Review

Grey mould of strawberry, a devastating disease caused by the ubiquitous necrotrophic fungal pathogen *Botrytis cinerea*

STEFAN PETRASCH¹, STEVEN J. KNAPP¹, JAN A. L. VAN KAN² AND BARBARA BLANCO-ULATE ¹,*

¹Department of Plant Sciences, University of California, Davis, Davis, CA USA
²Laboratory of Phytopathology, Wageningen University, Wageningen, Netherlands

SUMMARY

The fungal pathogen *Botrytis cinerea* causes grey mould, a commercially damaging disease of strawberry. This pathogen affects fruit in the field, storage, transport and market. The presence of grey mould is the most common reason for fruit rejection by growers, shippers and consumers, leading to significant economic losses. Here, we review the biology and epidemiology of the pathogen, mechanisms of infection and the genetics of host plant resistance. The development of grey mould is affected by environmental and genetic factors; however, little is known about how *B. cinerea* and strawberry interact at the molecular level. Despite intensive efforts, breeding strawberry for resistance to grey mould has not been successful, and the mechanisms underlying tolerance to *B. cinerea* are poorly understood and under-investigated. Current control strategies against grey mould include pre- and postharvest fungicides, yet they are generally ineffective and expensive. In this review, we examine available research on horticultural management, chemical and biological control of the pathogen in the field and postharvest storage, and discuss their relevance for integrative disease management. Additionally, we identify and propose approaches for increasing resistance to *B. cinerea* in strawberry by tapping into natural genetic variation and manipulating host factors via genetic engineering and genome editing.

Keywords: disease management, fruit ripening, fruit-pathogen interaction, plant breeding, plant defence, primary infection, secondary infection.

INTRODUCTION

Strawberry (*Fragaria × ananassa*) is an important soft fruit crop that is grown worldwide on more than 370 000 hectares (FAO STAT, 2014) and, for the United States alone, the total value of the annual strawberry production exceeds US$2.3 billion (USDA, 2016). Strawberries are beneficial to the human diet as a source of macro- and micronutrients, vitamins and health promoting antioxidants (Basu et al., 2014; Giampieri et al., 2015; Wang and Lin, 2000).

Strawberry is a perennial herbaceous plant with short stems (crowns) and densely spaced leaves. Strawberry produces complex accessory and aggregate fruit composed of achenes and a receptacle (Darrow, 1966). Achenes are small single-seeded fruit, whereas the receptacle is considered to be anatomically equivalent to floral meristem tissue (Hollender et al., 2012). *F. × ananassa* is an allo-octoploid (2n = 8x = 56) that originated as a synthetic hybrid between the octoploid species *Fragaria chiloensis* and *Fragaria virginiana* (Brinthurst, 1990; Edger et al. 2019; Darrow, 1966; Rousseau-Gueutin et al., 2008).

Strawberry is affected by several pathogens including fungi, bacteria, viruses and nematodes. The most economically impactful pathogens of strawberry are fungi, which can infect all parts of the plant and cause severe damage or death (Garrido et al., 2011). Amongst the fungal pathogens, the ascomycete *Botrytis cinerea* is considered the primary pathogen of harvested strawberries in the world leading to impactful economical losses to the strawberry industry. *B. cinerea* causes grey mould in fruit and senescing organs but can also affect vegetative tissues (Fig. 1). Under wet conditions, more than 80% of strawberry flowers and fruits can be lost if plants are not sprayed with fungicides (Ries, 1995).

THE PATHOGEN *BOTRYTIS CINEREA*

*B. cinerea* has no apparent host specificity and can infect more than 1000 plant species (Elad et al., 2016). The pathogen is found worldwide and causes disease in many fruit, flower and leafy vegetable crops (Boff, 2001; Carisse, 2016; Elad et al., 2007). *B. cinerea* is classified as a necrotroph, meaning that it prefers to infect and grow on damaged or senescing...
tissues, eventually causing tissue death. The inoculum (e.g. conidia) of the fungus is highly abundant and ubiquitous and usually comes from infected plant tissues (Jarvis, 1962). B. cinerea mainly enters the host via wounds or natural openings (Holz et al., 2007). Infections of non-senescing or unripe plant organs usually lead to limited damage and quiescent infections (Dewey and Grant-Downton, 2016; Jarvis, 1962). Different types of quiescence have been described: (i) delay of conidia germination or growth arrest after germination (Jarvis, 1994), (ii) endophytic symptomless growth in the apoplast (Barnes and Shaw, 2003; Sowley et al., 2010), (iii) colonization of abscising flower organs (e.g. petals) followed by growth into ovaries or receptacles where growth arrests (Bristow et al., 1986). Independent of the type of infection, the pathogen generally enters a short asymptomatic, biotrophic phase at the beginning of the disease cycle (Veloso and van Kan, 2018). An aggressive necrotrophic phase commonly succeeds the quiescent or asymptomatic phase once plant organs start to senesce or ripen, during which B. cinerea causes rapid decay of the infected tissues (Elad et al., 2007).

