 (*Vacciplant 1Lt – Anyfion Shop*, 2021).

Score

- Effectiveness received a score of **4**. This is based on the average disease control of *E. necator, B cinera* and *P. plasmopara* by Vacciplant®, which were 86%, 55% and 75%, respectively (Gianfranco Romanazzi et al., 2016.; Pugliese et al. 2018).
- Deduction scores, because of Vacciplant® does not tested in Dutch weather and in practice has only been tested in Italy for two years field trials, scoring **2** and **3** respectivly (Gianfranco Romanazzi et al., 2016.; Pugliese et al. 2018).
- Score for external effects is **3**. Vacciplant® can cause irritation if it contacts with the skin (*UPL | NL*, 2024).
- Easiness of application received a score of **3**, as recommended yearly application rate was 9 (Pugliese et al., 2018) and no special equipment was required (*UPL | NL*, 2024)

- <u>Costs</u> received a score of **3**. The price per hectare per growing season is €360-800 per hectare, based on the recommended application rate (Pugliese et al., 2018; *UPL | NL*, 2024) and price (*Vacciplant 1Lt – Anyfion Shop*, 2021).
- The <u>total score</u> of Vacciplant® is **7.7**.

3.4.4 Trichoderma atroviride SC1 - Vintec® (score = 7.0)

Allowance in the Netherlands

Trichoderma atroviride SC1 is the active compound in the commercialized biological control agent Vintec®. This product has been evaluated and it is allowed to be used in the Netherlands by professionals on several crops including wine grapes by means of spray application (ctgb, 2024).

Mode of action

Trichoderma atroviride strains are biological control agents produced by fermentation. It is active through various mechanisms of action. *T. atroviride* outcompetes pathogens for sugars, by producing non-volatile compounds which inhibit the growth and activity of pathogens (Card et al., 2009). It also inhibits pathogen growth through direct parasitism (Card et al., 2009). *Trichoderma atroviride* SC1 is mostly known for combatting trunk diseases in grapes.

Effectiveness against downy mildew, powdery mildew and grey mold

A study on tomato's sprayed with Vintec® showed that it was able to reduce disease incidence of *B. cinerea* by 92.86% in Dorjan region and 100% in the Bogdanci region (Rusevski et al., 2024). A total of four foliar application was performed with a hand pressure sprayer. The study was conducted in greenhouses located Macedonia, over the course of one year.

External effects

EFSA (2015) conducted a risk assessment on T. *atroviride* SC1. The report states that risk assessment for mammalian toxicity of secondary metabolites, and to non-target organisms could not be finalized due to knowledge gaps. Furthermore, not enough evidence was found to demonstrate that any toxins/secondary metabolites produced by the fungus will not occur in the environmental compartments in concentrations considerably higher than under natural conditions (EFSA, 2015).

Health risks are undefined in the safety sheet of *T. atrovide* SC1 based product Vintec® (Fytostat, 2023).

The effect of Vintec® on wine quality was not mentioned by the vineyard keepers nor found in scientific literature (interviews, 2024).

Products, application and costs

Trichoderma atroviride SC1 is commercially available as Vintec®, which is produced by Belchim Crop Protection (Lissieu, France). This product is also known to combat esca disease (interviews, 2024). *T. atroviride* LU132 and *T. atroviride* C65 are not commercially available.

Belchim crop protection (2020) recommends a usage dose of 0.15 kg/ha for Vintec®, and application rate of 8 sprays per year. Vintec® is sold for €75 per 50 grams (Farmagrishop.it, n.d.).

Score

- Effectiveness received a score of **4**. This is based on the average disease control of *B. cinerea* by Vintec® found by Rusevski et al. (2024). This was 96.4%.
- Deduction scores for the study by Rusevski et al. (2024) for not being tested on grapes, being tested in greenhouses, and the Macedonian climate were **3**, **2**, and **1** respectively.
- Score for external effects is **4**. No evidence is present for risks regarding health or environment for Vintec®.
- Easiness of application received a score of **3**, as recommended yearly application rate was 8 (Belchim crop protection, 2020) and no special equipment was required (Rusevski et al., 2024).
- Costs received a score of **1**. The price per hectare per growing season is €1800, based on the recommended application rate (Belchim, 2020) and price.
- The total score of *T. atroviride* SC1 is **7.0**.

3.4.5 Trichoderma harzanium strain T22 - Trianum-P (score = 6.8-8.3)

Allowance in the Netherlands

Trichoderma harzianum T22 is the active biological control agent in the commercial product Trianum-P. This product has been evaluated and it is allowed to be used in the Netherlands by professionals on several crops including wine grapes by means of soil application (ctgb, 2024).

Mode of action

Trichoderma harzianum T22 is a biological control agent, produced by protoplast fusion. It is mainly known for its ability to control soil-borne diseases, but it can also be effective in the control of fruit and foliar diseases (Harman, 2000). This strain is able to colonize all parts of the plant root system and to modulate the composition of the rhizosphere microbiome (Harman, 2000). These abilities are not particularly affected by soil type, plant species nor by geographical zone (Harman, 2000). The mechanisms by which *T. harzianum* T22 acts are multiple and include mycoparasitism, antibiosis, competition for nutrients and/or space, tolerance to stress through enhanced root and plant development, induced resistance, solubilization and sequestration of inorganic nutrients and inactivation of the pathogen's enzymes (Harman, 2000).

Effectiveness against downy mildew, powdery mildew and grey mold

A study on grapes treated with a foliar spray based on *T. harzanium* T22 demonstrated that it can provide significant control of *B. cinerea*, but also noted that successful biocontrol is more likely to occur if organisms are obtained from a site similar to that where biocontrol is desired (Harman et al., 1996). Between 9% and 52% of clusters were infected, whereas the untreated control had an infection rate of 62%. This study was conducted with field trials in New York for four years. The vines were sprayed five times per season.

External effects

A study on the ability of *Trichoderma* T22 to solubilize insoluble or sparingly soluble minerals, found that *T-22* was able to solubilize MnO_2, metallic zing and rock phosphate, making them available for plant take-up (Altomare et al., 1999). This study was conducted in vitro with a liquid sucrose-yeast extract medium.

A risk assessment report by the EFSA (2013) states that knowledge gaps remain, and it is not proven that *T. harzanium* T22 will not persist in the soil in concentrations higher than the natural background levels, possibly altering ecosystem functioning. Furthermore, not enough evidence was found to demonstrate that any toxins/secondary metabolites produced by the fungus will not occur in the environment in concentrations considerably higher than under natural conditions, possibly altering ecosystem functioning. Risk for birds, mammals, aquatic animals and bees was concluded to be low, while data on the risk for soil organisms was missing (EFSA, 2013).

The safety data sheet of *T. harzanium* T22 by Bioworks (2018) mentions eye irritation as possible hazard for humans.

A study on grapes treated with *T. harzanium* T22 found an 63% increase of yield (kg), as compared to the untreated control plot (Pascale et al., 2017). In order to evaluate the effect of T22 on the quality of grapefruits, total polyphenol content and antioxidant activity were also measured in this study. The antioxidant activity increased with the treatments of T22 respectively by 60.3% compared to control treatments. The polyphenol content increased in harvested fruits of plants treated with T22 (Pascale et al., 2017). This study was conducted with field experiments in Italy over the course of two years. *T. harzanium* T-22 was applied 10 times per trial (two trials total).

Negative effects of *T. harzanium* T22 application on grapes were not reported by vineyards keepers (interviews, 2024).

<u>Products, application and costs</u>

Trichoderma harzianum T22 is commercially available as Trianum-P (granulates based on *T. harzianum* T22), developed by Koppert BV (Berkel en Rodenrijs, Netherlands). Trianum-P can be applied via drench, drip-irrigation systems or by spraying onto the growing medium at the time of sowing. It can be applied with any spraying or drenching equipment. It should be applied as early as possible to the crop for optimal effect (Koppert BV, n.d.). Trianum-P performs well under different environmental conditions as *T. harzianum* T-22 grows in a wide temperature range (10-34°C), at a pH between 4 and 8, in many types of growing media (Koppert BV, n.d.). Application rates for Trianum-P typically range from 1-5 kg/ha (Koppert BV, n.d.). Trianum-P® is sold per 500 grams for €75.71 (*Trianum-P (500g),* n.d.).

<u>Score</u>

- <u>Effectiveness</u> received a score of **3**. This is based on the average disease control of *B. cinerea* on grapes found by Harman (1996), where *T. harzanium* T22 was applied as foliar spray. This is 50.5%, which led to a score of 3.
- Deduction score for <u>climate</u> is **2**, as the study by Harman (1996) is conducted in New York.
- The score for <u>external effects</u> is **3**, as eye irritation was the only negative effect with sufficient evidence.
- The <u>easiness of application</u> received a score of **4**. This is based on the application rate used by Harman (1996) and the knowledge that *T. harzanium* T22 can be sprayed with a normal back sprayer (interviews, 2024).

- Costs received **no score**, given that the average application rate for Trianum-P is for soil application and Harman (1996) used foliar application.
- The total score of *Trichoderma harzanium* T22 is **6.8-8.3**.

3.4.6 Trichoderma asperellum strain T34 - ASPERELLO® T34 Biocontrol/ T34 Biocontrol® (score = 6.1-8.6)

Allowance in the Netherlands

Trichoderma asperellum strain T34 is allowed in the Netherlands (ctgb, 2024). T34 Biocontrol® and ASPERELLO® T34 Biocontrol by Biocontrol Technologies are registered in the pesticides database of the ctgb for professional use (ctgb, 2024).

Mode of action

Trichoderma asperellum strain T34 is a biological control agent, where one of its main mechanisms of action against pathogen is by inducing plant resistance. More specifically, it produces volatile organic compounds (VOCs) such as 6-pentyl-2H-pyran-2-one and 2-pentylfuran, enhancing the plant defence by stimulating the deposition of callose and hypersensitivity against *P. viticola* (Lazazzara et al., 2021; Martínez-Medina et al., 2017). The decomposition callose in the stomata of the leaves reduces the possibility of zoospores to successfully entering the stomata (Lazazzara et al., 2021). Furthermore, the *T. asperellum* strain T-34 releases VOCs that activate iron uptake genes, such as MYB72, FRO2, and IRT1, enhancing root iron acquisition and promoting root growth (Martínez-Medina et al., 2017). Additionally, T-34 primes plants for stronger jasmonic acid (JA)-dependent defences, boosting resistance to pathogens. Moreover, the *Trichoderma* strains including T34, compete with pathogen fungi for space and nutrients (Lazazzara et al., 2021).

Effectiveness against downy mildew, powdery mildew and grey mold

A study that took place in a greenhouse conditions, where grape plants of Vitis vinifera cultivar Pinot Noir (downy mildew-susceptible) and V. riparia (downy mildew-resistant), where first sprayed 3 times in period of 3 days with conidia of *T. asperellum* strain T34 and then sprayed on with *P. viticola* (Lazazzara et al., 2021).

The results of the study of Lazazzara et al. (2021) showed that the direct application of *T. asperellum* strain T34 reduced the diseases severity of downy mildew significantly, at 72%. However, there is no other research supporting the diseases suppression of downy mildew or one of the other two diseases that are been researching in the project in grapevines.

External effects

According to Trillas et al. (2020), *T. asperellum* strain T34 is regarded as safe for the environment, human health, and crops. It enhances soil health by promoting microbial diversity and nutrient cycling, with minimal risk to non-target organisms or long-term ecological impact. Extensive studies confirm its non-toxic nature and compliance with stringent regulatory standards globally. However, a minor potential for allergenic risk to sensitive individuals can occur, but this risk can be mitigated with protective equipment.

Products, application and costs

T. asperellum strain T34 is commercially available and sold under two brand names, primarily for its use as a biological fungicide to protect plants from soil-borne pathogens.

- ASPERELLO® T34 Biocontrol (based on *T. asperellum* T-34, available in wettable powder forms) – developed by BioBest.

The product is approved to be used against grey mold in grapes. The ASPERELLO® T34 Biocontrol can be applied via foliar application and chemigation. Foliar applications can be performed with hand-held backpack or spray equipment. It should be applied as early as possible to the crop for optimal effect (Biocotrol Technologies, n.d.). ASPERELLO® T34 Biocontrol develops in soil temperature 15-35 but it performs well between temperatures of 20°C and 30°C, develops at pH between 4-9, in many types of growing media (Biocotrol Technologies, n.d.).

- T34 Biocontrol® (based on *T. asperellum* T-34, available in wettable powder forms) – developed by Biocontrol Technologies.

The product is approved to be used against botrytis in grapes. T34 Biocontrol® can be applied via drench, drip-irrigation systems or by spraying onto the growing medium at the time of sowing. It can be applied with any spraying or drenching equipment. It should be applied as early as possible to the crop for optimal effect (Biocotrol Technologies, n.d.). T34 Biocontrol® performs well between temperatures of 15°C and 35°C, at a neutral pH around 7, in many types of growing media (Biocotrol Technologies, n.d.).

