Norwegian University of Life Sciences

**Master's Thesis 2021 60 ECTS**
Department of Plant Sciences

# Aerated steam treatment against grey mould in strawberry transplants

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**Abstract**

Grey mould caused by *Botrytis* spp. is one of the most important fungal pathogens in strawberry and is often present in strawberry transplants in the form of latent infections. In the past, heat treatment has been effective in inhibiting several phytopathogens. However, despite its efficiency, heat treatment is often linked to adverse effects on plants. The 'Plant Sauna' is an insulated thermotherapy unit with the ability to provide uniform distribution of aerated steam at desired temperatures. The purpose of this study was to evaluate the efficiency of the 'Plant Sauna' in reducing the *Botrytis* population present in transplants of five strawberry cultivars: Falco, Favori, Murano, Sonsation and Soprano. The heat treatment included pre-treatment at 37°C for 1 h, followed by 1 h of cool-down at ambient temperature and eradicative treatment at 44°C for 2 h or 4 h. Eradicative treatment at 44°C for 4 h led to a 100% reduction in *Botrytis* populations for transplants of cvs. Soprano and Sonsation. While for transplants of cvs. Murano, Falco and Favori, the incidence of *Botrytis* spp. was reduced by 72 to 77 % compared to the untreated transplants. On the other hand, the incidence of *Botrytis* spp. in 2 h heat-treated transplants of cv. Soprano was < 8%. While for the rest of the 2 h heat-treated transplants, the incidence was not remarkably different from the untreated transplants. Furthermore, growth trials were conducted on transplants of cv. Sonsation to determine the effects of 'Plant Sauna'. The effect of the steam treatment on plant growth and yield was negligible. Aerated steam treatment was further extended for an in-depth study of its effect on *Botrytis* sclerotia. Eradicative treatment at 44°C lasted for 4 h only which killed 90 to 100% of the sclerotia. Isolates of *Botrytis* spp. were recovered from strawberry transplants and tested for fungicide resistance. Some of these isolates were resistant to up to five classes of fungicides. Overall, our findings indicate that aerated steam treatment in the 'Plant Sauna' is a safe and effective technique for reducing pathogen populations and the quantity of fungicide-resistant strains in strawberry transplants, with no or insignificant negative impacts on transplants.

**Acknowledgements**

My immense gratitude goes to my main supervisor Prof. Arne Stensvand and co-supervisor Katherine AG Nielsen their guidance, patience, and encouragement. Thank you for being super approachable and being by my side throughout this journey.

I want to thank Vinh Hong Le and Jafar Razzaghian at the Division for Biotechnology and Plant Health, NIBIO, Ås, for their assistance and technical support during the experiment and laboratory analysis. Thank you for making the learning process much easier.

I received three months internship for this thesis and got the opportunity to be part of several research projects, my biggest thanks to Arne Stensvand, Nina Johansen, and Nina Trandem for trusting me to be part of their research project.

A special thanks to Birgitte Henriksen for helping us continue our experiment during strict COVID-19 related regulations at NIBIO.

I would like to thank my lovely parents and my dearest brother for their endless love and support. It wouldn't have been possible without their encouraging words. Finally, big thanks to all my friends and fellow master students for the good times and moral support.

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# 1. Introduction

## 1.1. Strawberry plant and production

Strawberry is an economically important fruit crop belonging to the family Rosaceae and subfamily Rosoideae (Hummer & Hancock, 2009). Modern-day strawberry (*Fragaria* x *ananassa* Duchesne) is an allo-octoploid (2n = 8x = 56) that resulted from the hybridization of *Fragaria virginiana* and *Fragaria chiloensis* (Darrow, 1966). This incident occurred about 250-300 years ago in southern France, making *Fragaria* x *ananassa* one of the modern horticultural species (Darrow, 1966; Hancock, 2012). The strawberry plants are perennial herbaceous plants with short crowns and compound leaf with three leaflets. Plants reproduce both sexually via seed and vegetatively by runners. Fruits are aggregate, soft fruit which develops aroma and red colour with maturity.

Strawberry is cultivated worldwide in a broad range of environmental conditions, and despite challenging weather conditions, Nordic countries are not an exception. In 2019, global strawberry production was 8.88 million tonnes in 3.96 thousand hectares of land area (FAOSTAT, 2021). In case of Norway, the first introduction of strawberry into the country is believed to have occurred around the early 19$^{th}$ century (Nes, 1998). In terms of annual production, 9142 tons of strawberries were produced in 1330 hectares of land area in 2019 (Statistics Norway, 2019). Strawberries are cultivated in all parts of the country; however, the south-eastern region dominates this nation wise production (Haslestad, 2016). Furthermore, cultivation in semi- and controlled environment has made cultivation possible in country's northernmost parts. The strawberry production season is short, starting from the beginning of June to mid-September (Døving, 2017). The production season has been extended by introducing flower-initiated transplants and day-neutral plants in protected (high tunnels or greenhouses) production system (Nes et al., 2008b). The demand for fresh strawberries is year-round, but challenging climatic condition, prevalence of diseases and the higher cost of production seem to be a barrier to meet market demand (Davik et al., 2000). To meet this demand, strawberries are imported from southern European countries (Davik et al., 2000). In 2019, only 39.5% of total strawberry consumed was domestically produced (OFG, 2020).

During the main production season, strawberries are produced in the field, and the production systems are of two major types: Matted rows and ridges (Hancock et al., 2008). As in other Nordic countries, the most common type of strawberry production in Norway is matted row

(Davik et al., 2000). This production system is popular in Europe and North America, as it is well-adapted and relatively low-cost in cold climates with short summer (Black et al., 2002; Hancock et al., 2008). However, the area of production under protection is increasingly popular, which might help avoid freezing injury and provide year-round production (Sønsteby et al., 2004).

Propagation materials for field production are mostly domestically produced, but ready to flower planting material for protected production are imported from other European countries, predominantly from the Netherlands (Sønsteby & Heide, 2017). Initially, the import of these plant materials were strictly regulated (Davik et al., 2000). However, the regulations for strawberry transplants were loosened in January 2015 (Milford & Haukås, 2017). Currently, EPPO certification of strawberry transplants (PM 4/11 (2)) is the only criteria to be met by the transplants being imported (Mattilsynet, 2017). Along with the production system, healthy strawberry transplants are the foundation for sustainable production, and the possibility of the disease being introduced with imported plant materials is profoundly concerning.

Global strawberry production is severely affected by various diseases caused by fungi, viruses, bacteria and nematodes. Economically, fungal diseases are most important and can affect all plant parts (Garrido et al., 2011). Grey mould caused by *Botrytis cinerea* is a devastating fungal disease, as it can cause pre-harvest loss of flowers and fruits along with post-harvest fruit damage (Petrasch et al., 2019). Following the global trend, the most common fungal diseases in the Nordic strawberry production system is grey mould (Davik et al., 2000). In Norway, strawberry production is already limited by minimal production area and weather condition (Nes et al., 2008a), and grey mould has become an additional challenge in an already struggling Norwegian strawberry production system.

### 1.2. Grey mould in strawberry

#### 1.2.1. Taxonomy and general description

Several species of *Botrytis* cause grey mould (Botrytis fruit rot) in strawberry. In 1729, Pier Antonio Micheli coined the genus *Botrytis*. The genus *Botrytis* belongs to phylum Ascomycota, class Leotiomycetes, order Heliotiales and class Sclerotiniaceae. The genus includes 38 species, 28 of which are of phytopathogenic importance (Beever & Weeds, 2007; Dewey &

Grant-Downton, 2016). Although most species of *Botrytis* have a narrow host range, *Botrytis cinerea* and *Botrytis pseudocinerea* are polyphagous with a broader host range (Elad et al., 2016; Plesken et al., 2015). In all species of *Botrytis*, the conidiophores bearing macroconidia resemble grape clusters in the microscope, but shape and size vary across species (Walker, 2016). *Botrytis* spp. infect over 1400 species of vascular plants, including species belonging to 170 families of horticultural importance (Elad et al., 2016). They cause pre- and post-harvest fruit rots and blossom blights in strawberry, tomato, and other crops (Jarvis, 1977). Grey mould in strawberry is primarily caused by *B. cinerea*, but in recent studies, other species like *B. pseudocinerea, B. fragaria* and *Botrytis mali* have also been found to cause grey mould in strawberry (Dowling & Schnabel, 2017; Rupp et al., 2017a).

*Botrytis cinerea* (teleomorph: *Botryotinia fuckeliana* is the most common species causing grey mould in over 500 plant species (Hua et al., 2018). This ability to infect a broad range of horticultural crops makes it the second most important fungal pathogen in the world (Dean et al., 2012). In case of Norway, *B. cinerea* is the most crucial pathogen in strawberry production (Strømeng et al., 2009). It is a necrotrophic pathogen that induces cell death in the host and leads to fruit rot (Prins et al., 2000). The pathogen is responsible for massive post-harvest losses where senescent host tissue and a favourable environment can accelerate tissue rot (Williamson et al., 2007).

### 1.2.2. Life cycle and epidemiology

The infection cycle of *Botrytis* spp. can be divided into primary and secondary (Carisse, 2016; Jarvis, 1962). During primary infection source of *Botrytis* inoculum ranges from conidia, mycelium fragments to overwintering sclerotia (Jarvis, 1962). In the Norwegian perennial strawberry production system, mummified fruits, straw mulch, leaf residues and weeds harboured spores, sclerotia and dormant mycelium as the primary source of inoculum (Strømeng et al., 2009). Sclerotia, an overwintering structures formed under unfavourable weather conditions are composed of compact mycelium coated with β-glucan and melanin (Williamson et al., 2007). This extra layer of β-glucan and melanin protect sclerotia from microbial decay, dehydration, freezing weather conditions and UV radiation (Backhouse & Willetts, 1984; Williamson et al., 2007). With the approach of favourable environmental conditions in spring, these overwintering structures germinate to form mycelium. The mycelium then produces branched conidiophores and, upon maturity, bear clusters of conidia at the tip (Williamson et al., 2007). Climatic conditions such as temperature and relative

humidity affect the maturation of conidiophores and the number of conidia produced. Temperatures between 15-25°C with high relative humidity (> 90% RH) is favourable for conidia production, whereas production is lower above 30°C (Carisse, 2016). Although dispersal of spores is primarily by wind, water splash is an additional dispersal mode (Jarvis, 1962; Williamson et al., 2007).

Once the airborne conidia land on the host tissue, favourable environmental conditions are required for germination. Germination of conidia and mycelium growth occurs in a wide range of temperature; the optimum temperature of 20°C paired with high relative humidity (> 93 %) are most favourable (Jarvis, 1977). Appressoria formed after germination cannot penetrate host tissue with mechanical pressure. Therefore, penetration is either assisted by enzymes like cutinases, pectinases and proteases or happens through natural openings and wounds in the host tissue (Carisse, 2016; van Kan, 2006). The primary infection of immature plant parts usually leads to latent infection (Dewey & Grant-Downton, 2016; Jarvis, 1962). The latent infection is asymptomatic, and the *Botrytis* mycelium continues to grow inside the host apoplast and flowering organs (Barnes & Shaw, 2003; Bristow et al., 1986; Petrasch et al., 2019; Veloso & van Kan, 2018). With the start of senescence or ripening of these plant parts, the symptomless phase of infection is followed by a rapid necrotrophic phase. During this phase, enzymatic breakdown of the host cell wall succeeds rapid cell death and tissue decay (Elad et al., 2007; van Kan, 2006). In addition, there is rapid mycelium growth that results in either formation of sclerotia or conidiophores producing macroconidia (Schumacher & Tudzynski, 2012). The secondary infection cycle usually starts with the outgrowth of mycelia from adjacent infected plant parts (Braun & Sutton, 1988). This infection can cause an exponential increase in the fungal population and is capable of massive destruction (Mehli et al., 2005). Macroconidia are produced throughout the growing season, making *Botrytis* spp. polycyclic (Carisse, 2016; Mertely et al., 2018). Macroconidia are multinucleate, unicellular, aseptate, hydrophobic and hyaline (Jarvis, 1977).

Although the published information is little, *Botrytis cinerea* is a heterothallic fungus with a sexual cycle, where microconidia act as spermatia and spermatization of sclerotia produces apothecia (Beever & Weeds, 2007). Apothecia produces binucleate, unicellular and ovoid or ellipsoid shape ascospore. The description of the sexual lifecycle of *B. cinerea* is laboratory-based as apothecia are hardly ever found in field conditions (Beever & Weeds, 2007).

### 1.2.3. Signs and symptoms

In perennial production systems, young strawberry leaves are infected in early spring by primary *Botrytis* inoculum. This infection remains asymptomatic in the early stage, invasion and sporulation gradually increases with leaf senescence, and necrotrophic patches are visible (Carisse, 2016). Botrytis fruit rot starts with the infection of flowers which remains latent at the early stage (Bristow et al., 1986). However, under favourable conditions, the mycelium proliferates and causes soft fruit rot (Bristow et al., 1986). With flower development into fruit, symptoms become increasingly visible after 2 to 4 weeks of fungal infection. Initially, small brown lesions is spotted under the calyx, which later increases rapidly and cover whole fruit (Mertely et al., 2018). This initial stage of botrytis fruit rot is called blossom end rot (Figure 1a and 1b) (Mertely et al., 2018). With disease progression, mycelium with conidiophores bearing conidia are seen covering the fruits. Ultimately, grey cotton networks like mycelium cover the fruit completely and the fruit is mummified (Figure 1c) (Mertely et al., 2018). Water-soaked lesions with a network of sporulating mycelium can be seen in case of stem infection (Williamson et al., 2007).

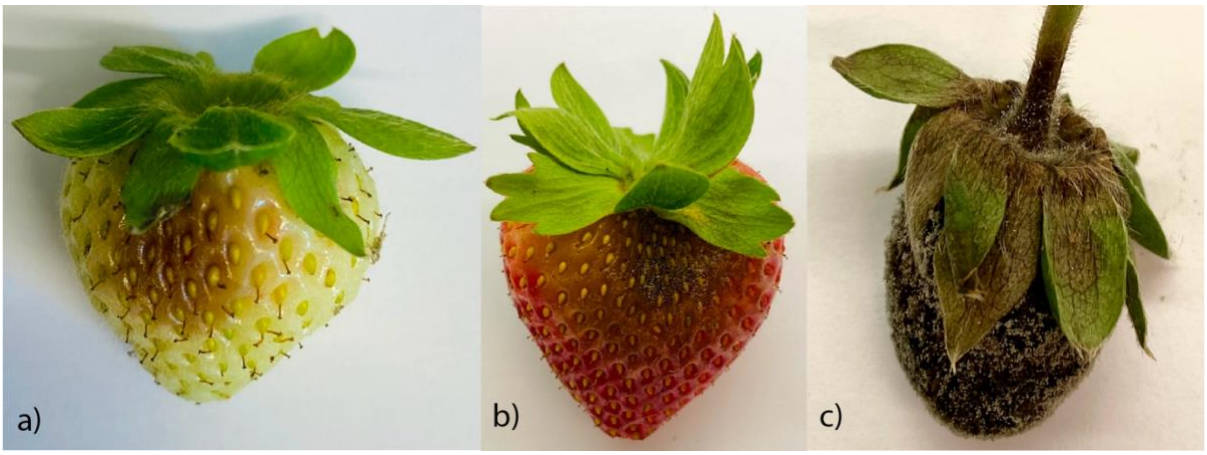


Figure 1: a) Blossom end rot on green fruit. b) Botrytis fruit rot on mature fruit. c) Mummified strawberry fruit with conidiophores and conidia.

### 1.3. Disease Control

Proper management of Botrytis fruit rot is a must for the financial sustainability of strawberry production. Over the years, several disease management practices have been developed for effective control of the *Botrytis* population.

#### 1.3.1. Agronomic and horticultural practices

In the perennial strawberry production system, crop debris and sclerotia are the most important source of *Botrytis* inoculum (Strømeng et al., 2009). Consequently, removing plant debris can significantly decrease major sources of primary inoculum (Daugaard, 1999). Furthermore, soil can harbour sclerotia and fungal spores too. In this case, polyethene and sterilized organic mulch can control the accumulation of these overwintering structures in soil (Daugaard, 1999). In addition, soil sterilization at 55°C for 15 min eradicates the inoculum of *Botrytis* spp. (McGovern & McSorley, 1997). Climatic conditions like surface wetness and high relative humidity play an essential role in disease development. As moisture promotes conidia germination, the selection of appropriate irrigation practice can significantly reduce the disease incidence. Drip irrigation instead of overhead irrigation and low plant density can suppress disease development by reducing wetness and humidity (Legard et al., 2005; Terry et al., 2007). Additionally, strawberry production in a high tunnel with the semi-controlled environment reduces Botrytis fruit rot (Nes et al., 2017). Such protective environments can further suppress the occurrence of airborne inoculum compared to the open cultivation system.

Fruit loss during secondary infection cycles can be more rapid (Mehli et al., 2005). Removing infected and senescent leaves, flowers and fruits from the field can help in avoiding the loss (Droby & Lichter, 2007). Harvested strawberry fruits are equally susceptible to infection (Petrasch et al., 2019). Suppressing activation of latent infections by avoiding bruises at the time of harvest, followed by rapid cooling and storage below 2.5°C, can minimize post-harvest loss (Nunes et al., 1995). During storage, relatively high humidity is crucial to avoid the desiccation of fruits. Nonetheless, high humidity can be favourable for the fungus as well. In this case, relative humidity around 85-90% can maintain fruit quality without accelerating fungal activity (Almeida et al., 2015).