B. cinerea’s infection mechanisms have been studied in model organisms and further characterized thanks to the availability of high-quality reference genome sequences (Amselem et al., 2011; Van Kan et al., 2017; Staats and van Kan, 2012). The fungus is known to actively promote plant susceptibility by employing a variety of virulence factors (Choquer et al., 2007; Nakajima and Akutsu, 2014; Petrasch et al., 2019). In early stages, B. cinerea deploys sRNAs and effector proteins to suppress premature host cell death and immune responses, which enables the fungus to establish inside the host and accumulate biomass prior to the necrotrophic phase (Veloso and van Kan, 2018). It was demonstrated that B. cinerea Dicer-like proteins DCL1 and DCL2 produce sRNAs that are secreted from fungal hyphae and translocated to the plant cell where they interfere with the host RNAi mechanisms to silence host immune response genes in Arabidopsis and tomato leaves (Wang et al., 2017b; Weiberg et al., 2013).

Some secreted virulence factors can lead to host cell death, like effector proteins, toxins and enzymes involved in reactive oxygen species (ROS) production (Schumacher, 2016). B. cinerea can also secrete oxalic acid that lowers the pH of the host tissues.
and stimulates the production and activity of fungal enzymes like pectinases, laccases and proteases (Fernández-Acero et al., 2010; Manteau et al., 2003; Prusky and Lichter, 2007; Sharon et al., 2007). Furthermore, oxalic acid accumulation leads to Ca\(^{2+}\) chelation, which in turn weakens the pectin structures of plant cell walls and inhibits the deposition of callose (Chakraborty et al., 2013). Other virulence factors are cell wall degrading enzymes (CWDEs) that enable *B. cinerea* to cause plant cell lysis and loosen walls to facilitate tissue penetration (Blanco-Ulate et al., 2016a). The fungus is known to produce plant hormones or hormone analogues that may disturb the host's cellular metabolism and immune responses. The relevance of these mechanisms for the capacity of *B. cinerea* to infect strawberry remains unknown.

**STRAWBERRY - BOTRYTIS CINEREA PATHOSYSTEM**

Grey mould in strawberries can result from *B. cinerea* infections of open flowers (primary infections) or by penetration of fruit receptacle tissues (secondary infections) (Bristow et al., 1986). In primary infections, *B. cinerea* infects flower organs during or right after flowering, allowing hyphae to grow into the receptacle (Fig. 2). The sources of primary inoculum range from overwintering sclerotia to conidia or mycelium from infected neighbouring plants (Jarvis, 1962). Infected senescent petals, stamens and calyces can facilitate primary infections in fruit (Powelson, 1960). Histological studies have shown that even though styles are frequently infected, fungal growth appears to be strongly inhibited and never reaches the receptacle. In contrast, fungal growth in colonized stamens can reach the receptacle in some cultivars (Bristow et al., 1986).

Following infection of the unripe receptacle by *B. cinerea*, fungal growth is usually arrested and a symptomless quiescent phase occurs. The mechanisms that lead to quiescent infections are not yet fully understood. Proanthocyanins (PAs) appear to induce *B. cinerea* quiescence in unripe fruit by restricting the activity of fungal enzymes like polygalacturonases (PGs) that are necessary for aggressive infection of hosts (50% inhibition in unripe fruit compared to 8% inhibition in ripe fruit). Even though PA content in fruit remains constant during ripening, increasing polymerization of PAs leads to lower inhibitory activity in ripe fruit (Jersch et al., 1989). Similarly, anthocyanins might delay *B. cinerea* infections or cause quiescence (van Baarlen et al. 2007). For instance, strawberries illuminated with white fluorescent light showed increased anthocyanin content and delayed

![Fig. 2 Botrytis cinerea disease cycle in strawberry. Sources of *B. cinerea* inoculum include infected leaves and sclerotia. Primary infections of flowers and secondary infections of fruit are depicted.](image-url)
development of grey mould (Saks et al., 1996). Reduced fruit decay has also been observed in raspberries with high pigmentation (Harshman et al., 2014) and in transgenic tomatoes that accumulate anthocyanins (Bassolino et al., 2013; Zhang et al., 2013). Other small phenolics, especially catechins, may have a role in quiescence. High levels of catechins inhibit fungal growth, and a decrease in catechins is correlated with a reduction of other antifungal compounds such as lipooxygenases (Prusky and Lichter, 2007). Interestingly, young and ripe fruit have low catechin concentration, suggesting that initial infections of young receptacles are possible because they do not yet accumulate enough catechins to stop colonization (Pühl and Treutter, 2008).