Score

- Effectiveness received a score of **3**, as disease reduction of *P. viticola* on grapes was 72%, surpassing the 50% threshold required for effectiveness (Lazazzara et al., 2021).
- Deduction score for testing conditions is **2**, as the product was tested only in greenhouse conditions and not in external field conditions.
- External effects received a score of **4**, as *T. asperellum* strain T-34 was regarded as safe for the environment, human health, and crops (Trillas et al., 2020).
- Easiness of application received **no score**, due to insufficient information regarding application rate requirements.
- Costs received the score **4.** The costs of 500 g of ASPERELLO® T34 Biocontrol is $264, and the average application rate required is 427.6 g/hectare.
- Total score of *T. asperellum* T-34 ranges from **6.1**-**8.6**.

3.4.7 Compost Tea (score = 5.6-8.6)

Allowance in the Netherlands

Compost teas have been investigated in the past for their suppressive properties against fungal diseases of a variety of plant species like apples, grapevines, tomatoes, and much more (Santos et al., 2010). However, in the European market, compost tea is primarily marketed as a bio stimulant or soil improvements rather than a registered fungicide, due to regulatory restrictions under EU Directive 2009/128/EC.

Mode of action

Compost tea, by definition, refers to a liquid produced by steeping compost in water, which extracts both soluble nutrients and microorganisms from the compost (St. Martin & Brathwaite, 2012). It is being used as an alternative to chemical pesticides and fertilizers, applied on the plants' foliage or in the soil to promote plant growth and health, suppress pathogens, and improve soil conditions (St. Martin, 2015).

There are two types of compost tea: Aerated compost tea (ACT) and Non-aerated compost tea (NCT) (Scheuerell & Mahaffee, 2002). The first method involves actively aerating the compost-water mixture during fermentation. This process promotes the growth of beneficial aerobic microorganisms, enhancing the effectiveness of the tea in suppressing pathogens and boosting plant health. While the second method does not include aeration in the process, and the compost-water mixture is left to ferment naturally for several days. This method is simpler and cheaper but can have slower microbial growth compared to ACT (Scheuerell & Mahaffee, 2002).

The beneficial properties of compost tea are derived from the variety of microorganisms that are contained in the mixture like bacteria, fungi, yeast, and other microbes that can suppress diseases (Kh et al., 2008; Evans et al., 2012). This microbial community developed in the tea plays a major role in disease control by: a) outcompeting harmful pathogens, b) producing antimicrobial compounds, and c) enhancing plant resistance (Scheuerell & Mahaffee, 2002; Evans et al., 2012). Moreover, compost tea also contains soluble nutrients that directly benefit plant growth, or the microorganisms in the tea can increase the availability and accessibility of nutrients to plants.

Effectiveness against downy mildew, powdery mildew and grey mold

In research experiments conducted in Tanzania and Australia, the beneficial effects of different compost teas against Botrytis and *E. necator* were investigated (Evans et al., 2012). The experiment was conducted on cultivar varieties Chardonnay and Riesling in two vineyards over two years. Both aerated compost tea (ACT) and standard fungicides were applied. The ACT in the study was produced from open-windrow compost containing cow or chicken manure, timber residues, and salmon culture residuals. When the compost cooled to 50°C, it was suspended at a 1:3 ratio with aerated, de-chlorinated water and brewed for 48 hours. In total, nine applications of ACT were applied each year. The results show that ACT was as effective as the standard fungicides, controlling the severity of powdery mildew and Botrytis at less than 1%, while the severity in the untreated plants reached 79% and 77%, respectively (Evans et al., 2012).

Furthermore, according to Evans et al. (2012), the culturable microorganism community on the leaves was higher 10 days post-application of ACT than before the application. It was shown that the culturable microorganism community took 21 days to return to the pre-application microbial population state. The plants sprayed with standard fungicides observed significantly fewer culturable fungi and yeasts than the ACT-treated plants.

Older research in Germany also showed the fungicidal properties of compost tea (Tränkner, 1992). Two different types of compost tea, horse manure (hm) and hm with antagonistic microorganisms, were used against the fungus *P. viticola*. The effectiveness in suppressing *P. viticola* was 75% for the horse manure compost and 90% for the horse manure with antagonistic microorganisms. However, this research was based on an unpublished PhD dissertation by Ketterer, N. (1990), and there is no further information on the experiment's duration, variety, or methodology.

However, the studies on the effectiveness of compost are having inconsistent results, because of no clear methodology regarding the type of compost used, the quantities applied, or the specific conditions under which the compost is tested (Bailey et al., 2006; Grubinger, 2010; Meghvansi & Varma, 2015). The lack of functional assays to identify key microbial groups from compost microbiomes is a major drawback (Lutz et al., 2020). This lack of standardized procedures makes it difficult to compare results across studies and draw definitive conclusions about its overall efficacy.

External Effects

The effects of compost tea are plentiful, extending beyond its antifungal properties. It is primarily beneficial for improving soil quality by enhancing soil microflora and optimizing the chemical and structural properties of the soil (Pilla et al., 2023). Additionally, compost tea supports plant growth and strengthens plant defence mechanisms (Eudoxie & Martin, 2019). Despite its benefits, compost tea generally has some limited negative effects.

On the downside, according to Pilla et al. (2023), non-aerated compost teas can pose a risk of sheltering harmful pathogens like *E. coli* and *Salmonella* if improperly prepared or applied, potentially contaminating crops and endangering human health. Over-application can lead to nutrient runoff, causing environmental pollution. Additionally, excessive or poor-quality compost tea can result in phytotoxicity, harming plant roots or foliage. These challenges highlight the need for proper preparation and careful application to ensure consistent and safe results.

Products, Application, and Costs

There are no compost tea products currently registered as fungicides in the European market. However, vineyard keepers at Der Linder have developed a systematic methodology for the use and application of compost tea, as reported during interviews. According to the vineyard keepers, the cost of producing compost tea is relatively low, ranging from €100 to €300, which contributed to a cost-effectiveness score of four in the MCA evaluation.

Score

- Effectiveness received a score of **0**, since the inconsistent results in studies on compost effectiveness arise from a lack of standardized methodologies regarding compost type, application rates, testing conditions, and functional assays to identify key microbial groups, hindering cross-study comparisons and definitive conclusions (Bailey et al., 2006; Grubinger, 2010; Meghvansi & Varma, 2015; Lutz et al., 2020).
- Deduction score, since the compost tea has not been tested in a Dutch climate scoring **1**, nor for more than two years in practice scoring **3**.
- External effects received a score of **4**, as compost teas do not negatively impact the environment, grapes, or human health when instructions are followed correctly (Pilla et al., 2023).
- Ease of application received a score of **3**, as the product requires more than six applications but does not need specialized equipment (Scheuerell & Mahaffee, 2002; Evans et al., 2012).
- Costs received a score of **4**, as it falls within the €100–€300 range reported by vineyard keepers interviewed in Wijchen.
- Total score for compost tea based on MCA is **5.6**-**8.6**.

3.5 Biological control methods not allowed in NL

3.5.1 Aureobasidium pullulans (Isolates 533, 547) (Score: =7.8-8.3)

Allowance in the Netherlands

No information suggests that these *A. pullulans* isolates are registered or available as commercial biocontrol products in the Netherlands. The study does not mention any formal approval or commercialization (Schena et al., 2003).

Mode of Action

Aureobasidium pullulans is an endophytic fungus found within the tissues of healthy fruit. Isolates 533 and 547 reduce postharvest rots by colonizing fruit surfaces and potentially inducing host resistance or outcompeting pathogens such as *B. cinerea and Monilinia laxa*. Since they reside naturally within the plant tissues, their presence provides a protective barrier, hindering pathogen penetration and spread without relying on synthetic chemical inputs (Schena et al., 2003).

Effectiveness Against Postharvest Rots

In a 2-year field investigation, these isolates proved highly effective at suppressing postharvest decay of sweet cherries and table grapes. They achieved total rot reduction ranging from 32% to 80% on sweet cherries and 59% to 64% on table grapes, placing them in the highest effectiveness bracket. An 80% reduction in disease means only about 20% disease occurrence remained, indicating substantial control under commercial orchard conditions (Schena et al., 2003).

External Effects

No negative effects on fruit quality, the environment, or human health were reported. Instead, the isolates appear environmentally friendly and compatible with existing orchard practices. Their natural origin and lack of adverse side effects make them suitable candidates for sustainable crop protection strategies (Schena et al., 2003).

Products, Application, and Costs

The study does not provide cost data or mention any established commercial formulations for these isolates. Applications were made as standard orchard sprays preharvest or quick dips postharvest. Such simple, conventional methods likely require no special equipment or management changes, contributing to their high ease-of-application rating (Schena et al., 2003).

Testing Conditions and Practice

Field studies were conducted in Apulia, southeastern Italy, a Mediterranean climate distinct from the Dutch climate. According to the scoring criteria, testing in Mediterranean conditions yields a lower climate relevance score. Nonetheless, the isolates were tested over two years and under practical orchard and storage conditions, demonstrating their real-world applicability. They were also directly tested on table grapes, making the results more applicable to grape industries worldwide (Schena et al., 2003).

Scores

- Effectiveness received a score of **4**, as disease reduction on grapes ranged between 59% and 64%, and in cherries up to 80% supressing the 50% threshold required for a top score (Schena et al., 2003).
- The deduction score for climate is **1**, as the study was conducted in a mediterranean climate(Italy), which differs from Dutch climate (Schena et al., 2003).
- The score for external effects is **4,** as no negative effects on fruit quality, environment, or human health were reported (Schena et al., 2003).
- The easiness of application received a score of **4**, since standard orchard sprays or dips were used, not requiring specialized equipment (Schena et al., 2003).
- The total score of *Aurebasidium pullanlns* (isolate 533, 547) is **7.8-9.3**

3.5.2 Myco-sin (score = 7.0)

<u>Allowance in the Netherlands</u>

The Myco-Sin, based on the literature review, shows insufficient data regarding its approval for use in the European Union. Currently, the only country that has approved its use is Switzerland. However, based on the safety data sheet available on the company's website, Myco-Sin adheres to Regulation (EC) No. 1907/2006 (REACH) and Regulation (EU) 2020/878 regarding chemical safety and classification standards (Andermatt Biocontrol Suisse AG, 2022, Regulation 2020/878). It is also classified and labelled under the CLP Regulation (EU) No. 1272/2008, which governs the classification, labelling, and packaging of substances and mixtures in the EU (Andermatt Biocontrol Suisse AG, 2022, Regulation 1272/2008). It couldn't be found in the ctgb Admissions of Netherlands.

<u>Mode of action</u>

The mode of action of Myco-Sin is multifaceted according to the product label in the website of Andermatt Biocontrol Suisse AG (2022), effectively protecting plants from pathogens and promoting overall health. It creates a highly acidic, which is unfavourable for fungal and bacterial pathogens, thereby inhibiting their growth and activity. The active ingredients, including sulfuric clay minerals and extracts from equisetum (horsetail), exert direct antimicrobial effects by releasing aluminium ions that prevent the germination and spread of fungal spores and bacteria.

Additionally, Myco-Sin enhances the plant's natural defence mechanisms by inducing resistance to pathogens, potentially activating systemic acquired resistance pathways (Myco-Sin, 2015, (Andermatt Biocontrol Suisse AG, 2022)). By coating plant surfaces, it provides physical protection against pathogen invasion while hardening the epidermis and cuticula, further strengthening structural defences. Moreover, Myco-Sin improves nutrient uptake and increases biological soil activity, contributing to overall plant vigour and resilience against pathogen attacks. In conclusion, Myco-Sin provides various modes of action against *P. viticola* and *E. necator*, offering farmers a safeguard against the development of resistance by these diseases.

<u>Effectiveness against downy mildew, powdery mildew and grey mold</u>

The Myco-Sin has not been tested in the Netherlands but has been tasted for over 4 years in Rovereto, Italy on the cultivar variety Cabernet Sauvignon on Kober 5BB rootstock (pergola trentina trellis system) and in Frick, Switzerland on cultivar varieties Riesling-Sylvaner and Chasselas on Kober 5BB rootstock (guyot trellis system) (Dagostin et al., 2011).

The effectiveness of the product in the specific paper were calculating different from how we are scoring in our project. The Dagostin et al. (2011) study bases the effectiveness on the efficacy to control the downy and powdery mildew on leaves and brunches. Where the efficacy of controlling the disease compare with the two controls of the study-without any applications and with standard treatment (copper hydroxile)-to check the significance. The Myco-Sin showed on average efficacy 83% on controlling the diseases in leaves and brunches. The were another treatment with Myco-Sin together with wettable sulphur were the efficacy of controlling the diseases increased on 91% average. Both treatments were significantly different from the control treatment without any application while there was no significant difference compared to control treatment with copper hydroxile.