### 1.3.2. Chemical control

In commercial production, agronomic and horticultural practices alone are not sufficient for proper disease management. Chemical control of *Botrytis* spp. with several fungicides is common in strawberry plant nurseries and fruit production farms (Petrasch et al., 2019). The Fungicide Resistance Action Committee (FRAC) have classified fungicides used in plant protection based on their mode of action, target site and chemical group (FRAC, 2020). Out of these classes of fungicides listed by FRAC, seven fungicide class, namely; anilinopyrimidines (Aps), benzimidazoles, dicarboximides, hydroxyanilides, phenylpyrroles (PPs), quinone outside inhibitors (QoIs), and succinate dehydrogenase inhibitors (SDHIs) are used against *Botrytis* spp. (Fernández-Ortuño et al., 2015; Hahn, 2014). In Europe, commonly used fungicides against *Botrytis* spp. are: azoxystrobin, trifloxystrobin and pyraclostrobin (QoIs); boscalid and fluopyram (SDHIs); fenhexamid (hydroxyanilides); cyprodinil, pyrimethanil and mepanipyrim (APs); and fludioxonil (PP). Dicarboximides like iprodione and methyl benzimidazole carbamates like benomyl and thiophanate-methyl are not used currently (Rupp et al., 2017b). Most of these fungicides are site-specific, and these fungicides cause disruption of extracellular protein formation, signal transduction, ATP-generation (cellular respiration), ergosterol biogenesis and amino acid biosynthesis in target organism (Fillinger & Walker, 2016).

Since *Botrytis* spp. are polycyclic pathogens and capable of causing latent infection, timing and frequency of fungicide application are vital for effective disease control. Quiescent infection during flowering can develop into Botrytis fruit rot and is highly destructive. For this reason, the protective dose of fungicides during flowering is most for earlier disease management (Mertely et al., 2002). Furthermore, rotation or a mixture of fungicides with different modes of action is highly recommended (Wedge et al., 2007).

However, the high genetic variability in the *Botrytis* population has resulted in several fungicide resistant strains, thus challenging the available chemical control measure. In addition, raising concerns about pesticide residue has increased the demand for sustainable ways of managing grey mould (Hjeljord et al., 2011). According to the European pesticide residue report 2019, multiple fungicide residue was detected in 63.6% of strawberry samples under study (EFSA, 2021).

### 1.3.3. Biological control

With the increasing cases of fungicide resistance and concerns about pesticide residues in food, biological control of *B. cinerea* is gaining popularity. Many biocontrol formulas used against *B.* cinerea are isolates of *Aureobasidium pullulans*, *Bacillus subtilis*, *Clonostachys roseum, Epicoccum purpurascens*, *Penicillium* sp. *and Trichoderma* sp. (Peng & Sutton, 1991; Sylla et al., 2015). These mycoparasites are effective antagonist as they can control host populations either directly by parasitism and releasing toxins or indirectly by limiting nutrients availability and activating host defence mechanisms in plants (Adikaram et al., 2002; Lima et al., 1997). The performance of these biocontrol agents is a result of multilayer interaction. Consequently, the control of *Botrytis* spp. varied a lot with the production system, pathogen population and formulation of biocontrol agent (Nicot et al., 2016).

Nonetheless, we cannot ignore inconsistent field performance. For example, the recommended dose of *Trichoderma* spp. and *Clonostachys roseum* were inadequate to control the disease at mean temperature during field trials in Norway (Hjeljord et al., 2011). Furthermore, difficulty in the formulation and application and limited availability of these biocontrol agents cannot be overlooked (Card, 2005). Despite all the challenges, this method can be an essential part of integrated pest management which can control *Botrytis* spp. (Jutsum, 1988).

## 1.4. Fungicide resistance in *Botrytis* species

The emergence of fungicide resistant population is a gradual process, which usually starts with mutation, causing alteration of target protein (Van Den Bosch et al., 2011). Subsequent fungicide application favours selection of resistant strains, leading to an ultimate outbreak of resistant population (Van Den Bosch et al., 2011). Furthermore, the ability of pathogens to build up resistance is highly dependent on the mode of action of fungicide (Damicone, 2014). Sensitivity to single-site fungicides is easy to lose than multisite fungicides, requiring multiple target protein alterations (Damicone, 2014). In addition, resistance build-up depends on the dose and number of fungicide application as well. A lower dose and number of fungicide applications can delay the resistance build-up (van den Bosch & Gilligan, 2008; Van Den Bosch et al., 2011). The Fungicide Resistance Action Committee (FRAC) has coded fungicide groups with numbers and letters according to their cross-resistance behaviour (FRAC, 2020).

In the mid-1900, the use of broad-spectrum fungicides against *Botrytis* spp., shortly followed fungicides with a systemic mode of action in the late 1960s (Morton & Staub, 2008). By the early 1970s, some of the strains of *B. cinerea* were recorded to have gained resistance against systemic fungicides (Brent & Hollomon, 1995). The genetic variability within the *Botrytis* population, short polycyclic lifecycle, and abundant sporulation contribute to making *B. cinerea* a high-risk pathogen for developing fungicide resistance (FRAC, 2020; Hahn, 2014). Furthermore, conidia of *Botrytis* spp. are air-borne; hence there is a higher chance of distant dispersal of resistant strains (Carisse, 2016; Mernke et al., 2011). Furthermore, fungicide's mode of action and application frequency plays an essential role (Hahn, 2014).

Several cases of fungicide resistance in *Botrytis* spp. have been registered in strawberry farms worldwide. In *Botrytis* isolates obtained from strawberry fields in Germany, fungicide resistance was common, and some strains were resistant to multiple fungicides belonging to the different chemical group (Leroch et al., 2013). Similarly, among *Botrytis* isolates collected from several strawberry farms across the USA, isolates were resistant to all seven FRAC code fungicides used against *Botrytis* (Fernández-Ortuño et al., 2014). In Norway, when isolates of *Botrytis spp.* collected from 19 strawberry field were tested for fungicide resistance, a larger fraction of isolates exhibited resistance to multiple fungicides like boscalid, pyraclostrobin, fenhexamid, pyrimethanil and iprodione (Strømeng et al., 2018). These studies suggest that several *Botrytis* isolates have gained resistance against almost all fungicides used in strawberry production worldwide.

### 1.5. Strawberry transplants as potential source of *Botrytis* inoculum

Latent infection of *Botrytis* spp. can remain undetected, and healthy-appearing transplants produced under certified guidelines can be a primary source of inoculum too (Oliveira et al., 2017). In addition, *Botrytis* isolates obtained have been found to show fungicide resistance (Oliveira et al., 2017; Weber & Entrop, 2017). Some of the fungicides used in nurseries to produce disease free transplants are also used by growers. Consequently, introduction of isolates resistant to these fungicides may lead to collapse of the strawberry production(Oliveira et al., 2017). Strawberry transplants imported to Germany form the Netherlands were found to carry isolates of *Botrytis* spp. resistant to one or more fungicide classes used in Germany (Weber & Entrop, 2017). In Norwegian context, after introduction of new regulation in 2015,

strawberry transplants are predominantly imported from the Netherlands (Milford & Haukås, 2017; Nielsen et al., 2020). In study by Nielsen et al. (2020), both domestically produced and imported transplants from the Netherlands were found to carry resistant *Botrytis* isolates. However, the incidence of resistant strains was higher in imported transplants. These resistant isolates possess the risk of surviving periodic field application of fungicides and multiplying, thereby causing an outbreak of resistant population.

### 1.6. Heat treatment as an alternative to control disease

With the increasing threat of fungicide resistance, the need for an alternative approach to reduce the pathogen population is crucial more than ever before. Thermotherapy can be the chemical-free alternative for disease management. Thermal treatment of horticultural crops has been practised successfully against numerous phytopathogenic microbes and pests (Brown et al., 2016; Hollings, 1965; Turechek et al., 2021). Few of the examples include reduction of infection caused by virus, bacteria, fungi, insects, and mites in numerous crops such as kiwi, sugarcane, apple, strawberry, sugarcane, potatoes, banana, and flowers (Alvindia, 2012; Lagopodi et al., 2009; Roistacher et al., 1957; Turechek & Peres, 2009; Viswanathan, 2001).

Heat treatment against *B. cinerea* has been found to kill the spores effectively. Although *B. cinerea* spores exhibit some level of heat tolerance, continuous heat at 44.3°C for 11 min was able to kill the spores (Ames, 1915; Smith, 1923). The ability of heat treatment to inhibit germination of spores varied with combinations of time and temperature of exposure (Lichter et al., 2003; Smith, 1923). It is therefore crucial to find the ideal combination of temperature and length of exposure at which *B. cinerea* can be killed with minimum damage to the plant.

Thermotherapy can be practised both on dormant and actively growing plants or plant parts (Hollings, 1965). In strawberry plants, heat treatments have been carried out since the 1920s to control *Aphelenchoides fragariae* by dipping strawberry runners in hot water at 45°C for 9 minutes (Staniland, 1953). Another study treated strawberry transplants against cyclamen mite by dipping transplants in hot water at 48-49°C for 5-7 min (Buchner, 1991). In the same experiment by Buchner, the given heat treatment delayed plant growth, and hot water temperature above 51-52°C for 7 min was detrimental for strawberry transplants. Conventionally, treatment of transplants was done by dipping in hot water. Although a popular

method for pathogen eradication, this technique adversely affects plant growth and yield (Buchner, 1991). Furthermore, there is a higher risk of cross-contamination associated with the use same hot bath for multiple batches of transplants (Turechek & Peres, 2009).

Aerated steam treatment in 'Plant Sauna' is an improved thermotherapy method, which uses aerated steam instead of hot water (Da Silva Jr et al., 2019). This improved method of thermotherapy is precise and can overcome the limitations of conventional hot water treatment (Turechek et al., 2021; Wang et al., 2019). In addition to the advanced technique of heat treatment, the right temperature at which the pathogen is killed without damaging the plant is a ticket to successful thermal treatment (Brown et al., 2016; Hollings, 1965). In the study by Turechek and Peres (2009), heated air treatment at 44°C for 4 h or 48°C for 2 h was least damaging to the plants and more effective against *Xanthomonas fragariae* than hot. Water treatment. Furthermore, the success of thermotherapy is dependent on the ability of the plant to tolerate heat. In this case, pre-treatment at moderately warm temperatures effectively activated heat shock proteins, thereby increasing the plant's tolerance to heat treatment (Brown et al., 2016; Lurie & Mitcham, 2007). Considering the role of temperature at several stages of thermotherapy specific sequence of heat treatment for strawberry transplant was developed (Wang et al., 2019). This protocol included pre-conditioning treatment at 37°C, an hour of cool down and eradicative treatment at 44°C for 4 h (Brown et al., 2016; Wang et al., 2019). Pre-treatment at 37°C is an essential part of the protocol because gradual heat treatment induces heat shock proteins to increase heat tolerance in strawberry plants (Brown et al., 2016; Gulen & Eris, 2003). This heat treatment method in strawberry is effective against several strawberry pathogens like *B. cinerea*, *Colletotrichum acutatum*, *Phytophthora* spp. (Da Silva Jr et al., 2019; Wang et al., 2017; Zuniga & Peres, 2017). Strawberry transplants, as mentioned before, are an important source of *Botrytis* inoculum. Therefore, the thermal therapy of these nursery stocks can be help reduce the pathogen population.

## 1.7. Objective and hypothesis

### 1.7.1. Aerated steam treatment of strawberry transplants

Although a popular method of pathogen eradication, hot water treatment came with few limitations (Buchner, 1991; Turechek & Peres, 2009). Improved aerated steam seemed to have the potential to overcome these limitations (Turechek et al., 2021; Wang et al., 2019). This study aims to evaluate the ability of aerated steam treatment to reduce populations of *Botrytis* spp. without adverse effect on plant health. The hypothesis is that *Botrytis* spp. latently infects strawberry transplants, and the aerated steam treatment of these transplants can reduce the inoculum without negatively affecting plant growth.

### 1.7.2. Aerated steam treatment of *Botrytis spp.* sclerotia

Earlier studies have focused on the aerated treatment of transplants infected with *Botrytis* (Zuniga & Peres, 2017) and hot bath treatment of *Botrytis* conidia (Zuniga, 2018). However, not much is known about the effect of heat treatment on *Botrytis* sclerotia. Here we aim to investigate the ability of aerated steam to kill sclerotia of *Botrytis* spp. The hypothesis is that the untreated sclerotia can readily germinate in the growth media, but the heat-treated sclerotia are killed and cannot germinate.

### 1.7.3. Fungicide sensitivity test

Preliminary studies show an increasing problem of fungicide resistance in *Botrytis* population (Fernández-Ortuño et al., 2014; Hu et al., 2016; Strømeng et al., 2018). This experiment aims to verify the presence of fungicide resistant strains in the *Botrytis* population obtained from strawberry transplants. The hypothesis here is that *Botrytis* spp. isolates obtained from strawberry transplants are resistant to fungicides used in Norwegian strawberry production.

## 2. Materials and methods

### 2.1. Aerated steam treatment on strawberry transplants

#### 2.1.1. Plant materials

Tray plants of five strawberry cvs. Soprano, Murano, Falco, Favori and Sonsation, were used in this experiment (Table 1). Transplants of all different cultivars were imported from the Netherlands and collected from an importer in Norway. The transplants were cold-stored at -1.5°C at the time of pick up. The plants were brought to Kirkejordet, Ås and kept at outdoor conditions in negative degrees temperature for 1-2 days before the experiment.

Table 1: General description of cultivars used in this experiment.

| Cultivar | Season | Country of origin |
|---|---|---|
| Soprano | ever-bearer | The Netherlands |
| Murano | ever-bearer | Italy |
| Falco | mid-season/ June-bearer | The Netherlands |
| Favori | ever-bearer | The Netherlands |
| Sonsation | mid-season/ June-bearer | The Netherlands |

#### 2.1.2. Steam treatment chamber 'Plant Sauna'

The 'Plant Sauna' is an insulated container that ensures the uniform distribution of aerated steam at the desired temperature (Figure 2). The 'Plant Sauna' used in these experiments was manufactured by Plantsauna AS, Lier, Norway. The 'Plant Sauna' is divided into two chambers by a horizontal wall; the lower anteroom is smaller than the upper treatment chamber. The treatment compartment was insulated with 12.5 cm thick walls, and the inner dimension was 150 ×94 × 112 cm. At the same time, the inner dimension of the lower compartment was 150 × 94 × 4.7 cm. The lower antechamber plays an essential role in the 'Plant Sauna', as it is where a 15 mm copper pipe running across its length delivers the steam. The copper pipe transport

steam from a 12 KW steam generator (10KOHM, Tylö AB, Halmstad, Sweden). Steam releases into the antechamber through 10 circular openings distributed across the length of the pipe. Opening's diameter ranges from 2.5 to 6 mm, and the diameter of the opening increases with the increase in distance from the steam generator. This arrangement ensures uniform dispersion of steam and maintains even temperature across the chamber. Once the antechamber is full, steam passes through 2 cm wide openings running across the length of the dividing wall into the treatment chamber.


Figure 2: Plant sauna. Picture Courtesy: Vinh Hong Le

The heating of the chamber was gradual. When steam at 100 °C meet cooler air in the anteroom, the air in the antechamber becomes warm and water saturated. This saturated, warm air then rises into the treatment chamber from two long slit openings. The saturated warm air then heats the cooler air in the treatment chamber. Steam production by the generator and temperature in the antechamber is synchronized with the help of an electronic sensor. The sensor was strategically placed in such a position that the temperature of the slit passage was not higher than the sensor's temperature. The steam production is halted when the treatment chamber touches the desired temperature, and the temperature distribution is even (± 0.5° C). When the temperature drop is sensed, the steam generation is resumed, and the desired temperature is

maintained throughout the experiment. Additional sensors (TinyTag TV4506, Gemini data loggers, Chichester, UK) were placed in two different corners of the treatment chamber, providing precise temperature supervision during the experiment period.

Usually, transplants going into the treatment chamber are colder than the saturated warm water inside, and condensation on the plant surface occurs. With increasing time of exposure, the temperature on the plant surface comes in alignment with the chamber temperature, making heat treatment of transplants possible. Also, transplants remain wet throughout the experiment hours. In our context, plant surface temperature was supervised by hanging sensors (TinyTag Plus 2 TGP-4017, Gemini data loggers, Chichester, UK) as close to the transplants as possible.

### 2.1.3. Experimental design and aerated steam treatment on strawberry transplants

For cv. Soprano, on 22 April 2020 and 23 April 2020, two independent experimental trials were conducted at SKP Kirkejordet Nord of the Norwegian university of life sciences (NMBU), Ås. In the first experiment, out of 240 transplants, two groups with 120 plants in each group was formed. The first experimental run one half and another was kept outside for the second experimental run. Both experimental runs had four replications (=blocks), each with five plants belonging to three different treatments. The treatments for this experiment included nontreated control, 37 °C/1 h conditioning treatment followed by an hour cool down and 44°C/2 h aerated steam treatment, and 37 °C/1 h conditioning treatment followed by an hour cool down and 44°C/4 h aerated steam treatment. This heat treatment sequence developed in Florida (Brown et al., 2016; Wang et al., 2019). During both trials, heat treatment of transplants took place inside 'Plant Sauna'.

As untreated controls, before starting the heat treatment in each experimental run, 40 transplants were randomly selected. Out of the 40 transplants selected, the plug of 20 transplants was removed, and for further investigations, the aerial parts of these transplants were packed in individual zip-lock bags (Figure 3a). At the same time, in 3.5L pots filled with peat soil (80% white moose H1-H4, 10% white moose H1-H4, 10% fine sand Go 'Jord, Degernes Torvstøfabrikk), the other half were potted (Figure 3b). For 80 transplants to be heat-treated, they were randomly and equally divided into four groups and placed into four 20 L IFCO tray (IFCO SYSTEMS GmbH, Pullach, Germany) (Figure 3c). Tinytag TGP-4500

loggers were fitted centrally in an IFCO tray with plants to record near plant temperature and relative humidity (Figure 3c). Then, the IFCO trays were placed into the 'Plant Sauna' chamber for pre-treatment at 37°C for 1 h and then removed to rest at room temperature for 1 h. Following the resting period, the trays with strawberry transplants were placed back in the steam chamber at 44°C. After 2 h of treatment, 2 IFCO trays with transplants were taken out from the steam chamber, and the remaining 40 transplants in 2 IFCO trays were treated for 2 h more. Like the control group of plants, for each heat-treated group, 20 plants were potted in 3.5L pots with peat soil (Go 'Jord, Degernes Torvstøfabrikk), and the aerial parts of the remaining 20 transplants per treatment were packed individually in zip-lock bags (Figure 3a). All the zip-lock bags were then grouped into respective treatment groups, kept in 55L, 600x400x285 mm box (Nopla AS, c/o Lycro AS) and stored at -20°C walk-in freezer at NIBIO (Norwegian Institute of Bioeconomy Research), Ås.