B. cinerea quiescence is complex and involves additional factors besides the accumulation of phenolic compounds. It has been proposed that quiescence in unripe fruit is initiated by: (i) lack of nutrients such as sugars (e.g. mono- and disaccharides) from the host, (ii) presence of preformed antifungal compounds, (iii) unsuitable environment for fungal virulence factors (Prusky and Lichter, 2007). In unripe strawberries, factors from all three categories are present, including lack of available sugars (Knee et al., 1977), preformed antifungal compounds (Hébert et al. 2002; Terry et al., 2004), and high activity of PG-inhibiting proteins (PGIPs) (Mehli et al., 2005). Induction of the necrotrophic phase in ripe strawberries could be triggered by changes in biochemical composition of the host tissues associated with the ripening process, such as increased sugar content, volatile production and alteration of plant defences (Neri et al., 2015; Prusky and Lichter, 2007). These modifications promote not only fungal growth but also host susceptibility, e.g. via the release of oxalic acid and efflux of toxins (Prusky and Lichter, 2007).

During secondary infections, the fungus initiates the necrotrophic phase without quiescence (Holz et al., 2007). The sources of conidia for secondary infections can also be diverse, from senescent leaves to infected fruit (Fig. 2). Conidia from B. cinerea-infected flower parts are major sources of secondary inoculum (Bristow et al., 1986). It has been estimated that more than 64% of the strawberry infections result from organic fragments that are in contact with the fruit, such as petals and stamens (Fig. 1D; Jarvis, 1962). Contrary to other fruit (e.g. raspberries), senescent flower parts often adhere to strawberries long enough to retain water films for at least 8 h, which is the time needed for B. cinerea conidia germination (Jarvis, 1962).

Secondary infections can also result from nesting, which corresponds to direct penetration of mycelia growing on neighbouring plant organs such as infected leaves and fruit (Fig. 1F; Braun and Sutton, 1988). Generally, secondary infections proceed rapidly and B. cinerea can complete its germination and infection as fast as 16 h post-inoculation (hpi) with a rapid increase in fungal biomass at 48 hpi (Fig. 3; Mehli et al., 2005). Early responses of strawberries to infection include higher expression of the defence genes FaPGIP and FaChi 2-1 (Class II Chitinase), whereas lower expression of the reference gene DNA Binding Protein – FaDBP indicates extensive cell death induced by B. cinerea at late stages of infection (Mehli et al., 2005).

RELEVANCE OF RIPENING PROCESSES TO BOTRYTIS CINEREA INFECTIONS OF STRAWBERRIES

Fruit ripening influences the susceptibility of strawberry fruit to B. cinerea (Fig. 4). Strawberries are mostly resistant to infection in their unripe stage, where they restrict fungal growth by causing quiescence. However, in the ripe stage, strawberries are highly susceptible and decay rapidly. Fruit susceptibility to fungal disease increases as ripening progresses; hence, B. cinerea appears to promote susceptibility in unripe fruit by activating specific ripening-related processes (Blanco-Ulate et al., 2016b). In tomato fruit, master transcriptional regulators of ripening have been shown to have different roles in disease susceptibility. For example, the activity of the tomato transcription factor NON-RIPENING (NOR) favours B. cinerea infection (Cantu et al., 2009). Strawberries are non-climacteric fruit with a ripening programme different from that of climacteric tomatoes. Thus, a deeper understanding of strawberry ripening regulation and how B. cinerea may modulate particular ripening events are pivotal to characterize the dynamics of the strawberry-B. cinerea pathosystem.

Recent transcriptomic studies of developing strawberries point out that ripening events start between the ‘large green’ and ‘white’ stages, and involve changes in cell wall composition, sugar metabolism, hormone biosynthesis and responses, pigmentation and antioxidant levels (Guo et al., 2018; Sanchez-Sevilla et al., 2017; Wang et al., 2017a). Moreover, a general decrease of oxidative phosphorylation processes has been observed during strawberry ripening (Sanchez-Sevilla et al., 2017; Wang et al., 2017a). Normal strawberry ripening involves a variety of biochemical and physiological processes, some of which are discussed below in the context of B. cinerea interactions.