External Effects

- Environmental Effects: Myco-Sin can negatively affect aquatic organisms due to aluminium ions. Care must be taken to prevent residues or containers from polluting waterways or soil (Andermatt Biocontrol Suisse AG, 2022).
- Human Health Risks: It can cause skin irritation, severe eye damage, and respiratory irritation. The use of personal protective equipment (PPE), including gloves, goggles, and a P3 respirator mask, is required (Andermatt Biocontrol Myco-sin is a biological control product produced by the Swiss company Andermatt. The product consists of 65% of sulfuric clay/alum and 0.2% horsetail extract (*Equisetum arvense*) (Myco-Sin, 2015). Can be used against downy mildew and powdery mildew in grapes (Dagostin et al., 2011).

The product can be used preventively before expected precipitation, starting from May until middle of August, after it is recommended to avoid any further applications due to phytotoxicity risks (Myco-Sin, 2015). The company recommends to prepare the spray solution by pre-dissolving Myco-Sin with 0.3 wettable sulphur at least 30 minutes before use by mixing 1 kg of the product in 4–8 litres of warm water using a whisk or electric mixer (Andermatt Biocontrol Suisse AG, 2022). Fill the spray tank halfway with water, then stir the pre-dissolved mixture thoroughly again and add it to the tank through a sieve while continuously agitating. Finally, fill the tank with the remaining water. Spray the entire contents in one session to avoid sedimentation, ensuring proper mixing and coverage. Is recommended for good coverage of the vineyard to dissolve the product in 800 litters per hectare of water. The application can be applied every 8-12 days, adjusted after rainfall.

The Myco-Sin can be bought from the official website of the Andermatt, as a package of 5 kg is cost 77 Swiss Francs (SF) or a package of 25 cots 275.5 SF, where in euro based on the exchange rate of European Central Bank on 2nd December 2024 are around 83€ and 296€, respectively (Myco-Sin, 2015, European Central Bank, 2019).

Score

- Effectiveness received a score of **4**, as the average disease control of downy and powdery mildew on grapes was 83% using only Myco-Sin, and increased to 91% when combined with wettable sulfur (Dagostin et al., 2011).
- Deduction score for testing conditions is **1**, due to the product was tested in Switzerland and Italy but not in a Dutch climate.
- External effects received a score of **1**, as Myco-Sin can negatively impact the environment and human health (Andermatt Biocontrol Suisse AG, 2022).
- Ease of application received a score of **3**, as the application rate is around seven applications per season (Dagostin et al., 2011).
- Costs received a score of **4**, as 4 kg of Myco-Sin is needed per hectare, costing approximately €66 (Andermatt Biocontrol Suisse AG, 2022).
- Total score for Myco-Sin is **7**.

3.5.3 Trichoderma harzanium strain T39 - TRICODEX™ (score = 6.9 -8.4)

<u>Allowance in the Netherlands</u>

Currently, *T. harzanium* strain T39, marketed under the trade name TRICHODEX™, is not available for commercial use in the Netherlands (ctgb,2024). While its use has been documented extensively in other countries like Italy, France, and Australia, it has yet to be officially registered and therefore cannot be used in the Netherlands (O'Neill et al., 1996).

<u>Mode of action</u>

The active component of TRICHODEX™ is a strain of *T. harzianum* strain T39, which contains fungal mycelium and conidia. This biocontrol agent employs several mechanisms to combat fungal pathogens such as *B. cinerea*, *Sclerotinia sclerotiorum*, and powdery mildew (Pertot et al., 2008). One primary mode of action is direct antagonism, where *T. harzianum* parasitizes fungal pathogens by producing extracellular enzymes and antifungal antibiotics that disrupt the pathogens' cell walls. Additionally, it competes with pathogens for essential nutrients and space, effectively starving them of resources necessary for growth and reproduction (Elad, 1994).

Another crucial mechanism is induced resistance, where *T. harzianum* primes host plants for an enhanced defensive response by activating induced plant defences. Furthermore, the fungus reduces the growth and spore dispersion capabilities of pathogens, limiting their ability to spread. The specific mode of action depends on the targeted pathogen. For instance, powdery mildew is primarily controlled through induced resistance, while *B. cinerea* is suppressed through competition and the enzymatic restraint of pathogenicity factors. These multifaceted actions make TRICHODEX™ a versatile and effective biocontrol agent in integrated pest management systems (Elad, 1994).

<u>Effectiveness against downy mildew, powdery mildew and grey mold</u>

TRICHODEX™ has been extensively tested in different environmental conditions and crop systems such as this large-scale study involving 133 experiments conducted across 19 countries, including France, Italy, and Australia. TRICHODEX™ achieved a 36.3% to 72.7% reduction in *B. cinerea* infections (O'Neill et al., 1996).

Another study found that that TRICHODEX™ was able to induce plant defences caused a reduction in downy mildew severity in grapevines. These trails were done in greenhouse settings (Perazzolli et al., 2011).

Lastly this study by Pertot (2008) found that regular application of TRICHODEX™ in combination with limited fungicides was shown to be as effective as fungicide-only strategies in greenhouses against powdery mildew. This reduces most of the amount of chemical fungicides needed to effectively combat this pathogen, unfortunately a little bit of the chemical fungicide is still needed to get the same amount of infection reduction as when only chemical fungicide is used (Pertot et al., 2008)

Products, Application, and Costs

TRICHODEX™ was commercially developed by Makhteshim Chemical Works as a water-dispersible powder containing *T. harzianum* strain T39. Application is recommended at the beginning of the vegetation season to suppress grey mold. The suspension requires a sprayer for foliar application, despite its effectiveness, frequent application is necessary, with biweekly sprays showing better control than weekly or longer intervals. However, the product is no longer commercially available, and cost data are not accessible (Rajkovic et al., 2013**)**.

External Effects

TRICHODEX™ is environmentally safe, posing no harm to humans, mammals, birds, or beneficial insects. Its incorporation into integrated pest management (IPM) programs has been widely recognized, especially in organic farming systems, due to its lack of harmful residues or phytotoxicity (Rajkovic et al., 2013).

Scores

- In terms of effectiveness, TRICHODEX™ is considered effective when combined with minimal fungicide use, but less potent as a standalone treatment, leading to a score of **3** (Neill et al., 1996; Perazzolli et al., 2011).
- A deduction score of **2** was given because it was not tested specifically in the Dutch climate, but extensive trials in countries with similar climates (O'Neill et al., 1996).
- It's use on grapevines has been well documented through extensive trials, resulting in a score of **4** (O'Neill et al., 1996).
- The product has also been tested extensively in practise settings, receiving another score of **4**.
- The product has no observed negative external effects on the environment or human health, earning a score of 4 (Rajkovic et al., 2013).
- A score of **3** for ease of application was given as its application requires a sprayer and frequent applications, which makes it less convenient (Rajkovic et al., 2013).
- The costs received **no score**, as the product is no longer available on the market, and commercial pricing is unknown.
- The total score for TRICHODEX™ is a range of **6.9-8.4**.

3.5.4 Galactomyces candidum JYC1146 (score = 6.8 -8.3)

Allowance in the Netherlands

Galactomyces candidum JYC1146 is not admitted in the Netherlands (ctgb, 2024)).

Mode of action

Galactomyces candidum JYC1146 is a biological control agent that is produced by fermentation. It inhibits the growth of pathogenic fungi by producing VOCs (Chen et al., 2018). Furthermore, it has the ability to secrete chitinase (Chen et al., 2018), which plays an important role in suppression of fungal growth (Dahiya et al., 2006; Singh et al., 1999).

Effectiveness against downy mildew, powdery mildew and grey mold

A study on strawberries that were put near a *G. candidum* JYC1146 culture on a petri dish, found that it was able to inhibit *B. cinerea* infection. Disease severity after 8 days was 1, as compared to 8 for control (Chen et al., 2018). In this case, 1 meant <12.5% of area rotted; 8 meant between 87.5-100% area rotted. The study was conducted in a sealed plastic box with high relative humidity at 22^{o}C.

External effects

No risk assessment on specific strain *G. candidum* JYC1146 could be found. However, a safety assessment of *G. candidum* was done as it is widely used as adjunct culture in the maturation of cheese. The report concluded that infection due to *G. candidum* when ingested, is virtually nil (Pottier et al., 2007).

Products, application and costs

Galactomyces candidum JYC1146 nor *G. candidum* is commercially available.

Score

- Effectiveness received a score of **4**. This is based on the disease control of *B. cinerea* found by Chen et al. (2018). This was 75.4% (worst scenario: 12.5% area rotted with treatment, 87.5% for control).
- Deduction scores for the study by Chen et al. (2018) for not being tested on grapes and lab tests were **3** and **1** respectively.
- The score for external effects is **4**, as no evidence is present for risks regarding health or environment.
- Easiness of application received a score of **3** as *G. candidum* JYC1146 was only applied once by Chen et al. (2018), but special equipment is needed to make *G. candidum* JYC1146.
- Costs received no score, as *G. candidum* JYC1146 is not sold commercially,
- The total score of *G.candidum* JYC1146 is **6.8**-**8.3**.

3.5.5 Clonostachys rosea – Prestop (score = 6.8 – 8.3)

Allowance in the Netherlands

While products based on *Clonostachys rosea* (formerly *Gliocladium roseum)* are available commercially in some markets, there is no evidence of approval specifically for use on grapes in the Netherlands. The Dutch Board for the Authorization of Plant Protection Products and Biocides (Ctgb) does not list any *C. rosea* product approved for controlling grape pathogens (Ctgb, 2024).

Mode of Action

Clonostachys rosea is a naturally occurring fungal antagonist that targets *B. cinerea* and other fungal pathogens. It suppresses disease development through mechanisms like competition, hyperparasitism, and nutrient sequestration, all of which reduce pathogen infection and sporulation. Unlike chemical fungicides, it does not rely on toxic modes of action, making it more environmentally friendly(Hjeljord & Tronsmo, 1998; Morandi et al., 2003).

Effectiveness against downy mildew, powdery mildew and grey mold

Greenhouse and controlled environment trials on grapes indicate significant disease suppression. Although exact figures vary, a roughly 50% reduction in disease incidence is commonly reported, placing disease occurrence in the 25–50% range. Consequently, *C. rosea* scores a 3 for effectiveness (Hjeljord & Tronsmo, 1998). While *C. rosea* has been studied extensively in Europe, no direct references confirm field testing under Dutch or Belgian climatic conditions for grape cultivation, scoring 0 for tested-in-Dutch/Belgian climate. Trials have focused mainly on greenhouse conditions rather than multi-year field experiments. The fungus has, however, been tested directly on grapes, (Hjeljord & Tronsmo, 1998; Morandi et al., 2003).

Products, Application, and Costs

Products based on *C. rosea* (e.g., Prestop) are typically applied as foliar sprays using conventional vineyard equipment. Around 2–5 applications per season, timed to critical points like bloom or pre-harvest, may be recommended. These requirements do not necessitate special equipment but may involve some adaptation of spray timing and tank mixes. No cost data was available, resulting in a cost score of 0 .

External Effects

Clonostachys rosea is considered environmentally safe and poses no known risks to non-target organisms, grape quality, or human health. Thus, emphasizing its potential role in sustainable and integrated pest management (Zvedenec et al., 2007).

Score

- Effectiveness score is **3**, based on approximately 50% disease reduction in controlled environment trails on grapes (Hjeljord & Tronsmo, 1998).
- Score of the external effects is **4**, Since no negative impacts on the environment, grape quality, non-targeted organisms were reported (Zvedenec et al., 2007).
- The easiness of application scored **3**, considering that *C. rosea* can be applied with standard vineyard equipment and about 2-5 sprays per season without special requirements (Morandi et al., 2003).
- Costs received **no score**, as no cost data was available
- Practice scored **2** as testing focused on greenhouse conditions rather than extensive, multiyear field trials (Hjeljord & Tronsmo, 1998; Morandi et al., 2003).
- Total score of *C. rosea* is **6.8-8.3**.

3.5.6 Ulocladium oudemansii (score = 6.4)

Allowance in the Netherlands

Ulocladium oudemansii is not registered for use as a commercial biocontrol product in the Netherlands. While BOTRY-Zen (a product containing a related *Ulocladium* species is authorized in parts of the EU, it currently does not hold approval in the Netherlands (ctgb, 2024).

Mode of Action

Ulocladium oudemansii is a biological control fungus that antagonizes *B. cinerea,* the pathogen responsible for bunch rot in grapes. It operates primarily through competition and inhibition of fungal growth, effectively reducing the conidiophore production and subsequent inoculum levels of *B. cinerea*. Unlike chemical fungicides, it achieves disease suppression by colonizing necrotic tissue and outcompeting the pathogen, thereby providing a natural and environmentally friendly means of control. No phytotoxic effects or negative impacts on grape quality, human health, or the broader ecosystem were reported (Reglinski et al., 2005).

Effectiveness Against grey mold

Field and laboratory studies have shown that *U. oudemansii* significantly reduces botrytis bunch rot severity on grapes. Under high-humidity postharvest conditions, untreated bunches reached 77–83% disease severity, whereas those treated with *U. oudemansii* remained at about 37%, indicating a considerable reduction and placing it in the 25–50% disease occurrence bracket (Reglinski et al., 2005).