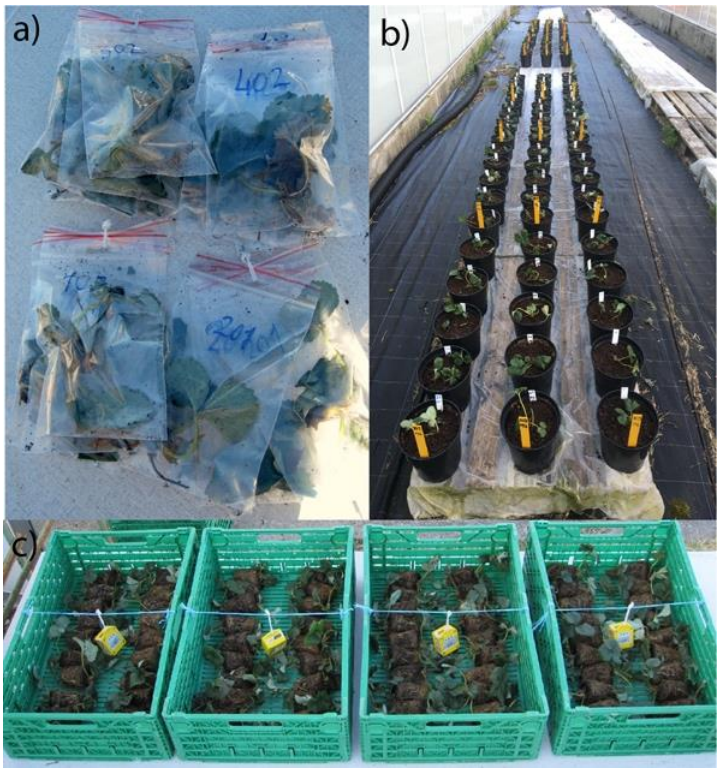


Figure 3: a) Aerial part of strawberry transplants packed for laboratory analysis. b) Potted strawberry transplants. c) Layout of transplants in IFCO trays. Picture Courtesy: Vinh Hong Le

After completion of each experimental trial, all potted plants were labelled with the treatment group. Plants within the treatment were then divided into four replicates (I, II, III, IV) with 5 plants and details and date of experiment added in a label. The pots were then placed on wooden pallets located at SKP Kirkejordet Nord, Ås on open ground between greenhouses, where the position of each pot was pre-determined in randomized block design with four blocks (Figure 3b). The experimental setup was open and exposed to precipitation, environmental temperature, and relative humidity.

Plants were irrigated with overhead shower as per weather demands. From the sixth week of potting the plant, fertigation was done once a week with nutrient-rich water (1.8-1.9 EC ,50:50 CALCINIT™ and KRISTALON™; YaraTera™, Yara Norge AS). Once fruiting started, plants were covered with a nylon net to prevent the loss of fruits to birds. There was no use of pesticides throughout the growth period.

Before the start of the warm steam treatments, tray plants of cultivars Murano (54 plants), Favori (54 plants), Falco (64 plants) and Sonsation (64 plants) were randomly divided into three equal parts for each cultivar. One part was considered the untreated control, and the remaining two parts were placed in two IFCO trays, ready for heat treatment. On 28 April 2020, four IFCO trays, two with transplants of cv. Murano and two with transplants Favori were treated in one experimental run. Whereas on 7 May 2020, transplants of cv. Falco and Sonsation were treated similarly. On both days of experiment, previous heat treatment pattern with 1 h pre-treatment, 1 h cool-down and 2 h/4 h eradicative treatment was followed. Plant parts including leaves, leaflets, petioles, flowers, and small fruits were collected from tray plants in each treatment. The collected plant parts were then stored at -20°C walk-in freezer at NIBIO, Ås. Transplants belonging to 3 treatment groups for these 4 cultivars were then transported to NIBIO, Apelsvoll for planting and further analysis of plant growth and growth (not included in this study).

### 2.1.4. Laboratory analysis

Laboratory analysis of freezer stored transplants was done to analyse the efficiency of aerated steam treatment to eradicate *Botrytis* spp. inoculum. These plant parts were incubated in several batches for analysis of *Botrytis* growth. Firstly, plant parts were taken out from the freezer,

placed on the disinfected lab bench, and allowed to thaw while inside the plastic bags. Once thawed, plant parts were carefully taken out, rinsed in running tap water, and placed between two filter papers to remove excess moisture. For incubation of these plant parts, 125 mm diameter Whatman$^{TM}$ filter papers were placed in 14 cm diameter plastic Petri dishes and uniformly moistened with 2ml distilled water to maintain high humidity. Finally, the leaves were quickly placed inside the Petri dishes. To reduce contamination, 5-6 of these Petri dishes were stacked and sealed in plastic bags (Figure 4a and 4b). The stacks were then allowed to incubate for 7 days at room temperature.

On the seventh day of incubation, plant parts were observed under a Leica MZ 12.5 Stereo microscope (14x) (Figure 4c) for the presence of conidiophores and conidia of *Botrytis* sp. (Figure 4d). Plant parts studied in the case of cv. Soprano were whole leaves with petioles. The total number of leaves incubated and the number of leaves with *Botrytis* per Petri dish were recorded for cv. Soprano. While for the other four cultivars, plant parts observed were whole leaves, leaflets, petioles, and even flowers and small fruits. Whereas for other four cultivars, different plant parts incubated per Petri dish, plant parts with *Botrytis* per Petri dish and the percentage covered by *Botrytis* conidiophores in each plant part (severity %) were recorded.

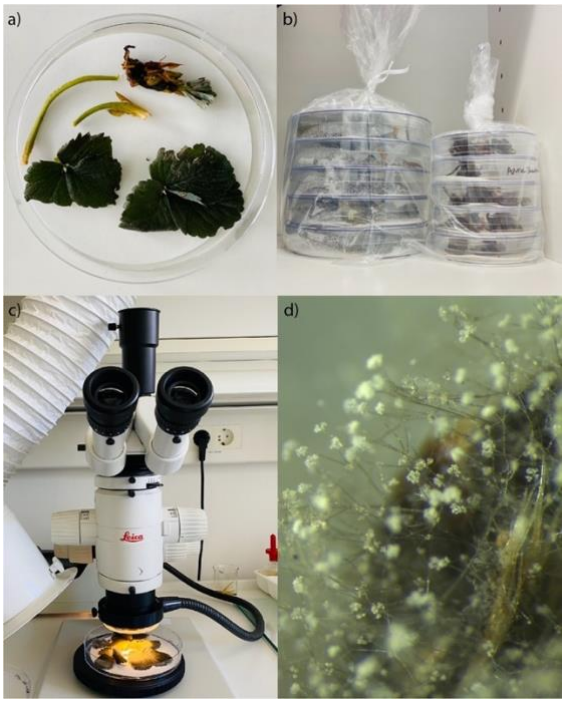


Figure 4: a) Incubation of strawberry plant parts over moistened filter paper inside petri dish. b) Incubation condition of strawberry plant parts. c) Observation under Leica MZ 12.5 Stereo microscope (14X). d) Confirmation of conidia and conidiophores of *Botrytis* spp.

### 2.1.5. Growth assessment of potted strawberry transplants of cv. Soprano

For assessment of growth parameters, the first day of flowering, number of runners, crowns, shoots, leaves, plant height, number of fruits, weight of fruits, shoot and root dry weight were recorded. Registration of first flowering was done from 25 May 2020 to 05 June 2020. Runners were removed once a week with clean scissors from 10 June 2020 until the end of harvest. Fruits were harvested twice weekly from 29 June 2020 to 13 July 2020. Harvesting was done per replica per treatment, and the number and weight of fruit were recorded.

The experiment was terminated on 13 July 2020, and the number of leaf, shoot, crown and runner per plant were recorded. Above-ground parts of plants were cut with a sharp knife, and length of the above-ground part was measured for plant height and then weighed to record fresh stem weight. Once the above-grown parts were removed, roots with soil were taken out of the pots. For accurate root assessment, soil particles were carefully washed off, and excess water was allowed to run off overnight in the greenhouse at ambient temperature before recording fresh root weight. After weighing fresh stem and root weight, shoot and root were packed individually in paper bags, labelled with plant number, and placed in a drying chamber (Model B8260/S, Termarks, Bergen, Norway) at 50°C and weighed on 15$^{th}$ day of drying for dry weight.

### 2.1.6. Storage of bulk spore isolates

Spores of *Botrytis* sp. were transferred to 9 cm Petri dishes with acidified potato dextrose agar (APDA, 9.5 ml of 1:10 tartaric acid diluted in distilled water per 500 ml PDA media), with the help of a sterile scalpel. The Petri dishes were laid flat, sealed inside plastic bags, and incubated at room temperature to avoid air contamination. On the 3$^{rd}$ day of incubation, they were checked for contamination, and clean Petri dishes were transferred to cabinet with continuous light, lighted by PHILIPS master TL-D 36W/840 1F (Poland) and OSRAM L36-73 (Japan), to induce sporulation.

Once there was complete sporulation, cryotubes (2ml; VWR® North American Cat. No. 10018-754, Canada) were labelled with a cultivar name, plate number and day of storage. For the storage of conidia, 600 µl of 20% autoclaved glycerol was pipetted into the labelled cryotubes.

The spores produced on the PDAS plates were transferred into prepared cryotubes with a sterile scalpel. The scalpel was disinfected by burning it off after dipping in alcohol. Then the scalpel was used to scrape conidia from a sporulating *Botrytis* culture, and the scalpel blade was stirred vigorously in the glycerol solution to collect the spores. The process was repeated 2 - 3 times. The scalpel was disinfected again and continued with the successive sample. After transferring the spores, the cryotubes were vortexed for 20 seconds to homogenize the solution and allowed to rest at room temperature for 1 h before storing in the freezer at -20°C.

### 2.1.7. Statistical analysis

Growth assessment and laboratory analysis data obtained from both experimental runs were grouped respectively and analysed using One-way analysis of variance (ANOVA) in R version 4.0.4 (2020-06-22). Here, "treatment" and "replication" (=block) served as the independent variables. The independent variable "treatment" was considered a fixed effect and "replication" (=block) as a random effect. Fisher's protected least significant difference (LSD) test at $P<0.05$ was used to obtain mean and grouping information.

## 2.2. Aerated steam treatment on sclerotia of *Botrytis* spp.

### 2.2.1. Sclerotia production

For sclerotia production, four single spore isolates collected from different municipalities in Norway were used (Table 2). These isolates were stored as spore glycerol solution at -20˚C. To produce mycelium, stored isolates were allowed to thaw at room temperature and mixed with a vortex for 20 seconds to make the solution homogenous. Then, 5µl of solution from each isolate was transferred by pipette to 4-cm diameter Petri dishes with acidified potato dextrose agar (APDA) and incubated at 20°C. Once the mycelium grew to the edge of the Petri dishes, isolates were ready to be used for sclerotia production. This preparation was done twice for two batches of sclerotia production.

Table 2: General description of *Botrytis* spp. isolates used to produce sclerotia.

| Single-spore isolate (2016 strawberry) | Municipality | Isolates |
|---|---|---|
| 96/16-15.3 (Bc) | Søgne | *B. cinerea sensu stricto* |
| 96/16-16.4 (BS1) | Mandal | *Botrytis* group S 1 |
| 96/16-18.5 (Bp) | Marnardal | *B. pseudocinerea* |
| 96/16-19.10 (BS2) | Lyngdal | *Botrytis* group S 2 |

For each batch of sclerotia production, 60 PDA (20 g dextrose, 15 g agar and 4 g potato starch) plates per isolate were labelled with isolate number and day of mycelium transfer. Then, an APDA plate with mycelium of an isolate was opened inside a sterile laminar airflow bench at a time, and 60 agar pieces with mycelium were punched with the help of a sterile cork borer. A sterile scalpel was used to transfer each piece of agar with mycelium and place it in the middle of a PDA plate. The laminar airflow and equipment used were sterilized thoroughly between isolates. PDA plates for the other isolates were prepared and incubated accordingly. During the first batch of sclerotia production (29 June 2020), these PDA plates for each isolate were stacked, sealed in a Ziplock bag and incubated at 20°C in continuous darkness for the production of sclerotia inside the incubation chamber (Versatile Environmental Test Chamber, MLR-352H-PE, PHC Corporation, Japan). For the second batch of sclerotia production (10 August 2020), PDA plates with mycelium were prepared similarly but were incubated at 10°C in continuous darkness in the same growth chamber as described above. The temperature of incubation was changed due to poor sclerotia at 20°C. For both batches of production, sclerotia were harvested after 3 months of incubation.

### 2.2.2. Pilot test

Sclerotia produced for the first batch of sclerotia production were fewer and used for a pilot test. The PDA plates were covered with mycelium that was washed off under running tap water to uncover the sclerotia. These sclerotia were then harvested with forceps. Excess agar on sclerotia was washed out under running tap water, and the sclerotia were then dried in between filter papers. Once surface dried, 9 sclerotia were wrapped inside 5x5 muslin cloth. For each

isolate, 4 such muslin bags were prepared, and sclerotia inside these bags were allowed to dry at room temperature. Consequently, sclerotia fused, and this could be a problem while running the actual test. After 72 h of drying, 50% of sclerotia per isolate were surface sterilized in 1% NaOCl solution followed by a rinse in distilled water.

In contrast, the rest of the sclerotia per isolate were used without surface sterilization. For the germination test of sclerotia, PDA in a Petri dish was cut into eight equals parts with an agar cutter and 4 agar segments per petri dish were placed at an equal distance (Figure 5). Individual sclerotium was placed in each PDA segment and 4 sclerotia in each PDA plate (Figure 5). In total, 4 PDA plates per isolate were prepared. Where 2 PDA plates contained sterilized, and the rest contained unsterilized sclerotia. PDA plates were sealed inside plastic bags and incubated at 20°C, 85% RH and 12 h day/night cycle in the same growth chamber as described above. On the third day of incubation, 100% germination was observed for both sterilized and unsterilized sclerotia. However, unsterilized sclerotia were contaminated with bacterial colony.


Figure 5: PDA sections with single *Botrytis* sclerotia per PDA segment.

### 2.2.3. Harvesting of sclerotia for the experimental run

Sclerotia from the second batch of production were used for actual heat treatment. Heat treatment was done in two independent experimental runs on 18 November 2020 and 2 December 2020. Out of 60 plates with sclerotia per isolate, 30 plates were randomly selected, and sclerotia were harvested for the first experimental run. Unlike plates harvested for the pilot run, sclerotia were not covered by mycelium. So, mature sclerotia were harvested with sterile forceps without washing off the surface. Excess agar was removed by rubbing sclerotia between filter papers. Clean sclerotia were then collected in 12 cm diameter Petri dishes with 125 mm diameter Whatman™ filter papers and labelled with isolate number (Figure 6). Collected sclerotia were then transferred to a Retsch stainless steel sieve and rinsed under running tap water. The sieve was carefully cleaned and sterilized with 70% ethanol between isolates. The filter paper was cut and placed on top of a stainless-steel sieve for drying of rinsed sclerotia. Washed sclerotia from different isolates were spread on the four different sieves and allowed to dry at room temperature. This air-drying method was adapted to avoid clustering of sclerotia, which happened during the pilot test. Prior to the second experimental run, harvesting and drying of sclerotia from the remaining Petri dishes were done similarly. At the time of harvest, the morphology of sclerotia from different isolates varied largely (Figure 6 and Table 3).

Figure 6: Harvested sclerotia from four different *Botrytis* isolates.

Table 3: Morphology and dry weight of harvested *Botrytis* sclerotia.

| Isolates | Morphology at time of harvest | Weight per 100 dry sclerotia (g) | Unit dry weight(g) |
|---|---|---|---|
| 96/16-15.3 (Bc) | Large, irregularly round | 1.53 | 0.015 |
| 96/16-16.4 (BS1) | Smallest, spherical sclerotia with rough surface | 0.16 | 0.002 |
| 96/16-18.5 (Bp) | Flat, thin, and medium in size | 1.04 | 0.010 |
| 96/16-19.10 (BS2) | Great variation from small spherical to large flat sclerotia | 1.26 | 0.012 |

### 2.2.4. Experimental design and aerated steam treatment sclerotia produced

After 72 h of drying, 7 sclerotia were packed in 5×5 $cm^2$ muslin cloth, held with wire twist ties and then tagged with isolate number making muslin bags with sclerotia. For each experimental run, 32 bags per isolate were prepared and tagged with isolate numbers, and there were two experimental runs. Each experiment had two treatment groups; untreated (control) and heat-treated (treatment). The heat treatment was sequential with 37 °C/1 h conditioning treatment, followed by an hour cool down and 44°C/4 h eradicative treatment (Brown et al., 2016; Wang et al., 2019). On 18 November 2020 and 2 December 2020, aerated steam treatment of sclerotia was carried out inside 'Plant sauna' located at SKP Kirkejordet Nord, Ås.

On each day of the experiment, the individual treatment group had four replicas (I, II, III and IV), where each replica included four bags per isolate. For the steam treatment group of sclerotia, bags belonging to different replicas were hung on four different IFCO trays (= "replica") along with Tinytag TGP-4500 loggers (Figure 7a). These four IFCO trays were then placed inside 'Plant Sauna' for heat treatment. While for the control group, bags belonging to the four different replicas were grouped in four different 14cm diameter Petri dishes and placed in the vicinity of the 'Plant Sauna' (Figure 7b).