![Fig. 3 Progression of Botrytis cinerea infection in ripe strawberries. Inoculation was performed by wounding the fruit and adding a B. cinerea conidia suspension on the surface of the wound. Fruit are shown immediately after inoculation, and at 24 h to 96 h post-inoculation (hpi). Wounded controls are included.](image-url)
Cell wall modifications

Ripening is associated with the disassembly of the fruit cell walls, which leads to tissue softening. Cell wall degradation benefits *B. cinerea* as it reduces mechanical barriers to infection and spread, increases the possibilities of bruising (e.g. leading to more wounds for pathogen entry) and provides the fungus with access to simple sugars as a carbon source (Blanco-Ulate et al., 2016b; Blanco-Ulate et al., 2016a; Brummel and Harpster, 2001).

In strawberry, cell wall solubilization occurs early in fruit development when the walls start to swell (Knee et al., 1977). Cell wall solubilization correlates with an increase in fruit sugar content, resulting from polysaccharide breakdown. A decrease of acid-soluble pectins and the alcohol-insoluble fraction of cell walls occur during ripening, whereas the water-soluble content increases (i.e. enriched in non-covalent bound pectins). The degree of pectin solubilization and depolymerization is highly-related to strawberry fruit firmness (Rosli et al., 2004). Silencing of an endogenous pectin lyase (PL) gene in strawberry resulted in fruit with higher external and internal firmness, mostly due to low pectin solubilization, stiffer cell walls, and increased cell to cell adhesion (Jimenez-Bermudez et al., 2002; Santiago-Domenech et al., 2008). Besides PL, other enzymes that may have affected strawberry firmness include PGs, β-galactosidases, endoglucanases, α-arabinofuranosidases and β-xylosidases (Figueroa et al., 2010).

In addition to the fruit endogenous cell wall disassembly, *B. cinerea* secretes an extensive array of CWDEs that target most polysaccharides in the fruit cell walls, particularly pectins (Blanco-Ulate et al., 2016a). These CWDEs include fungal PGs, such as Bcpg2, a gene that is mainly active in the early penetration stage (Mehli et al., 2005). The expression of *B. cinerea* PGs is dependent on the host species, the plant tissue, temperature and the stage of infection (Blanco-Ulate et al., 2014; ten Have et al., 2001).

Cuticle changes

Another barrier for *B. cinerea* infection is the fruit cuticle. During fruit expansion and ripening the cuticle gets thinner, which makes strawberries more susceptible to initial penetration by germinating conidia. *B. cinerea* can penetrate the plant cuticle by secretion of cutinases (van Kan, 2006). Additionally, cuticle properties can result in higher incidence of cracks and other damages through which *B. cinerea* can enter the fruit without the need of cutinases (Holz et al., 2007). Studies on strawberry cuticles are scarce and only exist for leaf tissues (Kim et al., 2009). In tomato fruit, thicker and stiffer cuticles lead to higher resistance to initial
B. cinerea infections. Moreover, it is known that the chemical composition of the cuticle changes during tomato ripening, and this is likely to be the case in strawberry (Isaacson et al., 2009; Kosma et al., 2010).

**Sugar accumulation**

During ripening, the content of sugar in strawberries increases and therefore can serve as nutrients for B. cinerea. In unripe strawberries, the main sugars are glucose and fructose with low concentrations of sucrose. Sucrose levels increase rapidly during de-greening and red colouring (Jia et al., 2011). In tomato, it has been shown that the Cnr mutant, which does not accumulate high levels of sugars is still highly susceptible to B. cinerea infection (Blanco-Ulate et al., 2016b). This observation suggests that even though sugars may serve as a susceptibility factor, high sugar concentrations are not essential for B. cinerea infection. However, sugar content could still influence susceptibility to B. cinerea as specific sugars may serve as ripening initiation signals. For instance, sucrose regulates abscisic acid (ABA) levels in strawberries, which are necessary for normal ripening and could influence fruit susceptibility as described below (Blanco-Ulate et al., 2016b; Jia et al., 2011; Li et al., 2011). Like other ripening-related events, B. cinerea can alter neutral sugar and sugar acid levels in the infected host tissues, mainly by degradation and depolymerization of cell walls. This was reported for infections in tobacco and Arabidopsis leaves, where the fungus degrades pectins to release the monosaccharide galacturonic acid (Zhang and van Kan, 2013).