Products, Application, and Costs

Although no specific commercial formulation of *U. oudemansii* is mentioned as approved in the Netherlands, research trials applied multiple sprays (11 applications per season) in a commercial vineyard setting. This indicates a higher workload compared to certain other control measures. Estimations suggest that costs, based on product preparation rates, would fit into a moderate category (US$500–$1200 per ha) (Reglinski et al., 2005). The application method resembles standard spraying procedures, but the high number of sprays needed lowers the easiness-of-application score.

Testing Conditions and Practice

Ulocladium oudemansii has been tested on Chardonnay grapes in Hawke's Bay, New Zealand, a region with a temperate oceanic climate (Cfb), similar to the Northwestern European climate found in parts of the Netherlands or Belgium. The trials were conducted under practical field conditions in a commercial vineyard, providing reliable, practice-oriented insights (Reglinski et al., 2005).

External Effects

No negative external impacts were documented. On the contrary, being a naturally occurring antagonist, *U. oudemansii* supports a sustainable approach to disease management. It poses no known risks to beneficial organisms, the environment, or human health (Reglinski et al., 2005).

Scores

- Effectiveness received a score of 3, as disease severity was reduced from ~77-83% in untreated controls to ~37%, placing it in the 25-50% disease occurrence bracket (Reglinski et al., 2005).
- Score for climate is **3**, since trails took place in a temperate oceanic climate (Hawke's Bay, New Zealand) considered more similar to that of Northwestern Europe than a Mediterranean or subtropical climate (Reglinski et al., 2005).
- The score for external effects is **4**, as no negative impacts on the environment, haman health, or beneficial organisms were reported (Reglinski et al., 2005).
- The easiness of application recieved a score of **1**, due to the need for 11 sparys per season, making it more labour intensive than other methods (Reglinski et al., 2005).
- Costs recieved a score of **2**, with estimated cost ranging roughly between $500 to $1200 per ha , placing it in a moerate cost category (Reglinski et al., 2005).
- The total score of *U. oudemansii* is **6.4**

3.5.7 Bacillus licheniformis (score = 5.7 -7.2)

Allowance in the Netherlands

Bacillus licheniformis is not admitted in the Netherlands (ctgb, 2024).

Mode of action

Bacillus licheniformis N1 is a biological control agent, developed using fermentation culture. It is mainly known for controlling *B. cinerea* on soft fruits and vegetables. *B. licheniformis* N1 combats fungal diseases in plants through antagonistic activity: it produces antimicrobial compounds such lipopeptides, which directly inhibit fungal growth by disrupting their cellular processes (Bonmatin et al., 2003; Ogenena et al., 2007). In addition, lipopeptides play a role in inducing plant resistance against pathogens (Ogena et al., 2007).
The production of chitinase by biological control agents plays an important role in suppression of fungal growth (Dahiya et al., 2006; Singh et al., 1999). It is known that *B. licheniformis* N1 contains a gene which encodes a chitinase, but the gene was not functional due to a lack of expression (Lee et al., 2009). Lee et al. (2009) suggested that proper engineering of the expression of the chitinase gene may enhance the biocontrol activity of *B. licheniformis* N1.

Effectiveness against downy mildew, powdery mildew and grey mold

A study on tomatoes treated with *B. licheniformis* N1 showed that the disease control of *B. cinerea* on tomato flowers was 90.5% under production conditions, as compared to 77% with chemical fungicide (Lee et al., 2006). A wettable powder formulation, called *B. licheniformis* N1E*,* was used in this research. It was conducted in plastic-house artificial infection experiments and natural infection experiments under field production conditions in Korea. Spray application of *B. licheniformis* N1E was repeated three times during the growing season.

The same wettable powder formulation was used in another study by Kim et al. (2007) on strawberries and proved to significantly reduce disease severity of grey mold, especially when applied before *B. Cinerea* inoculation in pot experiments. The disease control value on strawberry leaves was 81% under production conditions, as compared to 61.5% with chemical fungicide (Kim et al., 2007). Production conditions entailed cultivation in a plastic farmhouse in Korea. Spray application of *B. licheniformis* N1E was repeated three times during the growing season.
A study on lettuce treated with wettable powder formulation *B. licheniformis* N1K showed a *B. cinerea* disease control value of 79.6% (Lim, 2001). The control effect was maintained at 77.5% for two weeks but showed a 10% decrement after four weeks. The experiments were conducted in a greenhouse in Korea, but application rates were unclear.

A study on strawberry plants found that *B. licheniformis* N1 forms bacterial aggregates on plans surfaces for at least 3 days, creating a biofilm that suppresses fungal inoculums (Kong et al., 2010).

External effects

No risk assessment on specific species *B. licheniformis* N1 could be found. However, EFSA (2014) conducted a risk assessment on the use of *Bacillus* species in animal nutrition. The report states that some species produce toxins, but further research is needed to evaluate if this is the case for *B. licheniformis* N1. Concerns are also associated with the production of surfactin like lipopeptides, although the relationship between these compounds and human illness is not yet established (ESFA, 2014).
The study by Lee et al. (2006) also found a growth promotion by *B. licheniformis* N1E, which increased the number of tomato fruits, as compared to fungicide treatment and non-treated control. Studies on grapes treated with *B. licheniformis* N1, and consequently effect of on wine quality, were not found in scientific literature.

Products, application and costs

Bacillus licheniformis is commercially available as water soluble fertilizer. It can be applied through irrigation or foliar spray. For soil application, it is recommended to mix 0.5 kg/ha *B. licheniformis* fertilizer with 500 kg organic manure/fertilizer (marktnature.com n.d.). For foliar spraying, no recommendations on amount are given but it is recommended to spray at an interval of 7 to 10 days (*B. Licheniformis, 100 billion CFU/Gram, Water Soluble Biological Fertilizer*, n.d.). 1 kg *B. licheniformis* fertilizer is sold for €72.66 (*Bacillus Licheniformis, 100 billion CFU/Gram, Water Soluble Biological Fertilizer*, n.d.).

Score

- Effectiveness received a score of **4,** this is based on the average disease control of *B. cinerea* found by Lee et al. (2006) and Kim et al. (2007), because Lim (2001) tested on non-soft fruit lettuce. This was 85.8%, which led to a score of 4.
- Deduction scores for the studies by Lee et al. (2006) and Kim et al. (2007) for not being tested on grapes, tested in field trials, and Korean climate were **2**, **3**, and **1** respectively.
- The score for external effects is **3**, as concerns are expressed by the EFSA (2014) on the possible production of surfactin like-lipopeptides by *Bacillus* species.
- Easiness of application received a score of 4. Application rates used by Lee et al. (2006) and Kim et al. (2007) were low, and no special equipment was necessary.
- Costs received **no score**, as no dosage recommendations for foliar spraying are given, and Lee et al. (2006) and Kim et al. (2007) used foliar application.
- The total score of *B. licheniformis* N1 is **5.7-7.2**.

3.5.8 Paenibacillus polymyxa (score = 4.9-6.4)

Allowance in the Netherlands

Currently, the use of *Paenibacillus spp*., including *P. polymyxa,* as a biocontrol agent is limited by regulatory approvals in the Netherlands. While some formulations containing *Paenibacillus* strains have been patented and are commercially available in other regions, the Dutch Board for the Authorization of Plant Protection Products and Biocides (Ctgb) does not list specific approvals for these strains in viticulture (Dobrzyński & Naziębło, 2024)

Mode of Action

Paenibacillus spp. suppress fungal phytopathogens through diverse mechanisms, including the production of hydrolytic enzymes (e.g., chitinases, glucanases, cellulases) that degrade fungal cell walls (Dobrzyński & Naziębło, 2024). They also produce antifungal lipopeptides, such as fusaricidin and paenimyxin, which disrupt pathogen membranes and inhibit fungal growth (Dobrzyński & Naziębło, 2024). Additionally, *Paenibacillus* spp. release volatile organic compounds (VOCs) (Dobrzyński & Naziębło, 2024) and induce systemic resistance (ISR) in plants by activating defense pathways (Dobrzyński & Naziębło, 2024). These multifaceted mechanisms make them highly effective biocontrol agents.

Effectiveness

Paenibacillus spp., particularly *P. polymyxa,* have shown effectiveness against *B. cinerea* in greenhouse and field trials. For instance, strains producing fusaricidin and chitinases significantly reduce grey mold severity (Dobrzyński & Naziębło, 2024). However, there is no specific evidence of their activity against downy mildew or powdery mildew in grapevines, highlighting a gap in research on these pathogens (Dobrzyński & Naziębło, 2024).

External Effects

While *Paenibacillus* spp. are environmentally friendly and compatible with sustainable farming practices, concerns about their impact on native soil microbiota and non-target organisms exist. Some strains are associated with opportunistic pathogenicity in humans and animals, emphasizing the need for safety evaluations before widespread agricultural use (Dobrzyński & Naziębło, 2024).

Products, Application, and Costs

No cost data or specific product formulations were provided. Application involved preparing a fermentation broth and applying it to seedlings or roots. Although exact spray frequencies and special equipment were not specified, using standard spraying or drenching methods is likely feasible. Without cost information, the affordability remains uncertain (Tian et al., n.d.).

Testing Conditions and Practice

Paenibacillus spp. have been primarily tested in greenhouse and field conditions for crops like tomatoes, cucumbers, peppers, and strawberries (Dobrzyński & Naziębło, 2024). For instance, *P. polymyxa* significantly reduced Fusarium wilt in cucumbers under greenhouse experiments. Field trials have also been conducted for certain strains, but testing on grapevines, especially under Dutch climatic conditions, remains lacking (Dobrzyński & Naziębło, 2024).

Scores

- Effectiveness received a score of **3,** as *P. polymyxa* significantly reduced *Botrytis cinerea* severity in greenhouse and field trials on tomatoes and strawberries, but no evidence supports its activity against downy mildew or powdery mildew in grapevines (Dobrzyński & Naziębło, 2024).
- The score for climate is **1,** as trials were conducted outside the Netherlands in regions with different climatic conditions, such as greenhouse settings or Mediterranean climates (Dobrzyński & Naziębło, 2024).
- The The score for external effects is **2**, due to potential risks of disrupting native soil microbiota and concerns about opportunistic pathogenicity in non-target organisms, although *Paenibacillus spp.* generally align with sustainable farming practices (Dobrzyński & Naziębło, 2024).
- The easiness of application recieved a score of **3** , as standard methods such as foliar or soil treatments can be used for applying *P. polymyxa*, but application protocols for grapevines remain undefined (Dobrzyński & Naziębło, 2024).
- Costs received **no score**, since there is no price information on commercial product.
- The total score of *P. polymyxa* is **4.9-6.4**

3.5.9 Penicillium oxalicum (score = 3.9)

Allowance in the Netherlands

No information was found on the commercial registration or approval of *Penicillium oxalicum*-based products for grape disease control in the Netherlands. Products containing *P. oxalicum* (e.g., BioFungus) have been studied in other regions, notably Mediterranean climates, but no listings appear in the Dutch ctgb database.

Mode of Action

Penicillium oxalicum is a naturally occurring soil fungus that has been investigated for its biocontrol potential against various fungal pathogens. It suppresses diseases like brown rot (*M. laxa*) in peaches and Fusarium wilt in tomatoes, most likely by competing with pathogens for space and nutrients and possibly producing antifungal metabolites. Its activity has been validated under field conditions, suggesting compatibility with standard orchard practices (De Cal et al., 1997).

Effectiveness against downy mildew, powdery mildew and grey mold

Field trials on peaches demonstrated that *P. oxalicum* treatments reduced brown rot incidence by about 50%. While data on *B. cinerea* in grapes is lacking, the successful suppression of peach brown rot provides a reliable indicator of its biocontrol capability. A roughly 50% reduction in disease puts effectiveness in the 25–50% occurrence bracket, corresponding to a score of 3 (De Cal et al., 1997). Research on *P. oxalicum*'s effectiveness has taken place in Mediterranean climates (e.g., Spain), which differ from the temperate oceanic climate (Cfb) of the Netherlands or Belgium. According to the scoring criteria, testing in such distinct conditions earns a score of 1. Furthermore, the fungus was tested in field trials (rather than just laboratory or greenhouse experiments), awarding it a score of 3 for being tested in practice. However, no testing on grapes or soft fruits is reported; peaches are stone fruits, not soft fruits.

External Effects

No negative external impacts on plant quality, human health, or the environment were reported. *P. oxalicum,* being a naturally occurring fungus, appears safe and environmentally benign when used as a biocontrol agent.

Products, Application, and Costs

Specific details on spray frequency, equipment, or special management adaptations required for *P. oxalicum* application are not available. Without these data, ease of application and cost remain unknown,

Score

- Effectiveness received a score of **3**, based on field trials in peaches where *P. oxalicum* reduced brown rot incidence by about 50%, placing it in the 25–50% disease occurrence bracket (De Cal et al., 1997).
- Score for climate is **1**, as the testing occurred in a Mediterranean climate, which differs significantly from the Dutch or Belgian climate (De Cal et al., 1997). Tested on grapes scored **1**, since the data derives from peach trials rather than grapevines or soft fruits, limiting direct relevance to grape cultivation (De Cal et al., 1997). Practice received a score of **3**, acknowledging that trials were conducted under real field conditions rather than solely in laboratory or greenhouse settings (De Cal et al., 1997).
- The score for external effects is **4**, since no negative impacts on plant quality, human health, or the environment were reported, reflecting a benign environmental profile (De Cal et al., 1997).
- The easiness of application received **no score**, due to the lack of specific information on spray frequency, equipment requirements, or application methods (De Cal et al., 1997).
- Costs received **no score**, as no cost or economic data were provided.
- The total score of *P. oxalicum* is **3.9**.