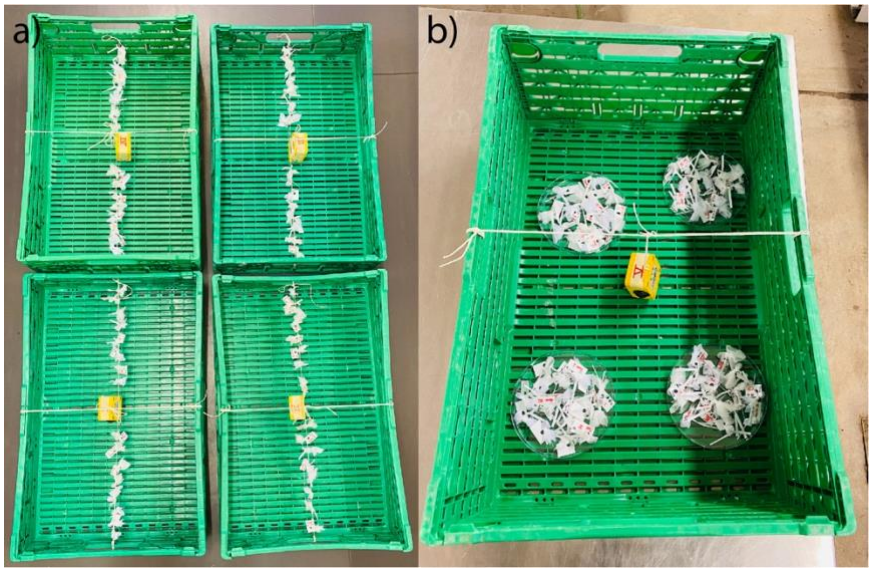


Figure 7: a) Arrangement of muslin bags with *Botrytis* sclerotia belonging to treatment group b) Arrangement of muslin bags with *Botrytis* sclerotia belonging to control group

### 2.2.5. Viability assessment of sclerotia

After completing the heat treatment, both treatment and control groups of sclerotia were transported back to the laboratory at NIBIO, Ås. Surface sterilization was done by dipping the bags with sclerotia in a 1% NaOCl solution and stirring for 1 minute. Bags were then rinsed by dipping in two different containers with distilled water. To avoid cross-contamination, bags belonging to the same treatment, replica and isolate were sterilized at once, and solutions were changed between different batches of sclerotia. After surface sterilization of sclerotia, they were transferred to growth media to induce mycelium growth. The PDA block used during the pilot test was inefficient in terms of time and space, so we used 24-well plates (Nunclon$^{TM}$ Delta Surface, Thermo Fisher Scientific, Denmark) instead (Figure 8). The 24-well plates were prepared by filling each well with 1 ml PDA. Plates were then labelled with treatment, isolate, and the replica of sclerotia. For each experimental run, eight 24-well plates per isolate were used.

To transfer sclerotia, the muslin bags were cut open with sterile scissors inside the laminar airflow chamber, and then surface sterilized forceps were used to transfer sclerotia into the designated wells. After transferring all 24 sclerotia, lids were placed back on and secured with

two tapes on opposite sides (Figure 8). The plates were then incubated in a growth chamber as described earlier at 20°C, with 12 h light/12 h dark day cycle and 85% RH.

For the experiment conducted on 18 November 2020, assessments of germination of sclerotia started within 24 hours of incubation and continued daily until the 7$^{th}$ day of incubation. Assessments were resumed from the 10$^{th}$ day and lasted until day 14. However, for the second experiment conducted on 2 December 2020, assessments started from the 5$^{th}$ day of incubation and continued uninterrupted until day 14. Growth registration for both experimental runs was based on observation under a Leica MZ 12.5 Stereo microscope (14×). Day of visible mycelial growth and day of sporulation were recorded for the incubated sclerotia.

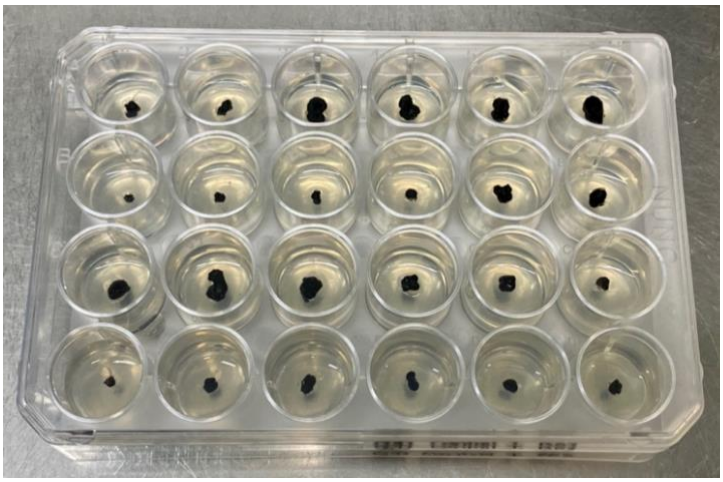

Figure 8: 24-well plates with *Botrytis* sclerotia on PDA growth media.

### 2.2.6. Statistical analysis

For each day of the experiment, the design was randomized block design with “treatment” and “replication” / “box” (=block) as the independent variables, where “treatment” was considered a fixed effect and “box” (=block) was a random effect. Since the number of sclerotia that survived the aerated steam treatment in each experimental run was meagre, survivability data for sclerotia of each *Botrytis* spp. isolate in two experimental runs were pooled. While doing so, it was assumed that all the observations recorded were independent to justify a small number of viable sclerotia. A Chi-square test ($\chi^2$ test, $P \leq 0.05$) of independence was carried out to see if there was difference between isolates in terms of the ability of sclerotia to

overcome aerated steam treatment using R version 4.0.4 (2020-06-22). Post-hoc analysis of the Chi-square test was done with Fisher's Exact Test.

### 2.3. Fungicide sensitivity test

#### 2.3.1. Fungal isolates

Bulk isolates of *Botrytis* sp. obtained from the incubation of transplants from three strawberry cultivars were used for this experiment (Table 4).

Table 4: Number and source of bulk isolates of *Botrytis* used for fungicide sensitivity test.

| Cultivar | Treatment | | |
|---|---|---|---|
| | 4 h | 2 h | 0 h |
| Murano | 5 | 10 | 12 |
| Falco | 5 | 6 | 6 |
| Favori | 4 | 7 | 5 |

These isolates were previously collected and stored in cryotubes with 20% glycerol solution and stored in the freezer at -20º C. To produce the spores for the experiment, the isolates were allowed to thaw at room temperature and stirred with a vortex mixer for 20 seconds to make the solution homogenous. Then 5µl of the homogenous solution from each cryotube was pipetted to a 4-cm diameter Petri dish containing PDA. Petri dishes were then stacked, sealed in a Ziplock plastic bag, and transferred to an incubation chamber (Versatile Environmental Test Chamber) at 20ºC continuous darkness. Once the mycelium grew to the edge of the Petri dish, it was transferred to continuous light, lighted by PHILIPS master TL-D 36W/840 1F (Made in Poland) and OSRAM L36-73 (Made in JP. 32w2) to induce sporulation.

#### 2.3.2. Fungicides used and their discriminatory concentration

Conidia from the bulk isolate of *Botrytis* sp. were tested for resistance to seven fungicides belonging to six chemical classes (Table 5), classified by FRAC (2020).

Table 5: List of fungicides used in fungicide resistance test.

| Common name | Group name | Product name | FRAC Code |
|---|---|---|---|
| Boscalid | SDHI (Succinate-dehydrogenase inhibitor) | Cantus®, BASF | 7 |
| Fenhexamid | KRI fungicides (KetoReductase Inhibitors) | Teldor® WG 50, Bayer | 17 |
| Fludioxonil | PP-fungicides (PhenylPyrroles) | Geoxe® 50 WG, Syngenta | 12 |
| Fluopyram | SDHI (Succinate-dehydrogenase inhibitor) | Luna® Privilege, Bayer | 7 |
| Pyraclostrobin | QoI-fungicides (Quinone outside Inhibitors) | Comet® Pro, BASF | 11 |
| Pyrimethanil | AP - fungicides (Anilino Pyrimidines) | Scala®, BASF | 9 |
| Thiophanate-methyl | MBC - fungicides (Methyl Benzimidazole Carbamates) | Topsin® WG, Nisso Chemical Europe | 1 |

Nutrient stocks used to prepare modified growth media with fungicides were: Malt extract solution (ME;15 g malt extract was added to 1 L distilled water), Malt extract agar (MEA; 6 g agar to 400 ml of malt extract solution prepared), Yeast Bacto Acetate solution (YB; 5 g yeast extract, 5 g Bacto peptone and 10 g sodium acetate to 500 ml distilled water), Yeast Bacto Acetate agar (YBA; 3 g agar in 200ml of YBA solution) and Czapek-Dox solution (CZA; 3.5 g Difco™ Czapek-Dox Broth and 1.5 g agar were added in 100ml distilled water). Furthermore, the discriminatory dose is the concentration of active ingredient of fungicide present in the amendment media. Previously determined discriminatory dose and growth media

for pyraclostrobin, fludioxonil, thiophanate-methyl, boscalid and fenhexamid was used (Fernández-Ortuño et al., 2014; Schnabel et al., 2015; Weber & Hahn, 2011). Discriminatory dose and media for fluopyram were adapted from another study (Weber et al., 2015). Pyrimethanil and cyprodinil are common names for the fungicides belonging to the same chemical group, Anilino Pyrimidines (Latorre et al., 2002; Myresiotis et al., 2007). Hence like cyprodinil, discriminatory dose (4ppm) and media (CZA) was used for pyrimethanil as described by Weber and Hahn (2011).

First, fungicide stock was prepared by dissolving fungicide products in 100 ml of respective stock solutions (Table 6). Then amended growth media with fungicide was prepared by adding fungicide stock solutions to 100ml of specific growth media (Table 7). For pyraclostrobin (QOI fungicide), 1.012 ml of pyraclostrobin stock solution and 101 µl of an alternative oxidase inhibitor SHAM (salicyl hydroxamic acid) stock solution was added to 100ml of MEA. SHAM stock solution was prepared by dissolving 0.1 g salicyl hydroxamic acid in 1 ml methanol. The prepared amended growth media with fungicides were poured into the respective row in two 24-well plates (Nunclon$^{TM}$ Delta Surface, Thermo Scientific, Denmark), labelled "A" and "B" as described by (Fernández-Ortuño et al., 2014) (Table 8 and Figure 9). In the control row, growth media (CZA) was used without added fungicide.

Table 6: Amount of fungicide and growth media used to produce fungicide stock solution.

| Fungicide active ingredient | Stock solution | Amount of product added to 100 ml stock solution | Active ingredient concentration in product | Concentration of stock solution (ppm) |
|---|---|---|---|---|
| Pyrimethanil | CZ | 250 µl | 400 g / L | 997.5 |
| Pyraclostrobin | ME | 502 µl | 200 g / L | 999.0 |
| Fluopyram | YB | 200 µl | 500 g / L | 998.0 |
| Fludioxonil | ME | 0.2 g | 500 g / kg | 1000.0 |
| Boscalid | YB | 0.2 g | 500 g / kg | 1000.0 |
| Thiophanate-methyl | ME | 0.143 g | 700 g / kg | 1001.0 |
| Fenhexamid | ME | 0.2 g | 500 g / kg | 1000.0 |

Table 7: Volume of fungicide stock solution and growth media used to prepare amended growth media.

| Fungicide active ingredient | Growth media | Volume of stock solution to add to 100 ml of medium | Active ingredient concentration of amended medium |
|---|---|---|---|
| Pyrimethanil | CZA | 0.403 ml = 403 µl | 4.0 ppm |
| Pyraclostrobin | MEA | 1.012 ml and 101 µl SHAM stock | 10.0 ppm SHAM 99.9 ppm |
| Fluopyram | YBA | 1.012 ml | 10.0 ppm |
| Fludioxonil | MEA | 0.05 ml = 50 µl | 0.5 ppm |
| Boscalid | YBA | 8.108 ml | 75.0 ppm |
| Pyraclostrobin | MEA | 1.012 ml and 101 µl SHAM stock | 10.0 ppm SHAM 99.9 ppm |
| Thiophanate-methyl | MEA | 11.099 ml | 100 ppm |

### 2.3.3. Fungicide resistance test:

The fungicide resistance test started once all the isolates of *Botrytis sp.* sporulated. The 24-well plates were placed inside sterile laminar airflow with the lid open to remove excess condensation. Transfer of conidia to 24-well plates was carried out under point suction. The transfer of conidia from Petri dishes with spores to designated columns of both plates "A" and "B" (Table 8) was done with the help of sterile toothpicks. Fresh toothpicks were used to make transfers to each well to avoid cross-contamination. After conidial transfer, plates were covered with lids, sealed in a double layer of plastic bags, labelled, and incubated in an incubation chamber (Versatile Environmental Test Chamber, MLR-352H-PE, PHC Corporation, Japan) at 20ºC in continuous darkness. Assessment of fungicide resistance was done on the fourth day of incubation (Figure 9). Resistance categories used for assessment were based on diametric growth of mycelium on 15mm diameter agar wells; sensitive (no mycelium growth), low resistant (< 20 % mycelium), moderately resistant (between 20-50%) and resistant (> 50 %)

(Schnabel et al., 2015). Visual assessment was done with the help of the Leica MZ 12.5 Stereo microscope (14x).

Table 8: Layout of two 24-well plates with different amended growth media in each row.

| Plate | Row | Fungicide active ingredient | Discriminatory concentration (a.i.) | Medium |
|---|---|---|---|---|
| A | A | Control (Cont) | - | CZA |
| | B | Pyrimethanil (S) | 4.0 ppm | CZA |
| | C | Boscalid (B) | 75.0 ppm | YBA |
| | D | Fluopyram (L) | 10.0 ppm | YBA |
| B | A | Fenhexamid (T) | 50.0 ppm | MEA |
| | B | Fludioxonil (G) | 0.5 ppm | MEA |
| | C | Pyraclostrobin + SHAM (C) | 10.0 ppm + SHAM 99.9 ppm | MEA |
| | D | Thiophanate-methyl (Tp) | 100.0 ppm | MEA |

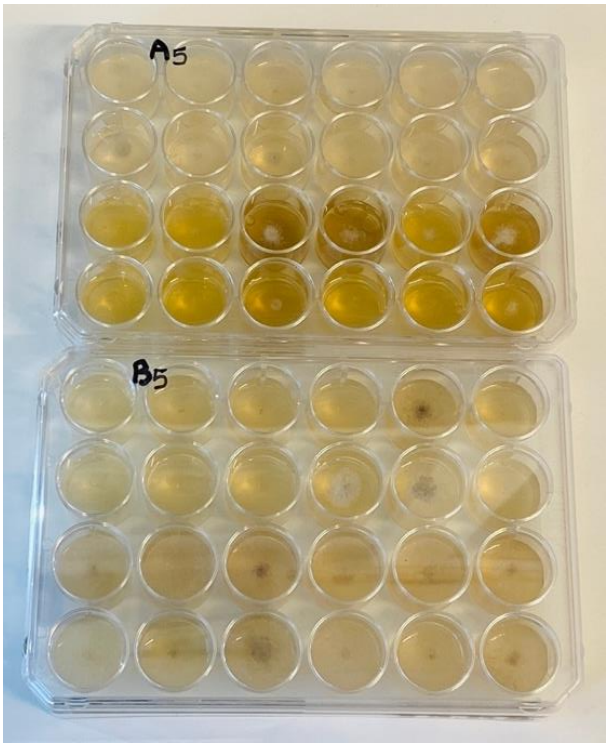


Figure 9: 24-well plates pair with one row of control and 7 rows of fungicides on 4th day of conidia transfer.

## 3. Results

### 3.1. Performance of Plant Sauna

#### 3.1.1. Performance during transplant trials

The temperature recorded by four sensors (TinyTag Plus 2 TGP-4017) hanged in the middle of the four boxes with transplants were used to draw temperature profile within the 'Plant Sauna' chamber (Figure 10). During the first trial (22 April 2020), it took around half an hour for plant materials in all the boxes to reach 37ºC. While in the second trial (23 April 2020), it took 15 minutes to reach the same temperature. Here, it is worth noticing that the soil around the root of transplants was still frozen on the first experiment and partly frozen during the second trial. Furthermore, the minimum temperature recorded at the beginning of the experiment on the first trial was ~20 ºC and on the second trial was ~25 ºC. The temperature built up to 30ºC was rapid in both trials, followed by a steady rise in temperature to 37ºC. Upon reaching 37ºC, the temperature remained more or less stable as desired. However, in the first trial, there was a rise in temperature above 37ºC, but it was short-lived. During the 1 h cool-down period, there was a rapid decline in temperature, and the minimum temperature recorded was near ambient air temperature (~22 ºC). For both experimental trials, all four boxes attended eradicative temperature (44ºC) at the same time, and once the target temperature was achieved, it remained stable throughout the treatment period. Nonetheless, retrieval of two boxes after 2 h treatment was followed by a slight drop in temperature.

#### 3.1.2. Performance during sclerotia trials

For both sclerotia trials (18 November 2020 and 2 December 2020), four boxes with sclerotia were equipped with four sensors (TinyTag Plus 2 TGP-4017) to monitor the temperature. An outline of the temperature inside the treatment chamber for each experimental run was drawn with the help of data collected by these sensors (Figure 11). The minimum temperature recorded at the beginning of both trials was ~20 ºC. During both experimental trials, it took the same amount of time for all boxes to reach 37 ºC and temperature build-up to 30 ºC was rapid. A speedy temperature drop followed Pre-treatment, and the minimum temperature recorded was near ambient air temperature (~22 ºC) during cool-down. After an hour of cool-down, 44 ºC eradicative treatment started. The increase in temperature at this stage was rapid, and once the aimed temperature was attained, it remained stable throughout the experiment period.