**Plant hormone biosynthesis and signalling**

ABA is the main hormone regulating and inducing ripening in strawberries (Jia et al., 2016; Li et al., 2011). ABA biosynthesis during fruit ripening is triggered by a decrease in pH, turgor changes, sugar accumulation, and the switch of sugars from mainly glucose and fructose to sucrose (Jia et al., 2011; Li et al., 2011). Effects of ABA on strawberry susceptibility to fungal disease have not been extensively studied, but down-regulation of the ABA biosynthetic gene β-glucosidase FaBG3 has been reported to result in fruit with limited ripening and higher B. cinerea resistance (Li et al. 2013). In tomato, ABA accumulation is related to higher pathogen susceptibility, probably via activation of senescence (Blanco-Ulate et al., 2013; Harrison et al., 2011; Lee et al., 2011). During strawberry ripening, the increase of ABA is correlated with a decrease of auxin, which induces early fruit growth and expansion but is known to inhibit ripening processes (Jia et al., 2011). The role of auxin in fruit susceptibility seems to depend on the plant species, as indole acetic acid (IAA) treatment in Arabidopsis leads to susceptibility, whereas IAA-treated tomato leaves and eggplant fruit show lower infection severity (Savatin et al., 2011; Sharon et al., 2007). Ethylene has a secondary organ-specific role in strawberry ripening, particularly in achenes and green and white receptacles (Knee et al., 1977; Merchante et al., 2013). Ethylene increases the susceptibility of tomato to B. cinerea by inducing ripening; however, its functions during strawberry infections are yet to be fully characterized. ABA, IAA and ethylene accumulation are altered by polyamine levels, which are positively correlated with fruit susceptibility to B. cinerea during strawberry ripening (Guo et al., 2018). Other hormones, such as brassinosteroids (BRs) and jasmonic acid (JA) are present at lower levels during strawberry ripening. BR positively regulates vitamin C levels, sugar and anthocyanin biosynthesis during ripening, while negatively regulating acidity and concentration of other phenolic compounds (Ayub et al., 2018). JA acts synergistically with ethylene by activating its biosynthesis in strawberries (Mukkun and Singh, 2009). Endogenous JA levels are modulated by methyl jasmonate (MeJA) and the JA carboxyl methyltransferase that lead to high levels in white fruit and a decline during ripening, antagonistically to ABA (Garrido-Bigotes et al., 2018; Preuß et al., 2014). In strawberry, JA appears to be involved in defence responses against B. cinerea. For example, strawberries treated with MeJA had a delayed and much slower progression of B. cinerea infections (Saavedra et al., 2017; Zhang et al., 2006).

**HIJACKING OF RIPENING REGULATION BY BOTRYTIS CINEREA**

As indicated previously, B. cinerea releases enzymes and metabolites that act as virulence factors but may also induce plant responses that are beneficial for fungal infection (van Kan, 2006). A relevant example of the manipulation of physiological processes in the host by B. cinerea is the interference with specific developmental processes. In tomato plants, B. cinerea infections modified host gene expression to increase susceptibility, such as the induction of senescence in leaves (Swartzberg et al., 2008). Moreover, infected unripe tomato fruit show premature expression of genes involved in ethylene synthesis during tomato ripening (Blanco-Ulate et al., 2013; Cantu et al., 2009). These findings suggest that B. cinerea could initiate ethylene production and thereby stimulate early ripening. As strawberries are non-climacteric fruit, ethylene production of B. cinerea may not have substantial effects on strawberry ripening; however, the fungus was also shown to induce genes involved in the biosynthesis of other plant hormones such as ABA. Moreover, B. cinerea can synthesize and secrete ABA that functions as a virulence factor (Siewers et al., 2004, 2006). Besides hormones, increased oxidative reactions caused by the pathogen may influence ripening progression (Bianco et al., 2009; Wang et al., 2017a).
MECHANISMS OF DEFENCE AND AVOIDANCE AGAINST BOTRYTIS CINEREA

Defence mechanisms can be divided into preformed and induced defences. In strawberries, preformed defence compounds are especially abundant in the unripe stage, as reviewed in the section on quiescence of *B. cinerea*. Even though plants accumulate defence compounds, *B. cinerea* has mechanisms to cope with these metabolites by efflux and detoxification of inhibitory substances. ATP-binding cassette (ABC) transporters are used by *B. cinerea* to facilitate the efflux of antifungal compounds, such as stilbenes (Schoonbeek et al., 2002; Schoonbeek et al., 2001). *B. cinerea* is capable of detoxifying inhibitory substances, like epicatechin by secretion of laccases (Amil-Ruiz et al., 2011; Staples and Mayer, 1995).