3.5.10 Trichoderma harzianum strain T-9 (score=0.4-7.4)

Allowance in the Netherlands

Base the Dutch Board for the Authorization of Plant Protection Products and Biocides and EU pesticides database no product is registered containing the strain *Trichoderma harzianum* T-9 (Ctgb, 2024; *EU Pesticides Database*, 2024).

Mode of action

Trichoderma harzianum T-9 acts by inducing the plant defense to increased production of pathogenesis-related (PR) proteins, such as chitinase and β-1,3-glucanase (Saksirirat et al., 2009). These enzymes degrade the cell walls of fungal pathogens, reducing the spread and severity of infections. In general *Trichoderma spp.* are competing with other microorganisms for space and nutrients (Harman et al., 2004).

Effectiveness against downy mildew, powdery mildew and grey mold

The is no available literature information about T. *harzianum* T-9 use as a biological control in grapevine for any fungal disease, neither exist any literature about the biological control of *T. harzianum* T-9 against *B. cinerea*, powdery and downy mildew in any crop. However, a study Saksirirat et al. (2009) in laboratory conditions in tomate leaf discs has tested the biological control properties of T. harzianum T-9 against bacterial spot (Xanthomonas campestris pv. vesicatoria (XCV)) and gray leaf spot (Stemphylium solani). The results of *T. harzianum* T-9 showed significant effectiveness in reducing bacterial spot symptoms by 69.32% and gray leaf spot by 7.52% on treated plants. The rest bibliography is focused on the antibiotic properties of *T. harzianum* T-9 against human pathogenic bacteria and enterobacteria (Weeraya Phupiewkham et al., 2015).

External effects

No external effects can be mentioned about *T. harzianum* T-9 as not enough literature exists to back up any negatives effects that may the fungi have to the environment, human health and grapevines.

It was mentioned by vineyard keepers that *T. harzanium* T-9 caused discoloration of the grapes skins, but did not influence the quality of the grapes (interviews, 2024).

Products, application, and costs

No commercial products exist in the Europe and in the world which are containing the strain of *T. harzianum* T-9.

Score

- There is no information about *T. harzianum* T-9 in grapes or soft fruits, nor for any of the three fungal diseases. However, it has been tested in tomato cells for bacterial and fungal diseases, showing 56% and 7% effectiveness, respectively, resulting in a score for effectiveness of **2** (Weeraya Phupiewkham et al., 2015).

- Deduction scores was applied because it has not been tested in Dutch weather, on grapes, or in practical applications, resulting in a score of **1 in each criterion**.
- No literature exists on the possible effects of *T. harzianum* T-9 on the environment, human health, or grapes. However, interviews with vineyard keepers report discoloration of grape skins after using T-9, so external effects scored **3**.
- Easiness of application received **no score**, as no information is available.
- Costs received **no score**, as no commercial products are available.
- The total score for the *T. harzianum* T-9 is **0.4**-**7.4**.

3.6 Non-microbial disease management strategies

Besides microbial biological control methods, non-microbial compounds or management strategies could contribute to managing diseases in Dutch viticulture. This subchapter will therefore answer the sub question: *"What are promising disease management practices, besides biological control measures, that could contribute to the efficient use of biocontrol measures?"*

A study by Dagostin et al. (2011) tested 112 different biological compounds. The research revealed that, while a broad range of compounds showed some level of activity, only a small number emerged as practically feasible solutions for viticulture application. This underscores the importance of screening through literature reviews and combining this with vineyard keeper interviews to come up with feasible solutions alongside microbial biocontrol agents.

3.6.1 Calcite

Allowance in the Netherlands

The mineral calcite, mainly consisting of calcium carbonate ($CaCO_3$), marketed as *Oenosan*, is approved for use as a foliar treatment in grapevines within the Netherlands (ctgb, 2024). It is a calcium-based product derived from natural limestones or seashells.

Mode of Action

When applied to grapevines, calcite provides multiple benefits. It improves the bioavailability of calcium, an essential nutrient that strengthens plant cell walls by inhibiting the degradation of pectin. Calcium limits the activity of polygalacturonases, enzymes responsible for fruit softening, thereby preventing cell wall breakdown. This structural reinforcement is particularly beneficial for disease-susceptible grape cultivars, which typically have more cracks in their berry skins than more disease-resistant varieties (Martins et al., 2021). The increased skin firmness acts as a physical barrier to fungal pathogens. Additionally, calcium plays a critical role in enhancing overall plant health, supporting growth and development, and reducing the likelihood of disease penetration.

Effectiveness against downy mildew, powdery mildew and grey mold

Through the strengthening of the physical structure and overall health of the grapevines, it helps mitigate the damage these fungal pathogens can cause, especially in varieties prone to disease (Maya-Meraz et al., 2023). Therefore, *Oenosan* is hypothesized to enhance grapevine resistance to powdery mildew, downy mildew, and grey mold. However, no available literature was found regarding calcium carbonate and resistance to downy mildew, powdery mildew and grey mold . However, antifungal activity of calcite is shown against pathogens that cause diseases in tomato plants (Motlhalamme et al., 2023).

External Effects

The foliar application of calcite has been shown to significantly increase grape cluster and berry weights without altering key maturity indicators like total soluble solids (TSS), pH, or titratable acidity (TA) (Maya-Meraz et al., 2023). Moreover, calcium-treated grapes exhibited a higher colour intensity, which is advantageous for grape quality. During vinification, wines produced from calcium-treated grapes demonstrated improved phenolic composition, enhanced antioxidant capacity, and better overall quality, including improved TA, TSS, and pH levels (Maya-Meraz et al., 2023).

Products, Application, and Costs

According to the website of Oenosan regular spraying equipment can be used and spraying can be done directly on the leaves. However, no specific details on spray frequency, or special management adaptations are available (Oenosan, Westkerk). Without this information, ease of application remains unknown. Oenosan is sold for €285 for 5 kg (Oenosan, Westkerk). The treatment plan (shown in appendix 9.4) of one of the interviewed vineyard keeper mentioned 300 grams of Oenosan with 300-liter water should be applied two weeks after bloom. However, as there is no scientific-based evidence this report will therefore not score the easiness of application.

Score

As no scientific-based literature was found regarding effectiveness or application instruction of calcite therefore it is decided calcite is not getting scored. Only the external effects could be assessed got scored a **4** as no external effects are shown on the environment nor the grape quality (Maya-Meraz et al., 2023).

3.6.2 Potassium bicarbonate (Score: 8.1)

Allowance in the Netherlands

Potassium bicarbonate-based biofungicides, sold under the brand names Vitisan and Armicarb, are approved for use in organic viticulture in the Netherlands (Ctgb, 2024). These products are permitted as an environmentally friendly solution for managing fungal diseases like grey mold and powdery mildew in grapevines.

Mode of Action

The primary mechanism of potassium bicarbonate involves altering the pH on the leaf and fruit surfaces. Potassium bicarbonate ($KHCO_3$) increases the alkalinity of the berry skins and leaf surfaces, creating an environment that is hostile to fungal growth and reproduction. This pH shift inhibits spore germination and fungal mycelium development (Arslan et al., 2006). Additionally, potassium bicarbonate exerts a desiccating effect on fungal spores and hyphae. The bicarbonate ions create an osmotic imbalance in fungal cells, causing mycelium cells to lose water and collapse.

Effectiveness against downy mildew, powdery mildew and grey mold

Research has demonstrated that potassium bicarbonate is effective in controlling powdery mildew on grape clusters, even achieving control levels that are comparable to standard chemical strategies (Rantsiou et al., 2020).

External Effects

While potassium bicarbonate is approved for use in organic viticulture and is considered an environmentally safe alternative, its application requires careful management. The product exhibits a narrow concentration range between ineffective and effective dosages. At higher concentrations, potassium bicarbonate can induce phytotoxicity, causing leaf damage and reduced plant vitality (Dagostin et al., 2011).

Products, Application, and Costs

Potassium bicarbonate is commercially sold as Vitisan and Armicarb and has a maximum set application rate of six times per year, with a maximum of 12 kg/ha. However, the advised standard dose is 5 kg/ha (ctgb, 2024). Whereas 25kg of Vitisan is sold online for €250 meaning an average cost of €50 per hectare.

Score

- For effectiveness a **4** was given as powdery mildew on grape clusters was controlled levels that are comparable to standard chemical strategies (Rantsiou et al., 2020).
- A score of **4** was given for application on grapes, as it was tested on grapes (Rantsiou et al., 2020). A deduction score of **2** was given for not testing under

Dutch climate conditions, but Italian climate conditions and a score of **3** for use in practice as it was only tested for one season a controlled field experiment.

- External effects got a score of **3**, as potassium bicarbonate is considered an environmentally safe compound however at higher concentrations, potassium bicarbonate can induce phytotoxicity.
- The product's ease of application received a **3** as a back sprayer is needed.
- Finally, the costs were given a **4**, because the average price of €50 per hectare, with a maximum application rate of 6 times per year will lead to €300 on average per hectare per growing season
- The total score of potassium bicarbonate is an **8.1**

3.6.3 5CSA (Score: 4.09+1.5)

Allowance in the Netherlands

5-chlorosalicylic acid (5CSA) is not commercially registered as a biocontrol product in the Netherlands. No indications of approval or commercialization are mentioned in the literature (Reglinski et al., 2005).

Mode of Action

5CSA functions as an elicitor of host plant defenses rather than as a direct fungicidal agent. By inducing systemic acquired resistance (SAR) and stimulating the plant's own defense pathways, it reduces the impact of *B. cinerea* infections. Its effectiveness is seen in the field, where 5CSA treatments increase plant resistance and reduce disease severity in grape bunches without relying on the direct inhibition of the pathogen. Unlike chemical fungicides, the emphasis is on strengthening the plant's immunity rather than targeting the pathogen directly (Reglinski et al., 2005).

Effectiveness against downy mildew, powdery mildew and grey mold

Field and postharvest evaluations have demonstrated that 5CSA effectively reduces *B. cinerea* severity. Under high-humidity conditions, untreated grape bunches experienced 77–83% disease severity, while those treated with 5CSA maintained levels around 41%. This reduction places 5CSA treatments in the 25–50% disease occurrence bracket, aligning with a moderate but significant control level (Reglinski et al., 2005).

External Effects

Although generally safe, 5CSA application did cause some minor negative effects on fruit appearance, specifically "flecking" on treated grape bunches. This is considered a slight negative effect on grape quality but no adverse impacts on ecosystem or health parameters were reported (Reglinski et al., 2005).

Products, Application, and Costs

No specific commercial formulations or cost details were provided. Field experiments required a large number of applications (over 10 sprays per season), indicating a greater labor input and lower ease of application. No cost estimates were given (Reglinski et al., 2005).

Testing Conditions and Practice

The experiments were conducted in Hawke's Bay, New Zealand, a region with a temperate oceanic climate (Cfb), comparable to the climate in parts of Northwestern Europe. Trials took place directly in commercial vineyards on Chardonnay grapes, providing realistic conditions that approximate the challenges faced in practical vineyard management (Reglinski et al., 2005).

Scores

- Effectiveness received a score of **3**, as the disease severity dropped from ~77-83% in untreated controls to about 41% in treated grapes. This places 5CSA's performance in the 25–50% disease occurrence bracket, showing moderate but meaningful disease suppression (Reglinski et al., 2005).
- Deduction score for the Climate is **3**, sine trials were conducted in Hawke's Bay , New Zealand (temperate oceanic climate, Cfb), which is similar to Northwestern European Conditions, increasing the relevance for Dutch viticulture (Reglinski et al., 2005). Practice scored **3**, because the trials were conducted in a commercial vineyard setting, reflecting practical and real-world conditions (Reglinski et al., 2005).
- The score for external effects is **2**, as minor "flecking" on fruits appeared, a slight negative impact on grape quality, though no adverse environmental or health effects were reported (Reglinski et al., 2005).
- The easiness of application received a score of **1**, due to the requirement of more than 10 sprays per season, thus requiring a high labor input (Reglinski et al., 2005).
- **Costs** received **no score** due to lack of information.
- The total score of 5-Chlorosalicylic acid (5CSA) is **4.1-5.6**

3.6.4 Preventive strategies

By integrating a range of preventative cultural and agronomic measures, Dutch vineyards can significantly reduce the susceptibility of grapevines to powdery mildew, downy mildew, and *B. cinerea*, and thereby limit their reliance on chemical interventions. For example, diligent removal of diseased and decaying plant materials, such as fallen leaves, dropped branches, and mummified berries, reduces overwintering inoculum, directly curtailing pathogen carryover into subsequent growing seasons (Elad et al., 2016). Managing the canopy structure through leaf thinning, shoot positioning, and optimal vineyard row orientation not only enhances ventilation and sunlight penetration but also ensures that foliage dries more rapidly after rainfall events, thus making conditions less conducive to both powdery and downy mildew development (Calonnec et al., 2008; Gessler et al., 2011; Pertot et al., 2017).