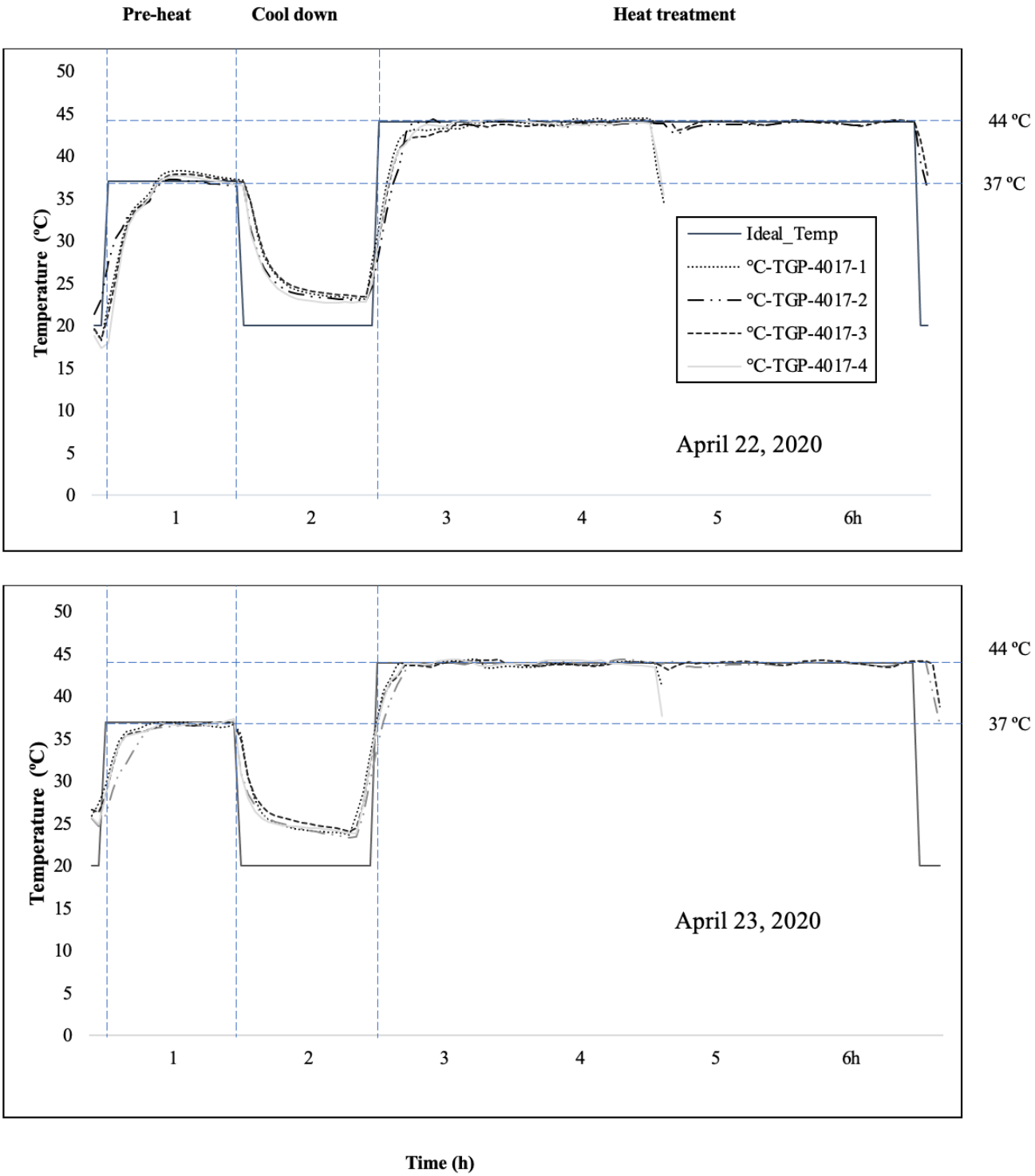


Figure 10: Graph representing temperature profile of boxes with transplants for two experimental runs. The data are collected by sensors (TinyTag Plus 2 TGP-4017) hanged on boxes with transplants.

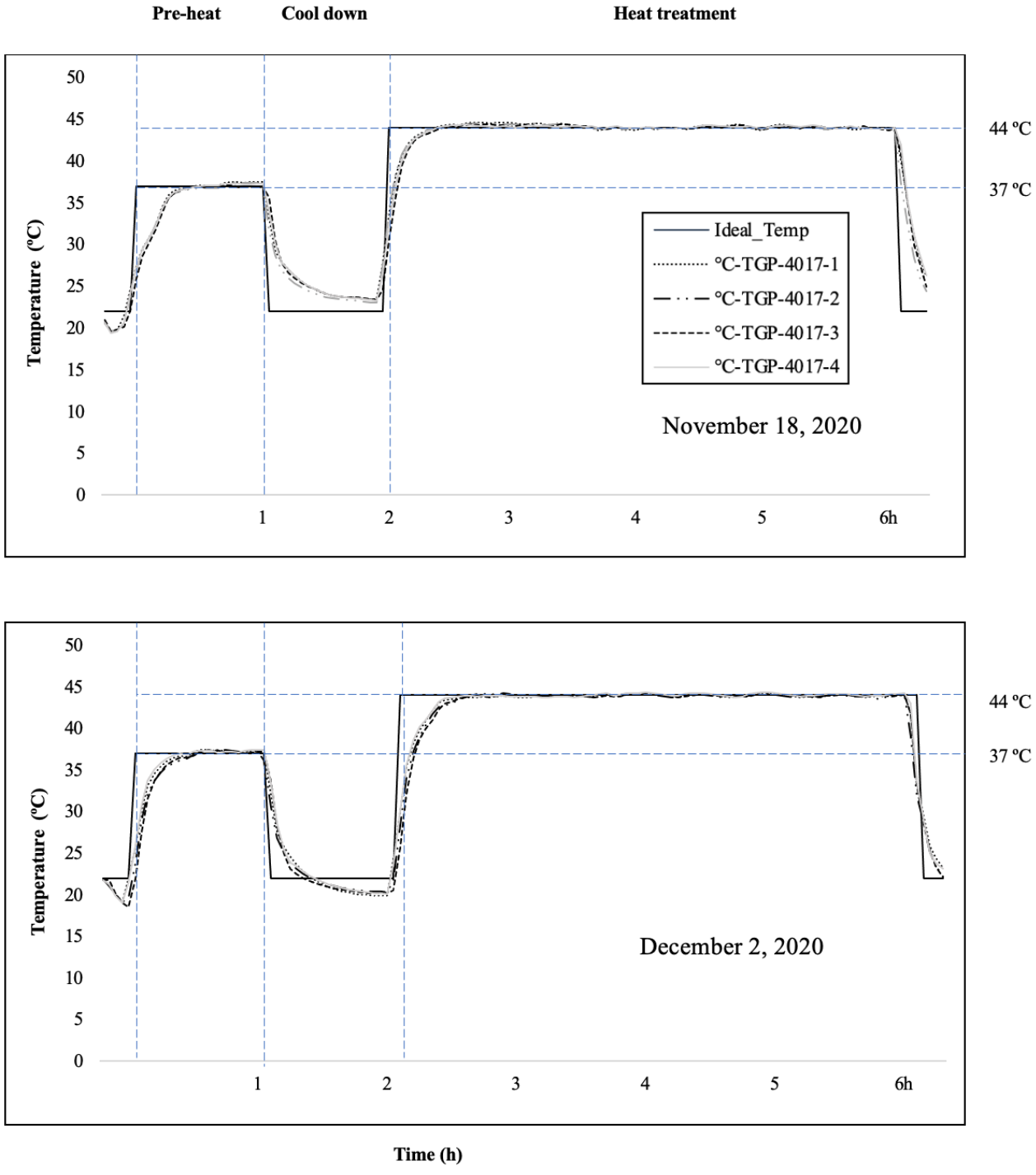


Figure 11: Graph representing temperature profile of boxes with sclerotia for two experimental runs. The data are collected by sensors (TinyTag Plus 2 TGP-4017) hanged on boxes with transplants.

### 3.2. Laboratory analysis

For cv. Soprano, the average incidence (%) of *Botrytis* spp. was calculated by dividing the number of leaves containing the pathogen by the total number of leaves incubated. The average incidence was highest (63.2%) in untreated transplants, followed by 4.8 % for 2 h treated. Whereas for transplants exposed to 4 h treatment, there was 0 % incidence of *Botrytis* spp. In figure 12, the bar represents incidence (%) of *Botrytis* spp. and treatments with different letters on their top are significantly different from each other. Here, in both groups of heat-treated plants, the incidence of *Botrytis* spp. was significantly lower than the control (P-value = 2.2e-16, Figure 12), and there was a 100% reduction of *Botrytis* population in 4 h heat-treated transplants.

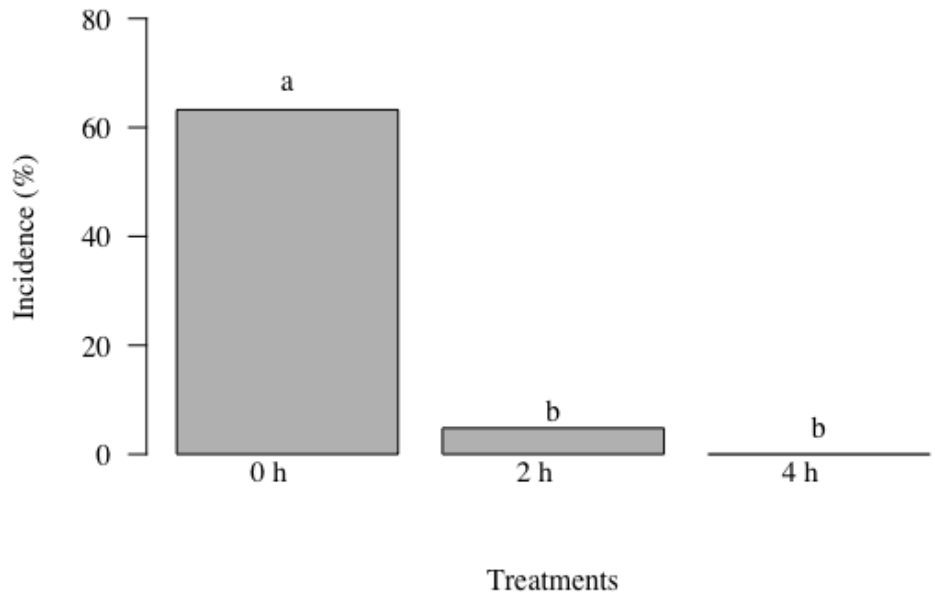


Figure 12: Percentage of leaves (incidence) with *Botrytis* spp. in 120 transplants of strawberry cv. Soprano, divided as one non-treated control (0 h) and two steam treated groups for 2 h and 4 h respectively.

Plant parts of the remaining four cultivars included single leaflets, whole trifoliate leaves, petioles, flowers, and small fruits. Data from the incubation of fruits and flowers were omitted to make the samples more uniform for statistical processing. For the remaining plant parts (leaflets or leaves), each part incubated was considered a unit and the Petri dishes used during incubation for each treatment group were considered the number of replicates for that treatment group. Average incidence (%) was calculated by dividing the number of plant parts containing *Botrytis* spp. by the total number of plant parts incubated. Furthermore, average severity (%) was calculated by dividing total severity (%) recorded by the number of units with *Botrytis* per treatment.

For cv. Murano, 4 h heat-treated plant material had significantly lower disease frequency than both 2 h heat-treated and untreated plant materials (Table 9). In addition, all treatment groups were significantly different from each other in terms of severity (%). In case of cv. Falco, there was no significant difference between the treatment groups, either in terms of incidence (%) or severity (%) (Table 9). Whereas for cv. Favori all three treatment groups were significantly different in terms of incidence (%) but not for severity (%) (Table 9). The result for cv. Sonsation was strikingly different as there were 0% incidence of *Botrytis* spp. in 4 h heat-treated transplants (Table 9). Furthermore, for the same cultivar, incidence (%) and severity (%) was highest for 2 h heat-treated transplants. Overall, compared to the untreated, in 4 h treated transplants of these four cultivars, the incidence of *Botrytis* spp. was reduced by 72 to 100%. However, the incidence of *Botrytis* spp. was not very far from the untreated for 2 h heat-treated transplants.

For further interpretation, data from cvs. Murano, Favori, Falco and Sonsation were pooled and analysed. Here, the incidence (%) was significantly less in the 4 h heat treated plant materials than the 2 h heat treated and untreated plant materials. Regarding severity, the 4 h heat treatment was different from untreated control only (Table 9).

Table 9: Incidence (%) and severity (%) of *Botrytis* spp. on leaves detached from steam-treated groups of four strawberry cultivars, either non-treated or steam treated for 2 or 4 h[a]

| | | Murano | | Falco | | Favori | | Sonsation | | Mean of four cultivars | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Incidence (%) | Severity (%) | Incidence (%) | Severity (%) | Incidence (%) | Severity (%) | Incidence (%) | Severity (%) | Incidence (%) | Severity (%) |
| **Treatments** | 0 h | 79.5 a[b] | 18.6 a | 53.7 a | 2.7 a | 37 ab | 1 a | 18.4 ab | 0.6 ab | 51.3 a | 7.1 a |
| | 2 h | 69.4 a | 6.3 ab | 27.6 a | 3.9 a | 45.3 a | 4.4 a | 30.5 a | 2.1 a | 44.9 a | 4.4 ab |
| | 4 h | 17.9 b | 2.3 b | 15.4 a | 1.3 a | 9.6 b | 0.5 a | 0 b | 0 b | 10.9 b | 1 b |
| | *P*- values | < 0.001 | 0.03 | 0.069 | 0.52 | 0.04 | 0.08 | 0.04 | 0.02 | < 0.001 | 0.025 |

[a]The data are the means of Petri dishes with unit of plant materials incubated per treatment group.

[b]Columns with mean values followed by different letters are significantly different at $P < 0.05$ by Fisher's LSD test

### 3.3. Effect of aerated steam treatment on plant growth in cv. Soprano

Once planted, strawberry plants were observed closely, and several growth parameters were recorded and analysed. Days to first flowering were calculated as the number of days from potting the tray plants to the day of first flowering. There was no significant difference between different treatment groups regarding days to first flowering (Table 10), and plants from all three treatments were found to bear first flowers after 32 to 44 days of transplanting (Figure 13). The number of runners pruned each week and the number of runners at the time of harvest were collectively used to determine the effect of heat treatments on runners, and no significant differences were found between treatments (Table 10).

Whole plants were harvested on 14 July 2020, and the number of leaves, number of shoots, number of crowns per plant and plant height were recorded. There were no significant differences between the treatment groups regarding the number of leaves, number of shoots, and crowns per plant. However, 2 h treated plants were significantly shorter than 4 h treated and untreated plants (Table 10).

Table 10*:* Agronomic performance of strawberry transplants of cv. Soprano after heat treatment[a].

| | | Days to first flowering | No. of runners per plant | No. of leaves per plant | No. of shoots per plant | No. of crowns per plant | Plant height |
|---|---|---|---|---|---|---|---|
| **Treatments** | 0 h | 37.2 a[b] | 3.7 a | 18.8 a | 2.6 a | 5.9 a | 392 a |
| | 2 h | 37.8 a | 3.6 a | 18.8 a | 2.5 a | 6.2 a | 377.7 b |
| | 4 h | 37.6 a | 3.2 a | 19.5 a | 2.7 a | 6.3 a | 396.5 a |
| | *P*- values | 0.7 | 0.2 | 0.7 | 0.6 | 0.68 | < 0.001 |

[a]The data presented are the means of four replicates, each with 5 potted plants per treatment in two experimental runs.

[b]Columns with mean values followed by different letters are significantly different at $P<0.05$ by Fisher's LSD test.

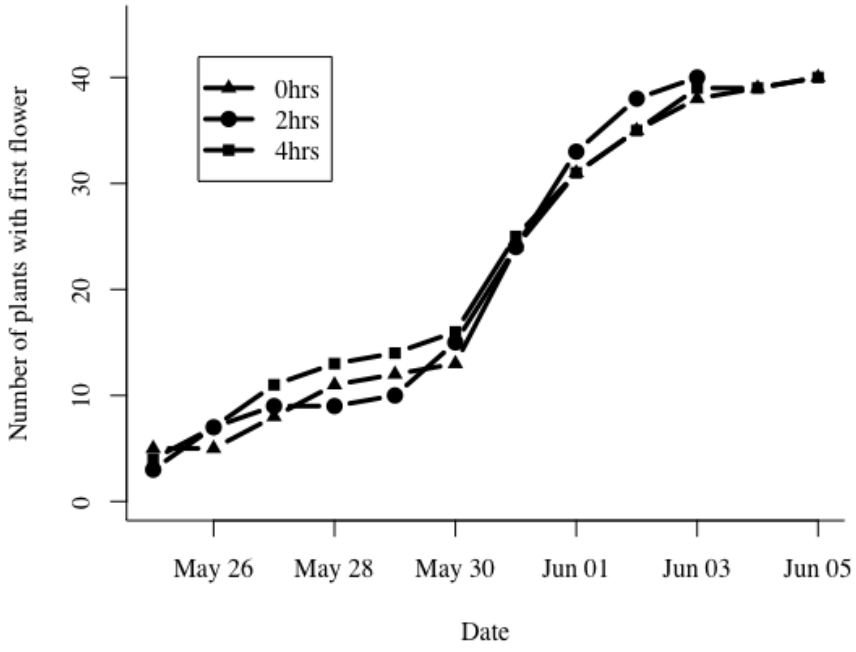


Figure 13: Cumulative number of strawberry plants with first flower per treatment of cv. Soprano on a particular day of registration.

### 3.4. Effect of aerated steam treatment on yield and dry plant mass

Strawberry fruits were harvested from the 8$^{th}$ week of planting; harvesting was done twice a week and five times altogether. Cumulative yield results for the different treatments are presented in Figure 14. There was no significant difference between treatment groups regarding total yield per replicate and the number of fruits per replicate (Table 11). Furthermore, there were no significant differences between the treatments regarding dry root weight per plant (Table 11). However, the dry shoot weight of 4 h treated plants are significantly less than the untreated group of plants (Table 11).