Active *B. cinerea* infections can result in a reduction of specific secondary metabolites. It has been reported that levels of flavan-3-ol, benzoic acid and phenylpropanoids drop in *B. cinerea*-infected strawberries (Nagpala et al., 2016). Strawberries respond to *B. cinerea* infection by triggering defences. In some cases, preformed and induced defences can overlap such as in the case of PGIPs. An endogenous PGIP appears to be constitutively expressed in fruit from various strawberry cultivars (Mehli et al., 2004). However, this PGIP and six additional ones show higher expression levels upon infection with *B. cinerea* (Schaart et al., 2005). Overexpression of FaPGIP1a and FaPGIP2a in cisgenic plants conferred enhanced resistance to grey mould (Schaart, 2004). Other enzymes induced by *B. cinerea* infections are chitinases. Expression of the chitinases FaChi2-1 and FaChi2-2 peaked 16 hpi in *B. cinerea*-infected strawberries (Mehli et al., 2005). Furthermore, heterologous expression of *Phaseolus vulgaris* chitinase ChSB in strawberry resulted in higher resistance to infection (Vellicce et al. 2006). Another study demonstrated that application of heat-inactivated cells of the yeast *Aureobasidium pullulans* promoted tolerance to *B. cinerea* in strawberries (Adikaram et al., 2002). This primed resistance is probably due to the fruit’s perception of chitin from the yeast leading to induction of chitinases or other plant immune responses. Moreover, fruit defence responses may be primed using mechanical stimulation as it was reported for strawberry leaves (Tomas-Grau et al., 2017).

Induced defences include accumulation of secondary metabolites and ROS. For instance, strawberries accumulate proanthocyanins around infection zones possibly to restrict fungal growth (Feucht et al., 1992; Jersch et al., 1989). The surroundings of infection sites generally display higher ROS production (Tomas-Grau et al., 2017). ROS can serve as an effective defence against pathogens but also can lead to cell death, which is considered beneficial for necrotrophic fungi (Prusky and Lichter, 2007).

*B. cinerea* itself produces ROS to induce host cell death, deplete plant antioxidants and increase lipid peroxidation (van Kan, 2006). It is therefore interesting that, in unripe tomato fruit ROS production leads to resistance against *B. cinerea*, whereas in ripe fruit it seems to promote susceptibility (Cantu et al., 2008, 2009). Future research will likely shed more light on the role of ROS in induced defences of strawberry fruit.

Basal immunity is activated upon fungal infection. Degradation of fruit cell wall pectins can produce demethylated oligogalacturonides that trigger basal immune responses (Amil-Ruiz et al., 2011). Expression of the *F. x ananassa* pectin methylsterase 1 FaPE1 in *Fragaria vesca* resulted in reduced methyl-esterification of oligogalacturonides in fruit. This reduced esterification activated basal defences via the salicylic acid (SA) signalling pathway that led to a higher resistance to *B. cinerea* (Osorio et al., 2011). Involvement of SA signalling in responses against *B. cinerea* was previously suggested when strawberry plants and fruit treated with SA showed decreased postharvest decay (Babalar et al., 2007). *B. cinerea* can suppress the expression of plant defence responses by hijacking the host sRNA regulatory pathways (Weiberg et al., 2013). In strawberry fruits, *B. cinerea* infections can alter the expression of microRNAs involved in the regulation of defence genes, including the plant intracellular Ras group-related LRR protein 9-like gene (Liang et al. 2018). Interestingly, *B. cinerea* can also take up plant sRNAs during its interaction with the host. For instance, transgenic plants expressing sRNA that targets *B. cinerea* DCL1 and DCL2 show significantly reduced fungal growth in strawberries (Wang et al., 2016). The suppression of fungal growth via host sRNA is not well understood, and it is yet to be demonstrated that this mechanism of defence naturally occurs in plants.