In addition, careful vineyard site selection and the use of disease-tolerant grape cultivars—tailored to local climatic and soil conditions—can further alleviate disease pressures. Resistant varieties often possess morphological or biochemical attributes that make them inherently less favorable for pathogen establishment, thereby diminishing the likelihood of severe outbreaks (Gessler et al., 2011). Taken together, these preventative measures serve as fundamental building blocks for integrated pest management (IPM) strategies that harmonize well with biological control solutions, fostering a more resilient and sustainable viticulture landscape in the Netherlands.

3.7 Communication insights

Understanding the communication preferences of vineyard keepers is essential for promoting the adoption of biological control methods. This subchapter will therefore answer the sub-question: This subchapter will therefore answer the sub question: *"How can effective biocontrol measures be communicated to vineyard keepers in such a way they are willing to adopt new practices?"*. This involves not only conveying the scientific and practical benefits of these methods but also ensuring the message aligns with the values, motivations, and practical realities of the audience. To achieve this, communication strategies must be clear, accessible, and tailored to vineyard keepers' specific needs and challenges.

3.7.1 The role of communication in behavioural change

The Theory of Planned Behaviour (TPB) provides a framework for understanding how communication can influence vineyard keepers' decisions to adopt biological control methods. TPB highlights the importance of three key factors in shaping behaviour (Ajzen, 1991):

1. Attitudes – Positive perceptions of biological control, such as cost savings or improved crop health, can increase willingness to adopt these methods.
2. Subjective Norms – Social pressures and norms, such as the perception that sustainable practices are valued within the farming community, can motivate adoption.
3. Perceived Behavioral Control – Belief in the feasibility of implementing biological control methods is crucial to action.

To leverage TPB effectively, communication strategies must emphasize the practical benefits of biological control, such as enhanced soil fertility and reduced dependency on synthetic inputs. Success stories of vineyard keepers who have successfully implemented these methods can serve as powerful tools for fostering positive attitudes and reinforcing social norms (Lapum et al., 2020). For example, field demonstrations can showcase how biological control works in real-world scenarios, building confidence and reducing perceived barriers to implementation.

3.7.2 Strategies for effective communication

The Transmission Model of Communication emphasizes the importance of effectively encoding and decoding messages to avoid misunderstandings caused by semantic or environmental noise (Shannon, 1948). This model underscores the critical need for clarity and simplicity in agricultural communication. Misunderstandings often arise when scientific jargon or overly technical information disrupts the encoding and decoding process. For vineyard keepers, messages must be presented in a straightforward, relatable manner to ensure they are not only understood but also actionable. This insight reinforces the need for practical, easily digestible communication tools. By presenting information at an appropriate literacy level and minimizing technical language, barriers to understanding can be reduced. Infographics, for instance, simplify complex information through a combination of visuals and concise text, ensuring messages resonate with vineyard keepers.

To bridge the gap between scientific research and practical application, communication efforts should employ tools and strategies that align with the principles of TPB and the Transmission Model:

- Infographics: These tools distil complex topics into visually appealing formats that are easier to process and remember. By incorporating visuals like diagrams, charts, and simple text, infographics can effectively communicate key points about biological control methods, such as application instructions or expected benefits (Gibbs et al., 2022; Smith, 2016). These infographics could be illustrated in a flyer or poster format.
- Field Demonstrations and Participatory Engagement: Hands-on activities allow vineyard keepers to experience biological control methods firsthand, increasing their confidence in adopting these practices. This aligns with TPB's emphasis on building perceived behavioural control. Additionally, collaborative pilot projects foster trust and mutual learning between farmers and scientists.
- Targeted Messaging: Messages should emphasize shared goals, such as enhancing environmental health and economic resilience, to foster collaboration between organic and conventional vineyard keeper. By addressing common concerns and promoting the universal benefits of biological control methods, communication can build a narrative of shared purpose within farming communities.

In conclusion, scientists can effectively communicate biological control methods to farmers by adopting clear, audience-focused strategies that consider farmers' values, motivations, and practical realities. Simplifying technical information, utilizing diverse communication channels, and emphasizing participatory engagement can enhance understanding and adoption of sustainable practices. By framing biological control as a feasible and beneficial approach, and by fostering collaborative efforts between stakeholders, scientists can empower farmers to integrate these methods into their agricultural practices, advancing both environmental and economic goals.

4. Discussion and limitations

The aim of this project was to evaluate potential biological control strategies for managing fungal diseases in Dutch vineyards, focusing on powdery mildew, downy mildew, and *B. cinerea*. This work serves as a foundation for potential biocontrol strategies to be researched and implemented in the next viticulture season, in order to provide scientific-based insights into the feasibility of the potential biological control methods in Dutch vineyards. In this paper, we present both strategies currently permitted under Dutch regulations and those not yet allowed, alongside all the approaches we researched, including those that received lower scores. The evaluation of these strategies was conducted using a defined Multi-Criteria Analysis (MCA) based on data obtained from existing literature. Therefore, the use of the MCA as primary evaluation method should be critically assessed. As the scoring in this research was depending on available literature, it is important to highlight that the reliability of our outcomes is only as reliable as the quality, and relevance of the existing studies. Whereas some biological control methods, like *T. harzanium* strain T39, contain more available literature than for example *P. oxalicum*. Therefore, due to the timeframe, we either had to make a selection or solely basing the scoring on a single or limited number of literature studies. This could have potentially led to missed information that could have influenced the scoring of this research. As there was often no available research with Dutch or identical climates, we created a deduction formula for the final score of each biocontrol strategy.

In this project, interviews were conducted with only a small sample of hobbyist vineyard keepers, all members being of the association Wijnbouwers der Lage Landen and organic growers. The limited sample size and reliance on convenience sampling may have introduced bias, potentially affecting the diversity and generalizability of the findings (Emerson, 2021). For instance, the perspectives of conventional and commercial growers, who might have different approaches and challenges regarding biological control methods, were absent. This could result in an incomplete representation of the Dutch vineyard landscape. Future research should aim to include a larger and more diverse group of participants, encompassing conventional and commercial growers, to better capture the range of opinions and practices in the Netherlands. If, for instance, more vineyard keepers with varied backgrounds were included, it might reveal alternative perspectives or identify additional challenges and opportunities in adopting biological control strategies. This could possibly affect the weight that were used in the MCA because conventional vineyard keepers may have a completely different view on biological control. Thus, expanding the participant pool in future studies would provide a more comprehensive picture of the situation in Dutch vineyards.

Furthermore, understanding the disease cycles of grey mold, powdery mildew, and downy mildew is crucial for developing effective biocontrol strategies. These pathogens have distinct life cycles that are influenced by different environmental conditions, highlighting the need for detailed management approaches. Their different environmental preferences and infection pathways present challenges as biological control strategies must align with these cycles to optimize timing and application. For example, targeting overwintering structures like oospores can reduce primary infection sources for downy mildew (Maddalena et al., 2022). Additionally, combining biocontrol agents with practices like pruning techniques and leaf removal a sustainable solution for vineyard disease management (Jacometti et al., 2010)

Additionally, despite the promising potential of biological control methods, their efficacy is often limited by external environmental factors such as humidity, pH, UV radiation, and rain fastness (Dagostin et al., 2011). These factors can significantly influence the performance of biocontrol agents in field conditions. However, as we were bound to limited available literature, we could not always score each biocontrol strategies under different climatic conditions. To ensure realistic scoring within the MCA framework, particularly for the criterion of 'effectiveness', each biocontrol strategy must be evaluated across a range of diverse field conditions in future research. This includes sunny or rainy days to test for efficacy in different weather conditions.

Also, in this report potential biological control methods that can be used in Dutch vineyards have been evaluated. A few of the methods were brought to our attention by the vineyard keepers that were interviewed, and the others were found by the literature research. Unfortunately, a lot of the methods that were found are not allowed to be used in the Netherlands on winegrapes due to its regulations. Furthermore, it would be a good investment to register some of these solutions as alternatives as more chemical fungicides are being banned. Two methods that were found are going to be discussed to give a bit more insight into to their advantages and disadvantages.

One of the allowed biological control methods in the Netherlands is compost tea. This method was brought to our attention by a few of the vineyard keepers that we interviewed. The compost tea's is made by steeping compost in water and then spraying it on the grapevines. Compost teas contain a lot of nutrients and microorganisms. The vineyard keepers use compost tea especially after they use *T. harzianum* to restore the microbiome of the grapevines (interviews, 2024). Some research was done on the use of compost tea, and they found that there was a reduction of infection with 77%-79% (Evans et al., 2012). A few of the interviewed vineyard keepers also used compost tea and they found similar results as Evans et al. (2012). However, these results are not comparable as it is not known what composition of manure was used by the interview vineyard keepers and therefore it is unknown what caused the reduction in infection. Unfortunately, there are no compost tea products currently on the European market.

However, the vineyard keepers can make the compost tea themselves, by buying compost and steeping it. But, because the composition of the manure they use is different to make compost tea the effectiveness can also widely vary. The different compost teas therefore can contain a different variety of microorganisms and nutrients levels. This is also why it is very difficult to test this method, because the composition of the manure that is used can almost never be the same and therefore the results will be difficult to interpretate. Unless a commercial product is made that has a standardized composition then testing this product could be possible and can potentially be used as a biological control method.

The biological control method *T. harzianum* strain T9 is a *Trichoderma* strain that is used by some of the interviewed vineyard keepers. However, no commercial product containing this strain is allowed in the Netherlands according to the ctgb. Furthermore, there is no existing literature on the effects of this strain *B. cinerea*, powdery and downy mildew in any crop. However, there was a study done in laboratory conditions on tomato leaves for two bacterial diseases that found a reduction in symptoms (Saksirirat et al., 2009). Because of the lack of literature found on this strain and because there is no commercial product with this biological control agent not allowed in the Netherlands this biological control agent would not be a good option to use in the field experiments in the Netherlands. Instead of this strain it would be better to test another *Trichoderma* strain which have more literature and has commercial products that are allowed to be used in the Netherlands. Furthermore, it is possible that the vineyard keepers used the T9 strain, but that it is not the best strain to use to combat the three fungal diseases. For example, the literature that was found showed some reduction in bacterial disease, which could mean that this strain is more effective against bacterial diseases than fungal diseases that they want to tackle.

Furthermore, this research primarily focused on individual control strategies. However, stacking multiple strategies has the potential to enhance disease control, was observed during interviews with vineyard keepers. In many vineyards, stacking is already a common practice, with frequent combinations such as *Trichoderma* applications followed by compost tea treatments, and sometimes an additional Oenosan treatment (interviews, 2024). However, it is important to highlight that interactions between stacked strategies can sometimes lead to unintended outcomes. These interactions may arise due to competition for resources, differences in environmental preferences, or antagonistic effects between microbial communities. As *Trichoderma* species are effective biofungicides that work by enzymatically breaking down other fungi, producing antimicrobial compounds that kill pathogens, and outcompeting harmful fungi for space and nutrients timing of application is important (Siddiqee, 2014). The simultaneous application of *Trichoderma* and compost tea could therefore result in reduced effectiveness of the microbes available in compost tea. Also, the use of biological compounds such as Vitisan can influence the BCA efficacy. Vitisan is known to increase the pH of the soil. However, most fungi, including Trichoderma, species grows better in acidic conditions (Singh et al., 2014). Therefore, simultaneous use might affect Trichoderma's control abilities.

Soil microbiomes are highly complex and dynamic systems where various microorganisms interact, often in ways that are precisely balanced and context specific. When new microbes, such as BCAs, are introduced, they can alter this set microbiome's equilibrium (Cunniffe et al., 2011). While the intended outcome is often an increase in beneficial microbes or the suppression of pathogens, unintended shifts may occur. The introduction of a biocontrol agent might inadvertently suppress beneficial microbes that are essential for nutrient cycling or disease resistance, thus diminishing overall plant health. In the study of Mawarda et al., (2020), a review of 108 studies showed that 86% reported changes in soil microbial communities after the application of microbial inoculants. Among studies focusing on the long-term effects of these inoculants, 80% found that the soil microbial communities did not show any change back to the initial composition. This demonstrates that clear understanding of the interactions between BCAs, plants, and the microbiome is crucial for their effective and sustainable use.