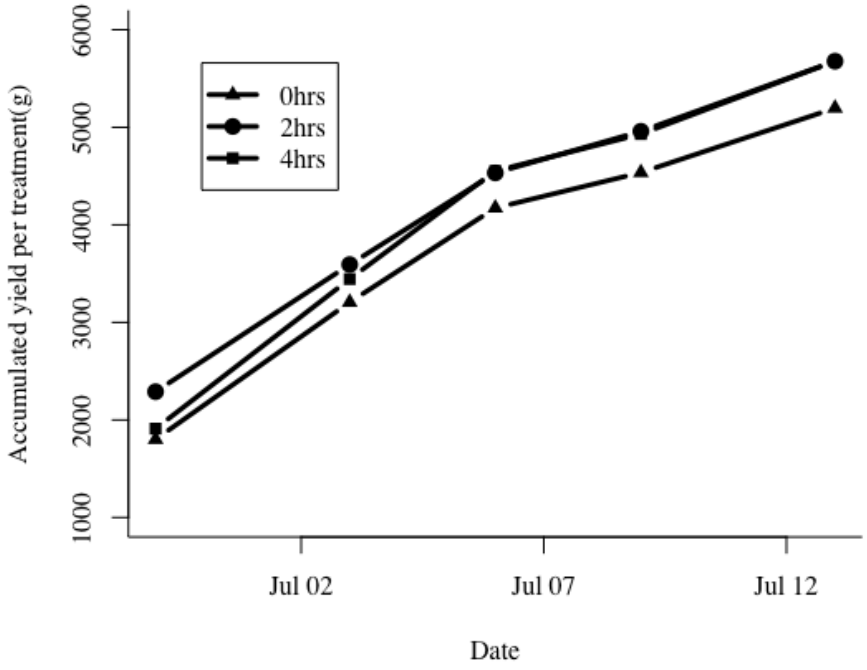


Figure 14: Cumulative fruit weight per treatment of cv. Soprano from two experimental runs.

Table 11: Number of fruit, fruit yield, shoot dry weight and root dry weight for three different treatment groups of strawberry cv. Soprano

| | | Fruit per treatment per replicate[a] | Yield (g) per treatment per replicate[a] | Shoot dry weight (g) per plant[b] | Root dry weight (g) per plant[b] |
|---|---|---|---|---|---|
| **Treatments** | 0 h | 64.1 a[c] | 649.6 a | 31.7 a | 8.9 a |
| | 2 h | 68.6 a | 709.6 a | 29.9 ab | 9.01 a |
| | 4 h | 75.1 a | 709.8 a | 27.7 b | 8.3 a |
| | *P*- values | 0.2 | 0.3 | < 0.001 | 0.3 |

[a] Data presented are the means of 5 plants per treatment per replicate for two experimental runs.

[b] Data presented are the means of four replicates, each with 5 potted plants for each treatment and two experimental runs.

[c] Columns with mean values followed by different letters are significantly different at $P<0.05$ by Fisher's LSD test.

### 3.5. Effect of heat-treatment on survivability of sclerotia of different *Botrytis* spp.

Sclerotia from four different isolates of *Botrytis* spp. were tested for their sensitivity towards aerated steam treatment. The germination data from the 14$^{th}$ day of the experiment was used to assess the effect of heat treatment. There was 100% germination of untreated sclerotia belonging to four different isolates of *Botrytis* spp. in both experimental runs (Figure 15). In contrast, the survivability of heat-treated sclerotia was nominal. For sclerotia of isolate *Botrytis cinerea* sensu stricto (Bc), 18 out of 192 heat-treated sclerotia germinated. The number of surviving sclerotia decreased drastically for other isolates, with 3 out of 192 heat-treated sclerotia for *Botrytis pseudocinerea* (Bp) and 2 out of 192 heat-treated sclerotia for *Botrytis* group S isolate 2 (BS2). Whereas for sclerotia of isolate *Botrytis* group S isolate 1 (BS1), none of the heat-treated sclerotia germinated. The survivability profiles of sclerotia of different isolates are presented in Figure 16. Hence, the effect of aerated steam treatment on the survivability of sclerotia was undeniable. Statistical analysis of the data from heat-treated sclerotia was done with the help of $\chi^2$ test to figure out the degree of susceptibility for each isolate. Here, the sclerotia of isolate BS1 were most sensitive to aerated steam treatment, whereas the sclerotia of Bc isolate were least affected ($\chi^2$ = 5.305 e-08, P≤0.05). This result was cross-validated with results from Fisher's Exact Test (1.683 e-07, P≤0.05).

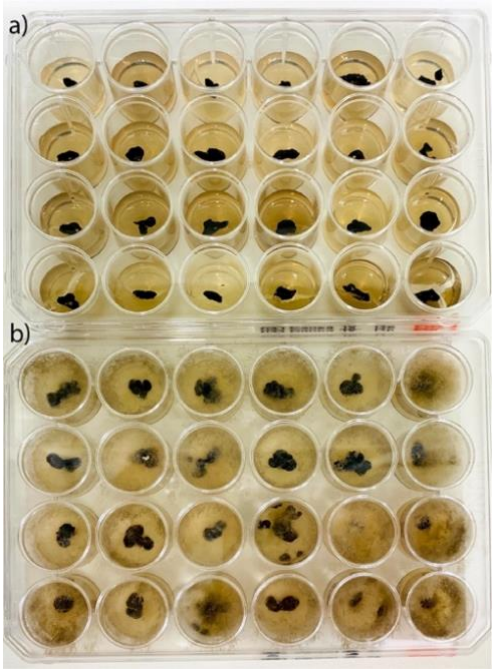


Figure 12: An overview of 24-well plates a) with treated sclerotia. b) with untreated sclerotia on 14$^{th}$ day of incubation.

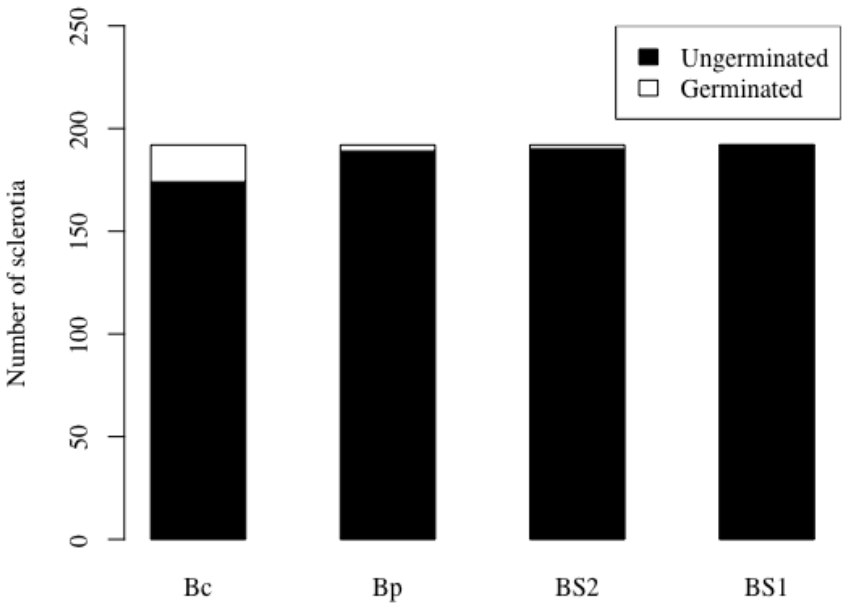


Figure 13: Survivability profiles of 192 heat-treated sclerotia per isolate from 4 different isolates of *Botrytis spp.* Bc= *Botrytis cinerea* sensu stricto, Bp= *Botrytis pseudocinerea*, BS1= *Botrytis* group S isolate 1, and BS2= *Botrytis* group S isolate 2.

### 3.6. Fungicide sensitivity test

Fungicide resistance was categorized as Resistant (R), Moderately Resistant (MR), Low Resistant (LR) and Susceptible (S). Table 12 represents an overview of the number of isolates showing different spectra of resistance to seven fungicides. Out of 62 isolates collected, highest percentage of isolates (90.3%) were resistance to pyraclostrobin, followed by thiophanate-methyl (41.9%) pyrimethanil (35.5%), boscalid (17.7%), fenhexamid (16.1%), fludioxonil (3.2%) and fluopyram (1.6%) as presented in Table 12. Moreover, out of 62 isolates tested, 6.5% of the isolate were susceptible to all the fungicides used, and the remaining showed resistance to several fungicides (Table 13). Here, 27.4% of fungicides used were resistant to single fungicide, 38.7% to two, 11.3% to three, 12.9% to four and the remaining 3.2% were resistant to five different fungicides (Table 13). However, none of the isolate tested showed simultaneous resistance to all seven fungicides used in this test.

Table 12: Resistance profile of bulk conidia isolates of *Botrytis* sp. collected from cvs. Murano, Falco and Favori.

| Fungicides | Isolates[a] | | | | | | | | | | | | Resistance (%)[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Falco | | | | Favori | | | | Murano | | | | |
| | R[b] | MR | LR | S | R | MR | LR | S | R | MR | LR | S | |
| Pyrimethanil | 2 | 3 | 1 | 10 | 5 | 7 | 1 | 6 | 15 | 10 | 2 | 0 | 35.5 |
| Boscalid | 4 | 6 | 6 | 0 | 0 | 4 | 12 | 3 | 7 | 5 | 11 | 4 | 17.7 |
| Fluopyram | 0 | 8 | 8 | 0 | 0 | 0 | 3 | 16 | 1 | 10 | 13 | 3 | 1.6 |
| Fenhexamid | 4 | 0 | 0 | 12 | 0 | 0 | 0 | 19 | 6 | 1 | 0 | 20 | 16.1 |
| Fludioxonil | 0 | 0 | 0 | 16 | 0 | 0 | 0 | 19 | 2 | 2 | 0 | 23 | 3.2 |
| Pyraclostrobin | 15 | 0 | 0 | 1 | 15 | 2 | 0 | 2 | 26 | 0 | 0 | 1 | 90.3 |
| Thiophanate-methyl | 5 | 0 | 1 | 10 | 3 | 0 | 0 | 16 | 18 | 1 | 0 | 8 | 41.9 |

[a] 16, 19 and 27 isolates recovered cvs. Falco, Favori and Murano, respectively were used.
[b] R= resistant, MR = moderately resistant, LR = low resistant and S = sensitive.
[c] percentage of isolates exhibiting complete resistance for a particular fungicide out of 62 isolates in total.

Table 13: Multiple resistance profile of *Botrytis* isolates obtained from different transplants.

| No. of fungicides resistant to | Isolates[a] | | | |
|---|---|---|---|---|
| | Falco (%)[b] | Favori (%) | Murano (%) | Total (%) |
| 0 | 6 | 15.8 | 0 | 6.5 |
| 1 | 50 | 47.4 | 0 | 27.4 |
| 2 | 19 | 36.8 | 51.9 | 38.7 |
| 3 | 6 | 0 | 22.2 | 11.3 |
| 4 | 13 | 0 | 22.2 | 12.9 |
| 5 | 6 | 0 | 3.7 | 3.2 |
| 6 | 0 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | 0 |

[a] Isolates are bulk conidia isolates obtained from incubation of respective strawberry cultivar's transplants.
[b] Percentage of isolates that were resistant to respective number of fungicides.

## 4. Discussion

In this study, strawberry transplants imported from the Netherlands were found to carry quiescent infections of *Botrytis* spp. Upon further investigation, isolates obtained from these transplants were resistant to several fungicides used against *Botrytis* species. Such initial inoculum can be a severe threat to commercial strawberry farms and should be addressed with innovative disease management practices. In our study, aerated steam treatment in the 'Plant Sauna' significantly reduced the primary inoculum of *Botrytis* spp. without a negative effect on plant growth and yield. Although the ability of aerated steam treatment to decrease *Botrytis* population from strawberry transplants was previously reported (Zuniga & Peres, 2017); this is, to our knowledge, the first report of this treatment being successfully used against sclerotia of *Botrytis* spp. These are the significant findings that prove the ability of thermotherapy in the 'Plant Sauna' to reduce the initial inoculum. This can be a much-needed technology aiding in integrated disease management of strawberry transplants.

### 4.1. Precision of the 'Plant Sauna'

The adapted protocol and the medium of aerated steam treatment (Plant Sauna) used in this experiment were precise and safe. Before the introduction of aerated steam treatment, thermotherapy by submersion of strawberry transplants in hot water was commonly used in commercial nurseries to control several pathogens, mites, and insects. However, this heat treatment practice could increase cross-contamination and in some cases, injure the transplants (Buchner, 1991; Turechek & Peres, 2009). To overcome this problem, aerated steam treatment as an enhanced means of thermotherapy came forward. The protocol used for thermal treatment in this experiment was previously approved by Wang et al. (2019). The essential part of the protocol is the pre-treatment of strawberry transplants which activate heat shock proteins to acclimatize the plants to a higher temperature (Brown et al., 2016; Turechek et al., 2021).

Along with the protocol used, precision and uniformity of treatment temperature are critical for successful heat treatment, with minimum damage to the plants. In our case, the 'Plant Sauna' steam generator generated steam at a uniform rate, and the heat was accurately developed and evenly distributed. Once the target temperature was reached, it remained stable without further increase in temperature. This precision is important for the wellbeing of the

plant throughout the treatment period. Furthermore, in 'Plant Sauna', the heated air inside the treatment chamber is water-saturated and the plant surface is wet, the plant surface temperature remains identical to the treatment temperature. Such homogeneity inside 'Plant sauna' assures a highly efficient condition for heat treatment.

### 4.2. Strawberry transplants as a source of primary inoculum of *Botrytis* spp.

In this study, the occurrence of latent infections of *Botrytis* spp. was observed in strawberry transplants. In a previous study conducted in Norway, latent infections of *Botrytis* spp. were observed in both imported strawberry transplants and those produced in Norway (Nielsen et al., 2020). These transplants carrying quiescent infections are likely a primary source of fungicide resistant *Botrytis* spp. (Boff et al., 2001; Oliveira et al., 2017; Weber & Entrop, 2017). In a study by Strømeng et al. (2009), senescing plant parts, straw mulch, sclerotia and decaying dicotyledon weeds were primary sources of *Botrytis* inoculum in the Norwegian perennial production system. Moreover, our results and the findings by Nielsen et al. (2020) indicate that nursery transplants entering Norwegian production systems can be considered one of the primary inoculum sources. This finding also highlights the importance of screening and detection of quiescently infected strawberry transplants, which in many cases can be easily disguised as healthy transplants.

### 4.3. Efficiency of aerated steam treatment to control primary inoculum of *Botrytis* spp.

In this experiment, the aerated steam treatment effectively reduced *Botrytis* inoculum in transplants of all five strawberry cultivars tested (cvs. Falco, Favori, Murano, Sonsation and Soprano). For all the cultivars tested, thermotherapy at 44 ºC for 4 h was undeniably effective in reducing inoculum than heat treatment at 44 ºC for 2 h. The conidia and other dormant structures of *Botrytis* spp. were probably either killed or deactivated upon exposure to a higher temperature. Similar results were obtained in a study in Florida, USA, where warm steam treatment of strawberry transplants significantly reduced the incidence of *B. cinerea* (Zuniga & Peres, 2017). Although *Botrytis* spp. are known to survive freezing temperature, higher temperatures have been found to have damaging effects on disease development and survivability of overwintering structures (Droby & Lichter, 2007; Jarvis, 1977; Oliveira et al., 2017). This heat susceptibility makes *Botrytis* spp. suitable candidate for heat treatment. In

addition to its efficiency against *Botrytis* spp., the aerated steam treatment effectively reduced the inoculum of *X. fragariae*, *C. acutatum* and *P. aphanis* in strawberry transplants (Da Silva Jr et al., 2019; Turechek & Peres, 2009; Wang et al., 2017). Since nursery transplants are significant sources of primary inoculum, heat treatment of these transplants before planting can reduce pathogen entering the production system (Da Silva Jr et al., 2019; Turechek et al., 2021; Zuniga & Peres, 2017). Thermotherapy is an old method of transplant sterilization, but hot water increased the risk of plant damage and disease cross-contamination, decreasing its popularity (Buchner, 1991; Turechek & Peres, 2009). 'Plant Sauna' has been able to overcome drawbacks associated with cross-contamination; to test the performance ability of transplants after being exposed to high temperature in 'Plant Sauna' is interesting.

### 4.4. Effect of aerated steam treatment on plant development and yield

Steam treated plants of cvs. Falco, Favori, Murano and Sonsation were brought to another research facility of NIBIO, and further investigations on growth and yield performance are not reported here. Plant growth performance of cv. Soprano, in terms of days to flowering, plant height, dry weight, number of shoots, leaves, crowns and fruit yield, was taken into consideration to determine the effect of heat treatment on agronomic traits of strawberry plants. There was no effect of heat treatment in most of the growth parameters, except for dry shoot weight. The dry shoot weight of 4 h heat-treated transplants was significantly lower than for the untreated control group of plants. However, there was no reduction in yield for the 4 h heat-treated plants. Our results are in alignment with other experiments where aerated steam treatment at 44 ºC for 4 h against several pathogens like *X. fragariae*, *C. acutatum* and nematodes did not show a detrimental effect on the growth and production of strawberry transplants (Khanal et al., 2020; Turechek et al., 2013; Turechek et al., 2021; Wang et al., 2017). In a study by Wang et al. (2019), heat treatment positively affected flowering and growth. However, our result could not conclude the same. The contrasting results could be due to the variation in transplants used, where we used tray transplants of previously mentioned cultivars compared to bare-root transplants of different cultivars used in the study by Wang et al. (2019). This study has highlighted the uniformity, safety, and precision of aerated steam-based heat treatment along with its ability to overcome multiple limitations associated with previous methods of thermotherapy.