VARIATION OF QUANTITATIVE RESISTANCE TO BOTRYTIS CINEREA IN STRAWBERRY

The diverse arsenal of infection mechanisms employed by *B. cinerea* explains its extremely wide-host range. It is therefore not surprising that entirely resistant strawberry genotypes do not exist (Bestfleisch et al., 2015; Bristow et al., 1986). Several authors have analysed field resistance of strawberries to *B. cinerea* by quantifying disease development without artificial inoculation. A multi-year study of three strawberry cultivars found a significant effect of year, cultivar and cultivar by year interaction on the incidence of *B. cinerea* infections (Rhainds et al., 2014, 2002). Moreover, there was a positive correlation between row density and disease. Other studies investigated field resistance in annual winter production systems and found that variation of *B. cinerea* incidence between years was larger than genotype differences within years (Chandler et al., 2004; Seijo et al., 2008). Even though field resistance assessments investigate conditions similar to commercial production, considerable variability between environmental conditions and years can interfere with the detection of genotype differences.
Due to the confounding effects of different non-genetic variables in field studies, assessment of postharvest resistance to *B. cinerea* infections has been pursued to determine genotype differences between strawberry cultivars or species. A large study of grey mould development during postharvest storage of non-inoculated fruit reported variation in disease incidence and speed of progression amongst cultivars, but no complete resistance was observed (Lewers et al., 2012). Another approach to reducing environmental effects in disease tests is to inoculate fruit with *B. cinerea* conidia suspensions. Bestfleisch et al. (2015) tested quantitative resistance in 107 accessions of wild and cultivated strawberry. In this study, two wild ecotypes of *F. virginiana* showed high resistance to *B. cinerea* infections and slow disease progression. Such high tolerance in wild species was also reported in *B. cinerea*-inoculated leaves and fruit of *F. chiloensis* accessions from Chile (González et al., 2009). In these wild accessions, *B. cinerea* grew much slower. Comparative studies of disease progression indicated that fruit from the cultivar Chandler developed lesions at 24 hpi, while fruit from an *F. chiloensis* ecotype developed symptoms at 72 hpi (González et al., 2013). Fruit were entirely covered with mould at 6 days post-infection (dpi) for the cultivar Chandler and at 9 dpi for the *F. chiloensis* ecotype.

Considering that some accessions, particularly wild ecotypes, show reduced grey mould incidence and progression, there might be genetic sources of resistance against *B. cinerea* that could be used to increase resistance in strawberry. However, information about resistance mechanisms is mostly based on assumptions or empirical data. Differences in ripening patterns have been suggested as a potential explanation for resistance. For instance, some strawberries ripen from inside to outside, leaving the skin, which is the entry point of infections, unripe and thus resistant for a longer time (Jersch et al., 1989). Some more tolerant cultivars remain white or unripe around the calyx (white shoulders), which is where many *B. cinerea* infections tend to initiate. Another mechanism of resistance could be the presence of fungal inhibitors or the induction of PR proteins. *FcpR5* and *FcPR10* are highly induced in resistant *F. chiloensis* accessions when compared to commercial *F. x ananassa* cultivars (González et al., 2013). Based on sequence homology, *FcPR5* probably possesses antifungal activity, and *FcPR10* is likely a ribonuclease. These findings reflect that even though efforts have been made to explore resistance mechanisms of strawberry to *B. cinerea*, very little is known. Therefore, more research is necessary to better understand the biology of strawberry interactions with *B. cinerea* infections using diverse germplasm accessions.

**CURRENT AND NEW MANAGEMENT APPROACHES FOR BOTRYTIS CINEREA IN STRAWBERRY PRODUCTION**

Many disease management strategies have been implemented for the control of *B. cinerea* in strawberry as further described below. However, even combined approaches are only capable of reducing disease incidence and severity but cannot completely prevent or eliminate grey mould in strawberries (Feliziani and Romanazzi, 2016).

**Agronomic and horticultural practices**

Historically, *B. cinerea* infections in strawberry production have been managed by agronomic and horticultural practices, such as removal of senescent plant material to avoid inoculum buildup (Daugaard, 1999). Preventing contact of fruit with soil (e.g. covering the planting beds with polyethylene foils) is another common practice to avoid *B. cinerea* infections, as most of the inoculum is present on the ground and soil moisture promotes conidia germination (Daugaard, 1999). Selecting the right irrigation system could help reduce grey mould incidence; mainly, the use of drip irrigation and micro-sprinklers results in limited inoculum spread and reduction of water films on the fruit (Dara et al., 2016; Terry et al., 2007). As canopy characteristics influence microclimates (e.g. humidity, airflow, contact between plants), nitrogen fertilization can lead to dense canopies and favour grey mould (Daugaard, 1999). Similarly, shorter plant spacings promote higher incidence of *B. cinerea* in the field (Legard et al., 2005). Additionally, plastic tunnels can avoid airborne inoculum and *B. cinerea* incidence is lower in non-fungicide treated tunnels than in fungicide treated fields (Xiao et al., 2001), but tunnels favour powdery mildew and complicate harvest. In summary, cultural practices are essential to limit preharvest *B. cinerea* infections of strawberries, especially in organic agriculture.