Lastly, as with many disease management practices, just applying biopesticides is not enough to provide maximum disease control. However, the main focus of this report was on finding potential biocontrol strategies, but maximum control is not possible without good hygiene and sanitation measures. Sanitation measures, such as reducing bunch compactness and rotting fruits are crucial. But also, leaf removal improving the fruit zone microclimate, making it less favorable for fungi to establish through exposure to sunlight and increased air movement around bunches (Thomas et al., 1988). Besides these practices, pruning and removing bleached canes infected with *B. cinerea* or other pathogens is an important step to reduce the inoculum for the next growing season (Mundy et al., 2022). As plant residues such as debris and dead leaves are potential inoculum sources, especially since *B. cinerea* survives and overwinters in dead plant material, it is highly important to remove all dead plant material and debris (Mundy et al., 2022; Molitor et al., 2015). As noted during the interview's sanitation is often overlooked or inconsistently implemented, this report really highlights the importance is good sanitation measures, education and training.

5. Advice

Commissioned by Wageningen Research Open Teelten (OT), this advice explores how Dutch vineyards can embrace sustainable solutions in the face of emerging opportunities and challenges. More information and references can be found in the research report (chapter 1.1).

Recent climate changes have made northern European regions like the Netherlands increasingly suitable for viticulture. Despite improved climatic conditions, Dutch viticulture faces significant challenges from diseases such as grey mold, downy mildew, and powdery mildew. Amateur growers are significantly impacted. They often lack the resources for chemical pesticide usage and prioritize sustainable farming, reducing reliance on active chemical compounds. While biological control methods are proven effective in other regions, such as France and India, their application in Dutch viticulture remains underexplored. Environmental differences, including soil type, humidity, and temperature, can significantly influence the success of biocontrol systems. This report focuses on addressing the challenges faced by amateur viticulturists, with a primary emphasis on members of the Wijnbouwers der Lage Landen association. The Wijnbouwers der Lage Landen unites 900 members to share their passion and knowledge of grape growing and winemaking. They offer expert-led courses and facilitate group purchases of viticulture and winemaking supplies.

In order to contribute to the internal drive of ecological and sustainable viticulture in of the subgroup within this project will focus on the potential biological control approaches that can be utilized by Wageningen Research Open Teelten (OT) for future experiments. Therefore, this research will be based on the following research questions:

Main research question: *Which biocontrol measures are promising for disease control of powdery mildew, downy mildew and grey mold in Dutch vineyards, and could be evaluated on effectiveness in future experiments?*

Sub-questions:

- How does is the current Dutch viticulture looks like in terms of grape varieties, cultivations systems and diseases control measures?
- What are the lifecycles of the most prominent diseases powdery mildew, downy mildew and grey mold in vineyards in the Dutch climate?
- What are the current disease management regulations for vineyards in the Netherlands?
- What are preventive, early detective and active defense disease management practices, besides biocontrol measures, that could contribute to the efficient use of biocontrol measures?
- How can effective biocontrol measures be communicated to vineyard keepers in such a way that they are willing to adopt new practices?

To answer these questions, this study employed literature research, semi-structured interviews, and a multi-criteria analysis (MCA) to identify the most effective biological control methods. Literature reviews of articles, websites, and books provided an overview of existing methods, while the CTGB and EFSA databases were checked to verify if these methods are allowed in the

Netherlands or Europe. Semi-structured interviews were conducted with wine growers from the "Wijnbouwers der Lage Landen" association. These interviews followed a set of predefined open-ended questions, ensuring consistency while allowing in-depth exploration of responses. Insights from the literature and interviews informed the MCA, which evaluated biological control methods based on four main criteria: effectiveness, external effects, ease of application, and costs. For more details on how the MCA worked for a detailed explanation of how the MCA functions see section 2.3 of the Methods of in the report. A total of 20 methods were assessed, of which 12 scored above 5.5/10 (a passing grade) and are discussed in this advice. However, an exception was given to the Calcium carbonate method, because through the interviews, everyone mentioned that was using Oenosad and that was looking promising.

Results of the Research

Through the literature review that took place and the interviews with the vineyard keepers of der Lage Landen, sixteen promising biological control methods (BCM) were identified. However, from the twenty BCM, only 8 of them are approved for use in the Netherlands for grapes. We conclude them in the following BCM (Table 1), the list contains a variety of control methods that can be used, such as bacteria, fungi, elicitors, inorganic compounds and compost tea.

Table 7: 1Biological control methods that are allowed in the Netherlands to be used in grapevines, ranked based on the scored they got on MCA

Biological control method	Target diseases*	Final effectiveness	External effects	Easiness of application	Cost	Overall score
B. subtilis (3.4.1)	G*	2.85	4	3	3	8.1
Potassium bicarbonate (3.6.2)	B*, D*	3.3	3	3	4	8.1
L. digitata (3.4.3)	G, D, P	3.3	3	3	4	7.7
***T. atroviride* SC-1** (3.4.4)	G	2.3	4	3	1	7.0
***T. harzanium* strain T-22** (3.4.5)	G	2.7	3	4	-	6.8-8.3
***T. asperellum* strain T-34** (3.4.6)	P	2.1	4	-	4	6.1-8.6
Compost tea (3.4.7)	G, P	-	3	3	4	5.6-8.6
Calcium carbonate (3.6.1)	-	-	4	-	4	-**

*Abbreviations of diseases are D; downy mildew P; powdry mildew G; grey mold

**No final score was given as there was no scientific-based evidence available

In the following pages, the eight promising BCM is briefly explained what the advantages, disadvantages and application information of each method is based on the findings from the literature review and the label of the products. For more information you can follow the link to the chapter of each method that is being explained in more detail, the mode of action, the effectiveness against the diseases, the external effects, the products, the applications, the cost and how each method has scored.

B. subtilis is the biological control agent, that is contained into the product Serenade. (chapter 3.4.1)

Advantages

- The product has been tested for over 4 years in vineyards showing 75% effectiveness
- Has no negative effects to the environment, human health and grapevines
- Easy to apply, just need backpack sprayer

Disadvantages

- Has a medium cost per hectare per growing season

Application information

- Applicable for gray mold and powdery mildew
- Professional license needed
- Applications by foliar spraying
- Can be used from April to October
- 9 applications per growing season
- Minimum interval between applications is 5 days
- 5-8 liters of product per hectare diluted in 500-1000 liters of water per hectare

The second most promising BCM is the product Vacciplant®, which is based on laminarin an extracted substance from brown algae. (chapter 3.4.3)

Advantages

- Have been tasted in all three diseases
- Show up 94.5% reduction of powdery mildew symptoms in 6 years field trials against powdery mildew
- No effects on the environment or vines
- It is low cost
- You can do many applications

Disadvantages

- The trials against grey mold and downy mildew it was only in laboratory
- Requires personal protective equipment
- Maybe it high cost

Application information

- Applicable for grey mold
- Professional license needed
- Applications by foliar spraying
- Can be used all year around
- Maximum 20 applications per growing season
- Minimum interval between applications is 10 days
- Maximum dosage per application is 1 liters of product per hectare diluted in 200-1500 liters of water per hectare

Vintec, with the biological controagaint *Trichoderma atroviride* SC1(chapter 3.4.4).

Advantages

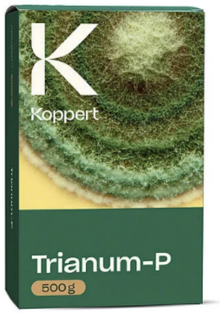
- Approved to be used against the grey mold
- In a greenhouse trial, it showed 96% reduction of grey mold in tomatoes

Disadvantages

- Has not been tested in any of the three diseases in the grapevines

Application information

- Applicable for grey mold
- Professional license needed
- Applications by foliar spraying
- Can be used from April to September
- 8 applications per growing season
- Minimum interval between applications is 7 days
- Maximum product dose per application is 0.2 kg per hectare diluted in 100-1000 liters of water per hectare
- For applications close to harvest period has to have one day gap before the harvest



Trichoderma harzianum strain T-22, the active ingredient in Trianum-P, is approved in the Netherlands for professional use on crops including wine grapes via soil application. (chapter 3.4.5)

Advantages

- Effective against *B. cinerea* with infection reductions from 62% to 9–52% in trials.
- Adaptable to diverse environmental conditions (10–34°C, pH 4–8).

Disadvantages

- Only drip applications to soil can take place, however the
- Can be applied only in the beginning of the year to reduce the overwintering population of the *B. cinerea*

Application information

- Professional license needed
- Drip applications
- All around the year
- 5 applications per growing season
- Minimum interval between applications is 70 days
- 15-30g per 1000 plants

Trichoderma asperellum T34, found in Asperello® T34 Biocontrol and T34 Biocontrol® (chapter 3.4.6).

Advantages

- Showed high % of controlling downy mildew in greenhouse conditions
- 1 application per season

Disadvantages

- Only available for soil applications
- Not tested in field trails
- Can be used only in protected/greenhouses in Netherlands

Application information

- Professional license needed
- Applications by foliar spraying
- Can be used all year around
- 1 application per growing season
- 5kg per hectare or 0.01 kg/m^3

Compost tea, a liquid derived from steeping compost in water (chapter 3.4.7).

Advantages

- No effects to the environment if it's applied to correctly
- Contains microorganisms that suppress pathogens and enhance plant resistance.
- Improves soil quality and promotes plant growth.
- Low cost

Disadvantages

- Non-aerated compost tea can cause harmful pathogens if improperly prepared.
- There is no available standardized procedure for preparing compost tea
- The lack of functional assays to identify key microbial groups from compost microbiomes

Application information

- No professional license needed
- There is not enough information about the application of compost tea as biological control against diseases

Potassium bicarbonate, marketed as Vitisan or Armicarb (chapter 3.6.2).

Advantages

- Has been tested against powdery mildew
- Has no effects on the environment, human health

Disadvantages

- No information available about the cost
- Risk of phytotoxicity at higher concentrations.

Vitisan application information

- Applicable for powdery mildew
- Professional licence needed
- Applications by foliar spraying
- Can be used from March to September
- 6 applications per growing season
- Minimum interval between applications is 3 days
- Maximum product dos is 12kg per hectare 5-8 liters. For the preparation of the product dilute 0.75kg of the product in 100L of water.

Armicarb application information

- No professional license needed
- Applications by foliar spraying
- Can be used from April to August
- 5 applications per growing season
- Minimum interval between applications is 7 days
- 1000 liters per hectare

Calcium carbonate is a natural foliar treatment derived from limestone or seashells, is sold in the market as Oenosan (chapter 3.6.1).

Advantages

- Enhances calcium bioavailability, strengthening plant cell walls.
- Derived from natural sources, making it environmentally friendly and sustainable.
- Potentially cost-effective and accessible for Dutch grape growers.

Disadvantages

- Insufficient data on application protocols (frequency, equipment, or management).
- Lack of direct evidence linking calcium carbonate to resistance against key grapevine fungal diseases.

Application information

- There is not enough information about the application of compost tea as biological control against diseases

The vineyard is a complex ecosystem, and based on the interviews, all three diseases can be found within it. Therefore, addressing each disease individually is not effective; a more holistic approach is necessary to improve the vineyard's health and establish a manageable routine for vineyard keepers. Consequently, we propose the following experiments to be conducted by Wageningen Research OT.

Recommended Treatments

Based on our research, we recommend experimenting with Serenade, Vacciplant®, Vintec, Vitisan, and Armicrab. However, a significant limitation is that none of these products are indicated by the CTGB for use against downy mildew. Despite this, Vacciplant® has been extensively tested in vineyards and shown promising results, achieving 75% effectiveness in laboratory experiments. Below, we outline four treatment plans to be implemented across different vineyard locations belonging to members of Der Lage Landen.

Treatment 1: Serenade

This treatment aims to evaluate the effectiveness of Serenade against *B. cinerea* and *E. necator*.

- **Timing**: The first application should occur in April, one week before bud break, during dry weather conditions.
- **Dosage**: Apply 8 liters of product per 1,000 liters of water per hectare.
- **Application**: Foliar spraying, with a total of 9 applications spaced at least 5 days apart, depending on the weather. Avoid application when rain is forecast.

Treatment 2: Vacciplant®

This treatment tests Vacciplant® against *B. cinerea, E. necator* and *P. viticola*.

- **Timing**: The first application should occur in April, one week before bud break, during dry weather conditions.
- **Dosage**: Apply 1 liter of product per 1,500 liters of water per hectare.
- **Application**: Foliar spraying, with a total of 16 applications from April to September, spaced 10 days apart. This schedule targets critical disease stages: the early infection stage and the July-September overwintering spore production period.

Treatment 3: Vintec

This treatment focuses on the effectiveness of Vintec against *B. cinerea*.

- **Timing**: The first application should occur in April, one week before bud break, during dry weather conditions.
- **Dosage**: Apply 0.2 kg of product per 1,000 liters of water per hectare.
- **Application**: Foliar spraying, with a total of 8 applications spaced 7 days apart, avoiding rainy weather.

Treatment 4: Vitisan

This treatment evaluates Vitisan's effectiveness against *E. necator*.

- **Timing**: The first application should occur in April, one week before bud break, during dry weather conditions.
- **Dosage**: Apply 0.75 kg of product per 1,000 liters of water per hectare.
- **Application**: Foliar spraying, with a total of 6 applications spaced 3 days apart.