### 4.5. Efficiency of aerated steam treatment against sclerotia of *Botrytis* spp.

Heat exposure of sclerotia from four different *Botrytis* isolates for 4 h at 44 ºC was highly effective, where germinability of the treated sclerotia was severely affected. Heat treatment was most effective against sclerotia from *Botrytis* group S isolate 1 (BS1), with complete deactivation of all the treated sclerotia. While, for sclerotia from the remaining three isolates, germination of heat-treated sclerotia was nominal, and the impact of heat treatment was evident. As sclerotia from isolate *Botrytis* group S isolate 1 (BS1) were the smallest, size might be an explain this result. Here, the small size may have facilitated heat penetration to the inner core. Similar results of nominal sclerotia germination were obtained by Zuniga (2018) when sclerotia were treated in a thermal bath. Heat treatment is effective against sclerotia of *Sclerotinia sclerotiorum* as well, but the temperature and time required for total inactivation was 120 ºC for 20 minutes (Dueck et al., 1981). Low temperatures are favourable for the survivability of *Botrytis* sclerotia; however, temperature above 30 ºC decreased their germination (Jarvis, 1977). These findings show susceptibility of sclerotia to a higher temperature.

In addition to the effect of heat treatment on the survivability of sclerotia, the original plan was to investigate its effect on days to germination and days to sporulation. A schedule of daily growth registration from day 1 to day 14 of the experiment was made. However, restricted entry at NIBIO due to COVID-19 related regulations, limited daily registration. For the first experiment (started on 18 November 2020), the 8$^{th}$ and 9$^{th}$ day registration was obstructed. While for the second experiment (started on 2 December 2020), registration started only from 5$^{th}$ day. Therefore, data from the final day of registration (day 14) was used to determine survivability of sclerotia only.

Although sclerotia are hardy overwintering structures, aerated steam treatment in the 'Plant Sauna' seemed to be more effective against sclerotia than against conidia and dormant mycelium of *Botrytis* spp. Symptomless endophytic growth in the apoplast during latent infection is typical in *Botrytis* spp. (Barnes & Shaw, 2003; Sowley et al., 2010). This inner cellular growth of mycelium might be least affected by the temperature outside and can be one reason for getting our result. Also, it would be interesting to test the efficiency of similar thermal treatment of sclerotia in the presence of plant materials and soil.

### 4.6. 'Plant Sauna' as much needed technology for production of clean transplants

In the present work, the frequency of resistance against pyraclostrobin was very high, followed in descending order of resistance by thiophanate-methyl, pyrimethanil, boscalid, fenhexamid, fludioxonil and fluopyram. In addition, some of the isolates tested were found to build up simultaneous resistance to up to five fungicides. This result is in accordance with other studies, where isolates were found to develop cross resistance to multiple fungicides (Fernández-Ortuño et al., 2015; Leroch et al., 2013; Li et al., 2014; Oliveira et al., 2017). The ever-increasing use of fungicides against grey mould and genetic variability of the *Botrytis* population has made it evident that some isolates are likely to gain resistance against certain fungicides. The increasing cases of multiple fungicide resistance are concerning.

A comparison was made in a study conducted in Norway, between domestically produced strawberry transplants with those imported from the Netherlands for quiescent infection (Nielsen et al., 2020). Fungicide resistant strains of *Botrytis* spp. accompanied transplants from both origins, but the frequency was higher in transplants imported from the Netherlands. In a similar investigation of fungicide resistance in Germany, strawberry transplants imported were found to carry fungicide resistant strains of *Botrytis* spp. (Weber & Entrop, 2017). Nurseries are known to use multiple sprays of pyraclostrobin, boscalid, cyprodinil and fludioxonil in their strawberry transplant production (Weber & Entrop, 2017), and the high frequency of fungicide applications is known to the favour selection of fungicide resistant strains of *Botrytis* spp. (Fernández-Ortuño et al., 2012; Hu et al., 2016; Weber & Entrop, 2017). This use of fungicide in nurseries might explain the high occurrence of resistant strains of *Botrytis* spp. in strawberry transplants. This problem needs to be addressed as strains of *Botrytis* spp. with multiple fungicide resistance can enter the production system along with transplants and decreases the efficacy of periodic fungicide applications (Amiri et al., 2014; Fernández-Ortuño et al., 2012; Weber & Entrop, 2017).

In this study, thermotherapy seemed detrimental to the *Botrytis* population regardless of resistance. This reduction in the initial pathogen population eases the burden of disease management during the production season. Despite its ability to reduce primary inoculum in strawberry transplants, the efficiency of the 'Plant Sauna' at a commercial scale may be questioned. To address this problem, a commercial scale Precision thermotherapy unit (PTU) was constructed (Turechek et al., 2021). This commercial PTU was able to treat transplants on

a large scale with negligible side effects. Due to its impressive results, some strawberry farms in the USA have already used the unit for sterilization of plant material (Turechek et al., 2021).

### 4.7. Conclusion and future perspective

Strawberry transplants are the foundation of the strawberry production system, and nurseries try to produce disease-free planting material. However, strawberry transplants carrying latent infection and can easily be disguised as healthy transplants. Fungicides are the most common means of disease control in nurseries. Increasing cases of fungicide-resistant populations and raising awareness of its adverse effects on the environment and human health, make use of fungicides less desirable. As heat is non-discriminatory against susceptible and resistant strains of the pathogen, aerated steam treatment in the 'Plant sauna' can be a much-needed method of disease management. In addition to its efficiency to eradicate *Botrytis* spp., aerated steam treatment has been effective against several other pathogens in strawberry transplants (Da Silva Jr et al., 2019; Turechek & Peres, 2009; Wang et al., 2017).

Reduction of the initial pathogen population in transplants can act as the first line of defence. Hence, 'Plant Sauna' can be part of an integrated disease management program for strawberry and used to produce disease-free strawberry transplants. So far, aerated steam treatment in the 'Plant Sauna' has been used to treat of limited plant species. It would be interesting to test if this technology can reduce the pathogen population without hampering plant growth and yield of other crop species. Nonetheless, the selected plant must have some level of heat tolerance. Heat treatment of plant propagules like corms, setts and tubers has been practised previously (Mackay & Shipton, 1983; Roebroeck et al., 1991; Viswanathan, 2001), and it would be interesting to see if thermotherapy in the 'Plant Sauna' would reduce pest populations in such propagules.

## 5. List of references


Adikaram, N. K., Joyce, D. C. & Terryc, L. A. (2002). Biocontrol activity and induced resistance as a possible mode of action for *Aureobasidium pullulans* against grey mould of strawberry fruit. *Australasian Plant Pathology*, 31 (3): 223-229.

Almeida, M. L. B., Moura, C. F. H., Innecco, R., Santos, A. d. & de Miranda, F. R. (2015). Postharvest shelf-life and fruit quality of strawberry grown in different cropping systems. *Embrapa Agroindústria Tropical-Artigo em periódico indexado (ALICE)*. doi: https://doi.org/10.5897/AJAR2015.10239.

Alvindia, D. G. (2012). Revisiting hot water treatments in controlling crown rot of banana cv. Buñgulan. *Crop Protection*, 33: 59-64. doi: https://doi.org/10.1016/j.cropro.2011.09.023.

Ames, A. (1915). The temperature relations of some fungi causing storage rots. *Phytopathology*, 5 (1): 11-19.

Amiri, A., Heath, S. M. & Peres, N. A. (2014). Resistance to fluopyram, fluxapyroxad, and penthiopyrad in *Botrytis cinerea* from strawberry. *Plant Disease*, 98 (4): 532-539. doi: https://doi.org/10.1094/PDIS-07-13-0753-RE.

Backhouse, D. & Willetts, H. (1984). A histochemical study of sclerotia of *Botrytis cinerea* and *Botrytis fabae*. *Canadian Journal of Microbiology*, 30 (2): 171-178. doi: https://doi.org/10.1139/m84-027.

Barnes, S. E. & Shaw, M. W. (2003). Infection of commercial hybrid primula seed by *Botrytis cinerea* and latent disease spread through the plants. *Phytopathology*, 93 (5): 573-578. doi: https://doi.org/10.1094/PHYTO.2003.93.5.573.

Beever, R. E. & Weeds, P. L. (2007). Taxonomy and genetic variation of Botrytis and Botryotinia. In *Botrytis: Biology, pathology and control*, pp. 29-52: Springer.

Black, B. L., Enns, J. M. & Hokanson, S. C. (2002). A comparison of temperate-climate strawberry production systems using eastern genotypes. *HortTechnology*, 12 (4): 670-675. doi: https://doi.org/10.21273/HORTTECH.12.4.670.

Boff, P., Kastelein, P., de Kraker, J., Gerlagh, M. & Köhl, J. (2001). Epidemiology of grey mould in annual waiting-bed production of strawberry. *European Journal of Plant Pathology*, 107 (6): 615-624. doi: https://doi.org/10.1023/A:1017932927503.

Braun, P. & Sutton, J. (1988). Infection cycles and population dynamics of *Botrytis cinerea* in strawberry leaves. *Canadian Journal of Plant Pathology*, 10 (2): 133-141. doi: https://doi.org/10.1080/07060668809501745.

Brent, K. J. & Hollomon, D. W. (1995). *Fungicide resistance in crop pathogens: how can it be managed?*: GIFAP Brussels.

Bristow, P., McNicol, R. & Williamson, B. (1986). Infection of strawberry flowers by *Botrytis cinerea* and its relevance to grey mould development. *Annals of Applied Biology*, 109 (3): 545-554. doi: https://doi.org/10.1111/j.1744-7348.1986.tb03211.x.

Brown, R., Wang, H., Dennis, M., Slovin, J. & Turechek, W. W. (2016). The effects of heat treatment on the gene expression of several heat shock protein genes in two cultivars of strawberry. *International Journal of Fruit Science*, 16 (sup1): 239-248.

Buchner, R. P. (1991). Hot water preplant dip for strawberry disease control. *The Strawberry into the 21st Century. A. Dale and JL Luby, eds. Timber Press, Inc. Portland, OR*: 217-218.

Card, S. D. (2005). *Biological control of Botrytis cinerea in lettuce & strawberry crops*: Lincoln University.

Carisse, O. (2016). Epidemiology and aerobiology of *Botrytis* spp. In Fillinger, S. & Elad, Y. (eds) *Botrytis – The Fungus, the Pathogen and its Management in Agricultural Systems*, pp. 127-148. Cham: Springer International Publishing.

Da Silva Jr, C. D., Le, V. H., Tadesse, B. A., Grieu, C., Wang, N.-Y., Peres, N., Turechek, W. W. & Stensvand, A. (2019). Aerated steam by the 'Plant Sauna' eradicates powdery mildew from strawberry transplants. *Plant Health 2019*.

Damicone, J. P. (2014). Fungicide resistance management.

Darrow, G. M. (1966). The strawberry history, breeding and physiology. *The strawberry. History, breeding and physiology.*

Daugaard, H. (1999). Cultural methods for controlling *Botrytis cinerea* Pers. in strawberry. *Biological agriculture & horticulture*, 16 (4): 351-361. doi: https://doi.org/10.1080/01448765.1999.9755238.

Davik, J., Daugaard, H. & Svensson, B. (2000). Strawberry production in the Nordic countries. *Advances in Strawberry Research*, 19: 13-18.

Dean, R., Van Kan, J. A., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P. D., Rudd, J. J., Dickman, M., Kahmann, R. & Ellis, J. (2012). The Top 10 fungal pathogens in molecular plant pathology. *Molecular plant pathology*, 13 (4): 414-430. doi: https://doi.org/10.1111/j.1364-3703.2011.00783.x.

Dewey, F. M. & Grant-Downton, R. (2016). *Botrytis*-Biology, detection and quantification. In Fillinger, S. & Elad, Y. (eds) *Botrytis – The Fungus, the Pathogen and its management in Agricultural Systems*, pp. 17-34. Cham: Springer International Publishing.

Døving, A., Nes, A. & Serikstad, G. L. . (2017). Jordbærdyrking i økologisk landbruk. *Dyrkingsrettleiing*.

Dowling, M. & Schnabel, G. (2017). First report of *Botrytis mali* causing gray mold on strawberry in the United States. *Plant Disease*, 101 (6): 1034. doi: https://doi.org/10.1094/PDIS-01-17-0054-PDN.

Droby, S. & Lichter, A. (2007). Post-harvest *Botrytis* infection: etiology, development and management. In Elad, Y., Williamson, B., Tudzynski, P. & Delen, N. (eds) *Botrytis: Biology, Pathology and Control*, pp. 349-367. Dordrecht: Springer Netherlands.

Dueck, J., Morrall, R. A. A., Klassen, A. J. & Vose, J. (1981). Heat Inactivation of Sclerotia of *Sclerotinia Sclerotiorum*. *Canadian Journal of Plant Pathology*, 3 (2): 73-75. doi: https://doi.org/10.1080/07060668109501385.

EFSA (European Food Safety Authority), C. C., Luis, Medina Pastor, Paula. (2021). The 2019 European Union report on pesticide residues in food. *EFSA Journal*, 19 (4): e06491. doi: https://doi.org/10.2903/j.efsa.2021.6491.

Elad, Y., Williamson, B., Tudzynski, P. & Delen, N. (2007). *Botrytis* spp. and diseases they cause in agricultural systems – An introduction. In Elad, Y., Williamson, B., Tudzynski, P. & Delen, N. (eds) *Botrytis: Biology, Pathology and Control*, pp. 1-8. Dordrecht: Springer Netherlands.

Elad, Y., Vivier, M. & Fillinger, S. (2016). *Botrytis*, the good, the bad and the ugly. In Fillinger, S. & Elad, Y. (eds) *Botrytis – the Fungus, the Pathogen and its Management in Agricultural Systems*, pp. 1-15. Cham: Springer International Publishing.

FAOSTAT. (2021). *Food & Agricultural Organization of the United Nations - FAOSTAT Database*. Available at: http://www.fao.org/faostat/en/#data/QC (accessed: 30.03.2021).

Fernández-Ortuño, D., Chen, F. & Schnabel, G. (2012). Resistance to pyraclostrobin and boscalid in *Botrytis cinerea* isolates from strawberry fields in the Carolinas. *Plant Disease*, 96 (8): 1198-1203. doi: https://doi.org/10.1094/PDIS-12-11-1049-RE.

Fernández-Ortuño, D., Grabke, A., Bryson, P. K., Amiri, A., Peres, N. A. & Schnabel, G. (2014). Fungicide resistance profiles in *Botrytis cinerea* from strawberry fields of seven

southern U.S. States. *Plant Disease*, 98 (6): 825-833. doi: https://doi.org/10.1094/PDIS-09-13-0970-RE.
Fernández-Ortuño, D., Grabke, A., Li, X. & Schnabel, G. (2015). Independent emergence of resistance to seven chemical classes of fungicides in *Botrytis cinerea*. *Phytopathology*, 105 (4): 424-432. doi: https://doi.org/10.1094/PHYTO-06-14-0161-R.
Fillinger, S. & Walker, A.-S. (2016). Chemical control and resistance management of *Botrytis* diseases. In Fillinger, S. & Elad, Y. (eds) *Botrytis – The Fungus, the Pathogen and its Management in Agricultural Systems*, pp. 189-216. Cham: Springer International Publishing.
FRAC. (2020). *FRAC Code List 2020: Fungal control agents sorted by cross resistance pattern and mode of action (including FRAC Code numbering)*. Available at: https://www.frac.info/knowledge-database/knowledge-database.
Garrido, C., Carbú, M., Fernández-Acero, F. J., González-Rodríguez, V. E. & Cantoral, J. M. (2011). New insights in the study of strawberry fungal pathogens. *Genes Genomes Genomics*, 5 (1): 24-39.
Gulen, H. & Eris, A. (2003). Some physiological changes in strawberry (*Fragaria* × *ananassa* 'Camarosa') plants under heat stress. *The Journal of Horticultural Science and Biotechnology*, 78 (6): 894-898. doi: https://doi.org/10.1080/14620316.2003.11511715.
Hahn, M. (2014). The rising threat of fungicide resistance in plant pathogenic fungi: Botrytis as a case study. *Journal of chemical biology*, 7 (4): 133-141.
Hancock, J. F., Sjulin, T. M. & Lobos, G. A. (2008). Strawberries. In *Temperate fruit crop breeding: germplasm to genomics*, pp. 393-437: Springer Science & Business Media.
Hancock, J. F. (2012). *Plant evolution and the origin of crop species*. 3rd ed.: CAB International.
Haslestad, J. (2016). 6,5% reduksjon i jordbærarealet fra 2015 til 2016. *Norsk Frukt og Bær*, 19 (6): 7.
Hjeljord, L. G., Strømeng, G. M., Tronsmo, A., Sønsteby, A. & Stensvand, A. (2011). Attempts to reduce strawberry grey mould (*Botrytis cinerea*) in Norway using fungal antagonists. *Eur. J. Plant Sei. Biotechnol*, 5: 78-85.
Hollings, M. (1965). Disease control through virus-free stock. *Annual Review of Phytopathology*, 3 (1): 367-396.
Hu, M.-J., Cox, K. D. & Schnabel, G. (2016). Resistance to increasing chemical classes of fungicides by virtue of "selection by association" in *Botrytis cinerea*. *Phytopathology*, 106 (12): 1513-1520. doi: https://doi.org/10.1094/PHYTO-04-16-0161-R.
Hua, L., Yong, C., Zhanquan, Z., Boqiang, L., Guozheng, Q. & Shiping, T. (2018). Pathogenic mechanisms and control strategies of *Botrytis cinerea* causing post-harvest decay in fruits and vegetables. *Food Quality and Safety*, 2 (3): 111-119. doi: https://doi.org/10.1093/fqsafe/fyy016.
Hummer, K. E. & Hancock, J. (2009). Strawberry genomics: botanical history, cultivation, traditional breeding, and new technologies. In *Genetics and Genomics of Rosaceae*, pp. 413-435: Springer.
Jarvis, W. (1962). The dispersal of spores of *Botrytis cinerea* Fr. in a raspberry plantation. *Transactions of the British Mycological Society*, 45 (4): 549-559. doi: https://doi.org/10.1016/S0007-1536(62)80015-1.
Jarvis, W. R. (1977). *Botryotinia* and *Botrytis* species: taxonomy, physiology and pathogenicity-A guide to the literature.
Jutsum, A. (1988). Commercial application of biological control: status and prospects. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, 318 (1189): 357-373. doi: https://doi.org/10.1098/rstb.1988.0014.

Khanal, C., Gu, M., Peres, N. A. & Desaeger, J. A. (2020). Steam-based thermotherapy for managing nematodes in strawberry transplants. *Journal of Nematology*. doi: https://dx.doi.org/10.21307%2Fjofnem-2020-095.

Lagopodi, A. L., Cetiz, K., Koukounaras, A. & Sfakiotakis, E. M. (2009). Acetic acid, ethanol and steam effects on the growth of *Botrytis cinerea* in vitro and combination of steam and modified atmosphere packaging to control decay in kiwifruit. *Journal of Phytopathology*, 157 (2): 79-84. doi: https://doi.org/10.1111/j.1439-0434.2008.01445.x.

Latorre, B., Spadaro, I. & Rioja, M. (2002). Occurrence of resistant strains of *Botrytis cinerea* to anilinopyrimidine fungicides in table grapes in Chile. *Crop Protection*, 21 (10): 957-961. doi: https://doi.org/10.1016/S0261-2194(02)00074-1.

Legard, D., MacKenzie, S., Mertely, J., Chandler, C. & Peres, N. (2005). Development of a reduced use fungicide program for control of Botrytis fruit rot on annual winter strawberry. *Plant Disease*, 89 (12): 1353-1358. doi: https://doi.org/10.1094/PD-89-1353.

Leroch, M., Plesken, C., Weber, R. W., Kauff, F., Scalliet, G. & Hahn, M. (2013). Gray mold populations in German strawberry fields are resistant to multiple fungicides and dominated by a novel clade closely related to Botrytis cinerea. *Applied and environmental microbiology*, 79 (1): 159-167.

Li, X., Fernández-Ortuño, D., Chen, S., Grabke, A., Luo, C.-X., Bridges, W. C. & Schnabel, G. (2014). Location-specific fungicide resistance profiles and evidence for stepwise accumulation of resistance in *Botrytis cinerea*. *Plant Disease*, 98 (8): 1066-1074. doi: https://doi.org/10.1094/PDIS-10-13-1019-RE.

Lichter, A., Zhou, H. W., Vaknin, M., Dvir, O., Zutchi, Y., Kaplunov, T. & Lurie, S. (2003). Survival and responses of *Botrytis cinerea* after exposure to ethanol and heat. *Journal of Phytopathology*, 151 (10): 553-563. doi: https://doi.org/10.1046/j.1439-0434.2003.00767.x.

Lima, G., Ippolito, A., Nigro, F. & Salerno, M. (1997). Effectiveness of *Aureobasidium pullulans* and *Candida oleophila* against postharvest strawberry rots. *Postharvest Biology and Technology*, 10 (2): 169-178. doi: https://doi.org/10.1016/S0925-5214(96)01302-6.

Lurie, S. & Mitcham, E. (2007). Heat Treatments for Postharvest Pest Control: Theory and Practice In *Physiological Responses of Agricultural Commodities to Heat Treatments*, pp. 79-104.

Mackay, J. M. & Shipton, P. J. (1983). Heat treatment of seed tubers for control of potato blackleg (*Erwinia carotovora* subsp. atroseptica) and other diseases. *Plant Pathology*, 32 (4): 385-393. doi: https://doi.org/10.1111/j.1365-3059.1983.tb02852.x.

McGovern, R. J. & McSorley, R. (1997). Physical methods of soil sterilization for disease management including soil solarization. In *Environmentally Safe Approaches to Crop Disease Control*.

Mehli, L., Kjellsen, T. D., Dewey, F. M. & Hietala, A. M. (2005). A case study from the interaction of strawberry and *Botrytis cinerea* highlights the benefits of comonitoring both partners at genomic and mRNA level. *New Phytologist*, 168 (2): 465-474. doi: https://doi.org/10.1111/j.1469-8137.2005.01526.x.

Mernke, D., Dahm, S., Walker, A.-S., Lalève, A., Fillinger, S., Leroch, M. & Hahn, M. (2011). Two promoter rearrangements in a drug efflux transporter gene are responsible for the appearance and spread of multidrug resistance phenotype MDR2 in *Botrytis cinerea* isolates in French and German vineyards. *Phytopathology*, 101 (10): 1176-1183. doi: https://doi.org/10.1094/PHYTO-02-11-0046.

Mertely, J. C., MacKenzie, S. J. & Legard, D. E. (2002). Timing of fungicide applications for *Botrytis cinerea* based on development stage of strawberry flowers and fruit. *Plant Disease*, 86 (9): 1019-1024. doi: https://doi.org/10.1094/PDIS.2002.86.9.1019.

Mertely, J. C., Oliveira, M. & Peres, N. A. (2018). Botrytis fruit rot or gray mold of strawberry. *Plant disease*, 2018 (1). doi: https://doi.org/10.32473/edis-pp152-2018.

Milford, A. B. & Haukås, T. (2017). Åpning for import av epletrær og jordbærplanter: Økonomiske årsaker og konsekvenser. *NIBIO Rapport*.

Morton, V. & Staub, T. (2008). A short history of fungicides. *The American Phytopathological Society* doi: https://doi.org/10.1094/APSNETFEATURE-2008-0308.

Myresiotis, C. K., Karaoglanidis, G. S. & Tzavella-Klonari, K. (2007). Resistance of *Botrytis cinerea* isolates from vegetable crops to anilinopyrimidine, phenylpyrrole, hydroxyanilide, benzimidazole, and dicarboximide fungicides. *Plant Disease*, 91 (4): 407-413. doi: https://doi.org/10.1094/PDIS-91-4-0407.

Nes, A. (1998). *Bærdyrking*. Oslo, Norway: Landbruksforlaget.

Nes, A., Gullord, M., Alsheikh, M. & Sween, R. (2008a). *Strawberry breeding in Norway: progress and future*. VI International Strawberry Symposium 842.

Nes, A., Sønsteby, A. & Heide, O. (2008b). *Performance of everbearing strawberry cultivars in a Nordic climate*. VI International Strawberry Symposium 842.

Nes, A., Henriksen, J. K., Serikstad, G. L. & Stensvand, A. (2017). Cultivars and cultivation systems for organic strawberry production in Norway. *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science*, 67 (6): 485-491. doi: https://doi.org/10.1080/09064710.2017.1296490.

Nicot, P. C., Stewart, A., Bardin, M. & Elad, Y. (2016). Biological control and biopesticide suppression of Botrytis-incited diseases. In *Botrytis–The Fungus, the Pathogen and its Management in Agricultural Systems*, pp. 165-187: Springer.

Nielsen, K. A. G., Stensvand, A. & Haslestad, J. (2020). Fungicidresistens hos gråskimmelsoppen i småplanter av jordbær. *Norsk Frukt og Bær*, 1 (23): 26-29.

Norwegian Food Safety Authority (Mattilsynet). (2017). Available at: https://www.mattilsynet.no/language/english/plants/plant_health/imports_of_strawberry_plants.20858 (accessed: 31/03/2021).

Nunes, M., Brecht, J., Morais, A. & Sargent, S. (1995). Physical and chemical quality characteristics of strawberries after storage are reduced by a short delay to cooling. *Postharvest Biology and Technology*, 6 (1-2): 17-28. doi: https://doi.org/10.1016/0925-5214(94)00048-W.

Oliveira, M. S., Amiri, A., Zuniga, A. I. & Peres, N. A. (2017). Sources of Primary Inoculum of *Botrytis cinerea* and Their Impact on Fungicide Resistance Development in Commercial Strawberry Fields. *Plant Disease*, 101 (10): 1761-1768. doi: 10.1094/pdis-02-17-0203-re.

Opplysningskontoret for frukt og grønt (OFG). (2020). Frukt og grøntstatistikk 2019. In *Opplysningskontoret for frukt og grønt,* . Available at: https://www.frukt.no/globalassets/materiell/totaloversikten/frukt--og-grontstatistikk-2019-m-aktive-linker-rettet32.pdf (accessed: 30 April 2021).

Peng, G. & Sutton, J. (1991). Evaluation of microorganisms for biocontrol of *Botrytis cinerea* in strawberry. *Canadian Journal of Plant Pathology*, 13 (3): 247-257. doi: https://doi.org/10.1080/07060669109500938.

Petrasch, S., Knapp, S. J., van Kan, J. A. L. & Blanco-Ulate, B. (2019). Grey mould of strawberry, a devastating disease caused by the ubiquitous necrotrophic fungal pathogen *Botrytis cinerea*. *Molecular Plant Pathology*, 20 (6): 877-892. doi: https://doi.org/10.1111/mpp.12794.

Plesken, C., Weber, R. W., Rupp, S., Leroch, M. & Hahn, M. (2015). *Botrytis pseudocinerea* is a significant pathogen of several crop plants but susceptible to displacement by fungicide-resistant *B. cinerea* strains. *Applied and Environmental Microbiology*, 81 (20): 7048-7056. doi: https://doi.org/10.1128/aem.01719-15.

Prins, T. W., Tudzynski, P., von Tiedemann, A., Tudzynski, B., Ten Have, A., Hansen, M. E., Tenberge, K. & van Kan, J. A. (2000). Infection strategies of *Botrytis cinerea* and related necrotrophic pathogens. In *Fungal pathology*, pp. 33-64: Springer.

Roebroeck, E., Jansen, M. & Mes, J. (1991). A mathematical model describing the combined effect of exposure time and temperature of hot-water treatments on survival of gladiolus cormels. *Annals of Applied Biology*, 119 (1): 89-96. doi: https://doi.org/10.1111/j.1744-7348.1991.tb04846.x.

Roistacher, C., Baker, K. & Bald, J. (1957). Hot-water treatment of gladiolus cormels for the eradication of *Fusarium oxysporum* f. gladioli. *Hilgardia*, 26 (17): 659-684. doi: https://doi.org/10.3733/hilg.v26n17p659.

Rupp, S., Plesken, C., Rumsey, S., Dowling, M., Schnabel, G., Weber, R. W. & Hahn, M. (2017a). *Botrytis fragariae*, a new species causing gray mold on strawberries, shows high frequencies of specific and efflux-based fungicide resistance. *Applied and Environmental Microbiology*, 83 (9). doi: https://doi.org/10.1128/AEM.00269-17.

Rupp, S., Weber, R. W., Rieger, D., Detzel, P. & Hahn, M. (2017b). Spread of *Botrytis cinerea* strains with multiple fungicide resistance in German horticulture. *Frontiers in Microbiology*, 7: 2075. doi: https://doi.org/10.3389/fmicb.2016.02075.

Schnabel, G., Hu, M.-j. & Fernández-Ortuño, D. (2015). Monitoring resistance by bioassay: relating results to field use using culturing methods. In *Fungicide Resistance in Plant Pathogens*, pp. 281-293: Springer.

Schumacher, J. & Tudzynski, P. (2012). Morphogenesis and Infection in *Botrytis cinerea*. In Pérez-Martín, J. & Di Pietro, A. (eds) *Morphogenesis and Pathogenicity in Fungi*, pp. 225-241. Berlin, Heidelberg: Springer Berlin Heidelberg.

Smith, J. H. (1923). The killing of *Botrytis cinerea* by heat, with a note on the determination of temperature coefficient. *Annals of Applied Biology*, 10 (3-4): 335-347. doi: https://doi.org/10.1111/j.1744-7348.1923.tb05682.x.

Sønsteby, A., Heide, O., Grimsby, S. & Grimsby, L. (2004). *Out-of-season strawberry production in Norway: Yield responses of cv. Korona to photoperiod preconditioning treatment*. V International Strawberry Symposium 708.

Sønsteby, A. & Heide, O. (2017). Flowering performance and yield of established and recent strawberry cultivars (*Fragaria× ananassa)* as affected by raising temperature and photoperiod. *The Journal of Horticultural Science and Biotechnology*, 92 (4): 367-375. doi: https://doi.org/10.1080/14620316.2017.1283970.

Sowley, E. N. K., Dewey, F. M. & Shaw, M. W. (2010). Persistent, symptomless, systemic, and seed-borne infection of lettuce by *Botrytis cinerea*. *European Journal of Plant Pathology*, 126 (1): 61-71. doi: https://doi.org/10.1007/s10658-009-9524-1.

Staniland, L. (1953). Hot-water treatment of strawberry runners. *Plant Pathology*, 2 (2): 44-48. doi: https://doi.org/10.1111/j.1365-3059.1953.tb00637.x.

Statistics Norway. (2019). Available at: https://www.ssb.no/en/jord-skog-jakt-og-fiskeri/statistikker/hagebruk (accessed: 06/04/2021).

Strømeng, G. M., Hjeljord, L. G. & Stensvand, A. (2009). Relative contribution of various sources of Botrytis cinerea inoculum in strawberry fields in Norway. *Plant disease*, 93 (12): 1305-1310.

Strømeng, G. M., Skårn, M. N., Ørstad, K., Stensvand, A., Nielsen, K. A. G. & Brurberg, M. B. (2018). *Fungicide resistance in Botrytis spp. from strawberry fields in Norway*.

International Congress of Plant Pathology (ICPP) 2018: Plant Health in A Global Economy: APSNET.

Sylla, J., Alsanius, B. W., Krüger, E. & Wohanka, W. (2015). Control of *Botrytis cinerea* in strawberries by biological control agents applied as single or combined treatments. *European Journal of Plant Pathology*, 143 (3): 461-471. doi: https://doi.org/10.1007/s10658-015-0698-4.

Terry, L. A., Chope, G. A. & Bordonaba, J. G. (2007). Effect of water deficit irrigation and inoculation with *Botrytis cinerea* on strawberry (*Fragaria* x *ananassa*) fruit quality. *Journal of Agricultural and Food Chemistry*, 55 (26): 10812-10819.

Turechek, W. W. & Peres, N. A. (2009). Heat treatment effects on strawberry plant survival and angular leaf spot, caused by Xanthomonas fragariae, in nursery production. *Plant disease*, 93 (3): 299-308.

Turechek, W. W., Wang, S., Tiwari, G. & Peres, N. A. (2013). Investigating alternative strategies for managing bacterial angular leaf spot in strawberry nursery production. *International Journal of Fruit Science*, 13 (1-2): 234-245. doi: https://doi.org/10.1080/15538362.2012.698181.

Turechek, W. W., Myhrene, O., Slovin, J. & Peres, N. A. (2021). The use of aerated steam as a heat treatment for managing angular leaf spot in strawberry nursery production and its effect on plant yield. *PhytoFrontiers*, 1 (2): 104-119. doi: 10.1094/phytofr-08-20-0012-r.

van den Bosch, F. & Gilligan, C. A. (2008). Models of fungicide resistance dynamics. *Annual Review Phytopathology*, 46: 123-147. doi: https://doi.org/10.1146/annurev.phyto.011108.135838.

Van Den Bosch, F., Paveley, N., Shaw, M., Hobbelen, P. & Oliver, R. (2011). The dose rate debate: does the risk of fungicide resistance increase or decrease with dose? *Plant Pathology*, 60 (4): 597-606. doi: https://doi.org/10.1111/j.1365-3059.2011.02439.x.

van Kan, J. A. (2006). Licensed to kill: the lifestyle of a necrotrophic plant pathogen. *Trends in Plant Science*, 11 (5): 247-253. doi: https://doi.org/10.1016/j.tplants.2006.03.005.

Veloso, J. & van Kan, J. A. (2018). Many shades of grey in *Botrytis*–host plant interactions. *Trends in Plant Science*, 23 (7): 613-622. doi: https://doi.org/10.1016/j.tplants.2018.03.016.

Viswanathan, R. (2001). Different aerated steam therapy (AST) regimes on the development of grassy shoot disease symptoms in sugarcane. *Sugar Tech*, 3 (3): 83-91. doi: https://doi.org/10.1007/BF03014568.

Walker, A.-S. (2016). Diversity within and between species of *Botrytis*. In *Botrytis–the fungus, the pathogen and its management in agricultural systems*, pp. 91-125: Springer.

Wang, N.-Y., Turechek, W. W. & Peres, N. (2017). *Heat treatment as an alternative to fungicide application for control of strawberry anthracnose caused by Colletotrichum acutatum*. 2017 APS Annual Meeting: APSNET.

Wang, N.-Y., Turechek, W. W. & Peres, N. (2019). Thermotherapy via aerated steam: a safe practice to strawberry with benefits in disease control. *Plant Health 2019*.

Weber, R. W. & Hahn, M. (2011). A rapid and simple method for determining fungicide resistance in *Botrytis*. *Journal of Plant Diseases and Protection*, 118 (1): 17-25. doi: https://doi.org/10.1007/BF03356376.

Weber, R. W., Entrop, A.-P., Goertz, A. & Mehl, A. (2015). Status of sensitivity of Northern German *Botrytis* populations to the new SDHI fungicide fluopyram prior to its release as a commercial fungicide. *Journal of Plant Diseases and Protection*, 122 (2): 81-90. doi: https://doi.org/10.1007/BF03356535.

Weber, R. W. & Entrop, A.-P. (2017). Recovery of fungicide-resistant *Botrytis* isolates from strawberry nursery plants. *European Journal of Plant Pathology*, 149 (3): 739-742. doi: https://doi.org/10.1007/s10658-017-1206-9.

Wedge, D. E., Smith, B. J., Quebedeaux, J. P. & Constantin, R. J. (2007). Fungicide management strategies for control of strawberry fruit rot diseases in Louisiana and Mississippi. *Crop Protection*, 26 (9): 1449-1458. doi: https://doi.org/10.1016/j.cropro.2006.12.007.

Williamson, B., Tudzynski, B., Tudzynski, P. & Van Kan, J. A. (2007). *Botrytis cinerea*: the cause of grey mould disease. *Molecular Plant Pathology*, 8 (5): 561-580. doi: https://doi.org/10.1111/j.1364-3703.2007.00417.x.

Zuniga, A. & Peres, N. (2017). *Heat treatment for management of Botrytis cinerea inoculum on strawberry*. 2017 APS Annual Meeting: APSNET.

Zuniga, A. I. (2018). *'Sensitivity of Botrytis cinerea to Succinate Dehydrogenase Inhibitor (SDHI) fungicides and to heat treatment'*: University of Florida. Unpublished manuscript.

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