For the remaining four promising methods, we cannot guarantee the same effectiveness as the previously discussed treatments due to insufficient literature and limited information to establish a consistent treatment routine against any of the three diseases. Nonetheless, we propose potential applications for some of these methods. Compost tea can be applied to all plants prior to the use of any other treatment, both in the soil and on foliage. This approach helps boost plant biota, provides essential nutrients, and occupies ecological niches that might otherwise be taken by pathogens.

Trianum-P, though not approved in the Netherlands for treating any of the three fungal diseases, is allowed for drip application with a maximum of five applications spaced 70 days apart. Therefore, it can be utilized at the outset of the treatment regimen to manage potential overwintering fungal spores that may persist in vine residues within the soil.

Oenosan is another product with insufficient supporting literature to validate its effectiveness against fungal diseases. However, its potential could be explored through an experimental setup that builds upon the methodologies already developed by the farmers of der Lage Landen (Appendix 9.4), ensuring a more scientifically consistent framework to assess its impact.

Lastly, the products T34 Biocontrol and Asperello T34 Biocontrol are restricted to greenhouse use in the Netherlands and cannot be applied to field crops. This restriction precludes their inclusion in field experiments to investigate their potential effectiveness.

However, adopting preventive cultural and agronomic measures can significantly reduce the disease pressure in Dutch vineyards. For instance, regular removal of infected plant debris and pruning residues, including fallen leaves and dropped branches, helps limit the spread of B. cinerea and other pathogens by reducing sources of inoculum. Ensuring proper canopy management, such as leaf thinning and shoot positioning, enhances air circulation and light penetration, thereby creating less favorable conditions for powdery and downy mildew development. Selecting disease-resistant grape cultivars, such as Johanniter, Solaris, Souvigner gris and Rondo, and implementing appropriate vineyard spacing also alleviates the need for chemical treatments, as these strategies can inherently lower disease incidence and severity. By integrating these preventative measures with targeted biocontrol solutions, growers can more effectively manage grapevine diseases and foster sustainable viticulture practices in the Netherlands.

Communicating Effective Disease Management to Vineyard Keepers

When the experimental phase of this research concludes and the most effective methods are identified, it will be essential to share these findings with vineyard keepers in a meaningful and impactful way. This involves tailoring communication to suit the distinct preferences of conventional and organic vineyard managers. The information must be presented in clear, straightforward language, following a guideline that ensures accessibility to all.

To enhance understanding, it is recommended to provide vineyard keepers with a well-structured overview in the form of a visually appealing infographic or flyer. This flyer will offer a concise summary of the three primary grape diseases: Gray Mold, Powdery Mildew, and Downy Mildew. By including images of grapes affected by each disease, the flyer will help vineyard keepers quickly identify and recognize these issues.

Additionally, the flyer should feature a management schedule outlining strategies to prevent or control these diseases. It should detail the use of key methods such as Serenade Mex, Vacciplant, Vintec, and Vitisan. Scientists should actively support vineyard keepers in integrating these practices into their operations, ultimately fostering healthier vineyards and contributing to environmental sustainability.

7. Conclusions

This research has successfully identified promising biological control methods for managing the most prominent diseases in viticulture—powdery mildew, downy mildew, and grey mold —based on their effectiveness, external effects, ease of application, and cost-efficiency. By studying pathogen life cycles, interviewing Dutch viticulture practitioners, and analysing biological control practices used on grapes, the study offers a well-rounded perspective on sustainable disease management.

Following a comprehensive search and evaluation, it is evident that while a wide range of biological control methods exist, only eight have been approved for use in the Netherlands. Of these, one remains prohibited for use in grape cultivation. This underscores the reality that biological control is still a developing field. Although significant research is being conducted to explore future biological agents, the market lacks standardized products and methodologies, presenting challenges to their widespread adoption and commercialization.

Furthermore, variations in regulatory approvals highlight additional barriers. Some products approved in the Netherlands have more limited applicability for specific diseases compared to their approvals in other countries. The strict regulations governing fungicides in the EU further constrain market entry for these products. In response, many products are reclassified under less-regulated categories such as biostimulants or soil improvement agents, facilitating their introduction to the market under more lenient requirements.

This analysis emphasizes the need for ongoing development of standardized biological control products and a more harmonized regulatory framework to support their effective and consistent use across the EU.

To conclude, the products selected for further evaluation through experiments by Wageningen Open Teelt (OT) on behalf of Wijnbouwers Der Lage Landen show strong potential for addressing key challenges in Dutch viticulture. Future experiments will refine these findings and ensure the recommendations are practical and suitable for the target group.

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9. Appendices

9.1 Interview guide

Vineyard specifics:

- Can you tell us a little bit about this vineyard and its history? Why did you start/take over the vineyard? How old is the vineyard? What was here before? How much time do you spend on the vineyard?
- Are you certified organic, or do you participate in any sustainability programs?
- What grape varieties are cultivated in this vineyard and what kind of wine do you make?
- On which kind of soil is this vineyard located? Are there site-specific properties worth mentioning?
- Did you observe something specific about the soil?
- Observation about the farm plot, areas that may have been water logging or shading.
- What kind of trellis system and training method do you use?
- How has climate change influenced the potential for Dutch wine production? How does the climate in your region impact the prevalence of fungal diseases in your vineyard? Have you noticed any changes in disease patterns or severity in recent years, possibly due to climate change?

Disease management

- What are the main diseases that are affecting this vineyard?
- How severe is this vineyard affected by powdery mildew, downy mildew and grey mold? What are the consequences?
- Do you observe any disease more often than the others?
- Do you know Fungus-Resistant Grapes (PIWIS)? What are your views on the use of this type of grapes in the Netherlands?
- Ask more about this maybe à link this to grape variety question
- What kind of pest and fungi control methods do you use? And how effective are these methods
- What is your application schedule?
- What role do monitor and forecasting play in your disease management decisions?
- How do you balance effective disease control with environmental sustainability?
- Do you use any other management practices (e.g., canopy management) alongside chemical treatments?
- Are you using biological control methods to combat downy mildew, powdery milder and grey rot, and if so, what kind of biological control methods are you using?

If yes:

- Which type do you use? And if trichoderma, which spiecies/branch?

- How effective are these methods? Have you observed any changes in disease pressure or vineyard health since adopting biocontrol methods?
- How do you apply these biocontrol agents (e.g., foliar sprays, soil applications), and what is your application schedule?
- What challenges have you faced in implementing biocontrol strategies (e.g., environmental conditions, variability in efficacy)? Did the transition to biocontrol require significant changes in your management practices or infrastructure? Were there any regulatory hurdles in adopting biocontrol agents (e.g., product approvals, certifications)?
- What benefits have you observed regarding environmental impact, such as reduced chemical residues or improved biodiversity?
- Have you noticed any impact on yield or grape quality after switching to biocontrol methods?
- How do the costs of biocontrol agents compare to conventional fungicides in your experience?

If not:

- Are you aware of biocontrol methods available for managing grapevine diseases?
- Have you ever considered or experimented with biocontrol agents in your vineyard?
- What factors have influenced your decision not to adopt biocontrol strategies (e.g., cost, availability, uncertainty)?

- What is the available budget for disease management/control for this vineyard? Are you getting subsidies?

Experience, motivation and acceptance

- Can you share any success stories or lessons learned regarding disease management in your vineyard? Have you adopted any innovative practices or technologies recently?
- What advice would you give to new grape farmers starting in a climate like the Netherlands'?
- Do you collaborate with other local farmers or participate in growers' associations?
- How important is community support and knowledge sharing in addressing vineyard challenges?
- Are there any local or regional initiatives that have helped you improve your practices?
- We are developing an advice on the use of biological control measures. What would be a good way to communicate this with vineyard keepers? And how not?

9.2 Informed Consent

Wageningen University & Research

Toestemmingsformulier voor de deelname aan het onderzoek

Title van het onderzoek

Uncorking the potential of biocontrol in the Dutch Vineyards

Onderzoekers

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Doel van het onderzoek
Onderzoeken van potentiële biologische ziektebestrijding strategieën in Nederlandse wijngaarden + inzicht geven in management opties.

Procedures
U bent uitgenodigd om deel te nemen aan dit onderzoek en het zal ongeveer 60 minuten van uw tijd innemen. De vragen in dit interviews zullen gebaseerd zijn op uw eigen wijngaard, ziektebestrijding in deze wijngaard, en uw eigen ervaringen & ideeën hierover.

Vrijwillige deelname
Uw deelname aan dit onderzoek is vrijwillig. U kunt zich op elk moment in het onderzoek terugtrekken.

Vertrouwelijkheid
Uw gegevens en de audio opname zijn alleen bedoeld voor onderzoeksdoeleinden en zullen niet worden verspreid. Uw gegevens zullen anoniem gebruikt worden en als het onderzoek is volbracht worden deze audio gegevens vernietigd.

Toestemming
Door het ondertekenen van dit formulier, stemt u in met uw deelname aan het interview onder de volgende voorwaarden:

- Uw deelname blijft vrijwillig
- U kunt zich op elk moment terugtrekken uit het onderzoek
- Het interview wordt audio- opgenomen
- Uw informatie word vertrouwelijk gebruikt
- Uw audio recording zal worden vernietigd nadat het onderzoek voorbij is

Ik geef toestemming om deel te nemen aan dit interview aan de hand van de audio- recorder.

Handtekening van deelnemer:
Datum:

9.3 Grape Varieties

Grape variety:	Used in:					
	The Netherlands	Vineyard 1	Vineyard 2	Vineyard 3	Vineyard 4	Vineyard 5
Pinot Noir / Blauer Burgunder	X					
Chardonnay	X					
Riesling	X					
Müller-Thurgau / Rivaner	X					
Dornfelder	X					
Pinot Gris	X					X
Pinot Blanc / Weisser	X					
Auxerrois	X					
Carnernet Blanc	X					
Calardis	X					
Johanniter	X	X	X	X		
Merzling	X					
Muscaris	X	X			X	
Solaris	X	X	X	X	X	
Souvignier Gris	X		X	X		
Monarch	X				X	
Regent	X		X	X	X	
Rondo	X		X	X	X	
Marechal Foch	X	X				
Carbarnet Cortis	X	X	X	X		
Muscat blue	X	X				

9.4 Treatment Plan of Vineyard Owner 2

Voorgenomen behandelplan schimmeldruk, seizoen 2025, Lelystad

Vanaf medio Maart, bij een dagtemperatuur van >10 gr.C : In Lelystad lopen we de 25 jaar oude stokken langs, en kwasten nog zichtbare kale witte snoeiwondplekken in met Vintec(=*Trichoderma Atroviride*). Doel : Het stoppen van Esca-aantasting.

- **Eind maart** : Bodemgericht spuiten met *Trichoderma Harzianum* T9, dosis 60 gram op 150 liter regenwater, enkelzijdig (= om en om het pad), om nog aanwezige schimmelsporen uit te schakelen.
- **2 weken voor de bloei** : Naar keuze ofwel Trichoderma (60gr. Op 150 lr) of anders compostextract (1,5 kg op 150 lr regenwater), dan wel 1lr heermoessap(zie helemaal onderaan deze tekst) op 150 lr regenwater, dan wel biokwark (Lidl, 2,5 kg op 100 lr water) spuiten, spuithoogte 2 m, enkelzijdig (= om en om het pad).
- **1 week na de bloei** : Lichte Tricho-bespuiting, 2x (want dubbelzijdig = dus ieder pad!) 30 gram op 150 liter regenwater, oftewel 60 gram op 300 lr.
- **2 weken na de bloei** : 300 gram Oenosan op 300 liter kraanwater spuiten over hele loofwand, beide zijden.

Verdeeld over de periode tot 1 september de Oenosan-behandeling nog 3x herhalen.

- **Vanaf vruchtzetting, doperwt-grootte**: 2x per week controle op primaire infecties van echte meeldauw (blauw-grijze vlekken op de trossen/bessen) en valse meeldauw (olievlekken bovenzijde blad, grijze weefsellaag onderzijde blad).
- Indien infectie aangetroffen : **Meteen** Trichoderma spuiten, 2-zijdig, 60 gram/300 liter. Controleer 5 dagen achtereen of de aantasting vermindert; indien vlekken niet verdwenen zijn, dan behandeling herhalen. Indien de aantasting na de 5^{e} dag verdwenen blijkt dan **meteen** compostextract spuiten, 1,5 kg op 150 liter, tweezijdig, dan wel heermoessap.

Belangrijk : De precisie van de genoemde behandel-opeenvolging en de intervallen hebben als doel om het sporuleren na een 1^{e} infectie tegen te gaan; lukt je dat, dan scheelt dat enorm in de schimmeldruk en -schade over het resterende seizoen.

- Blijf nu 2x per week controleren op nieuwe infecties. Zolang die uitblijven, elke 2 weken spuiten met compostextract dan wel heermoessap, zelfde dosis.
- Bij geconstateerde nieuwe infecties de Trichoderma/compost-extract/heermoessap- behandeling herhalen.

Niet vergeten: Plan ook de te herhalen Oenosan-behandelingen in, zie boven !