**Fungicides**

In modern production, pesticide applications are the most common management practice for *B. cinerea* control (see Table 1). In the previous two decades, the main pesticides used in strawberry production against *B. cinerea* belonged to the Fungicide Resistance Action Committee (FRAC) Groups 1 and 2, as well as captan (Sutton, 1990; Wedge et al., 2007, 2013). However, due to increasing fungicide resistance and new legal restrictions, producers have been forced to diversify their fungicide regimen (Vellicce et al., 2006; Wedge et al., 2013). The frequency and timing of fungicide applications are crucial for *B. cinerea* control. One application of fenhexamid (FRAC 17) at anthesis can be as efficient as multiple weekly applications (Mertely et al., 2002). Additionally, alternation and combination of different fungicides with different modes of action are recommended (Wedge et al., 2007).

Resistance of *B. cinerea* to fungicides is a real challenge in horticulture and fungicide resistance profiles can shift considerably even within a single season (Cosseboom et al., 2019; Konstantinou et al., 2015; Leroch et al., 2013; Wedge et al., 2007). A screen of 13 *B. cinerea* isolates in Louisiana (USA)
showed that all were partial to full resistance to FRAC 1 fungicides, and several of the isolates also had different levels of resistance to FRAC 2 fungicides (Wedge et al., 2013). A larger survey of 1890 B. cinerea isolates (189 fields in 10 states of the USA) revealed that 7 isolates from different locations were resistant to all single-action site FRAC fungicides groups that are registered for B. cinerea control (Fernández-Ortuño et al., 2015). B. cinerea resistance to fungicides is usually associated with overexpression of efflux transporters or with modification of fungicide targets. These resistance mechanisms are acquired via mutations and recombination that occur frequently in B. cinerea due to heterokaryosis, sexual reproduction and the presence of abundant transposable elements in its genome (Konstantinou et al., 2015). Efflux of fungicides or accumulation of altered fungicide targets has also been shown to lead to multi-resistances (Konstantinou et al., 2015; Rupp et al., 2017). The presence of resistant isolates against the most common multi-action site fungicides reinforces the need for innovative management practices. A new generation of RNA-based fungicides has been proposed, which relies on the application of sRNA or dsRNAs that target B. cinerea virulence genes to reduce fungal infections in strawberries (Wang et al., 2016). However, these RNA-based fungicides remain far from commercialization, which is why fungicide resistance management such as mixture and rotation of different fungicides or testing local isolates for resistance is necessary (Hahn, 2014).

### Biological control

To date, B. cinerea biocontrol products are mostly Bacillus subtilis-based, but their use in commercial strawberry production is limited because of their insufficient applicability in the field or supply chain (Pertot et al., 2017). Nevertheless, there is social and scientific interest in using biocontrol against B. cinerea as an alternative to chemical pesticides. Isolates of Colletotrichum gloeosporioides, Epicoccum purpurascens, Gliocladum roseum, Penicillium sp., Trichoderma sp. have displayed high efficiency in controlling B. cinerea and were reported to reduce grey mould incidence on strawberry stamens by 79%–93% and on fruit by 48%–76% (Peng and Sutton, 1991). Interestingly, in some experiments, the efficiency of biocontrol by these organisms exceeded the efficacy of control via the fungicide captan. Similar results were obtained for other microbes, such as the yeasts A. pullulans (Adikaram et al., 2002) and Candida intermedia (Huang et al., 2011), the filamentous ascomycete Ulocladium roseum (Boff, 2001; Boff et al., 2002a, 2002b), or the bacterium Bacillus amyloliquefaciens (Sylla et al., 2015).

Biocontrol via microbes can work via different modes of action, including competition for nutrients, secretion of antibiotic compounds and induction of host defence mechanisms like the up-regulation of chitinase and peroxidase activity (Adikaram et al., 2002; Ippolito et al., 2000; Lima et al., 1997; McCormack et al., 1994). Because biocontrol of B. cinerea

| FRAC code | FRAC group | Target site | Target action | Risk of resistance | Example |
|-----------|------------|-------------|---------------|--------------------|---------|
| FRAC 1    | Benzimidazoles | β-tubulin assembly in mitosis | Cytoskeleton | High | Benomyl |
| FRAC 2    | Dicarboximides | MAP/histidine kinase in osmotic signal transduction (os-1, Daf1) | Signal transduction | Medium to high | Iprodione |
| FRAC 7    | Succinate dehydrogenase inhibitors | Succinate dehydrogenase | Respiration | Medium to high | Boscalid |
| FRAC 9    | Anilinopyrimidines | Methionine synthesis | Amino acid and protein synthesis | Medium | Cyprodinil |
| FRAC 11   | Quinone outside inhibitors | Cytochrome Bc1 at Qo Site | Respiration | High | Azoxystrobin |
| FRAC 12   | Phenylpyroles | MAP/histidine kinase in osmotic signal transduction (os-2, HOG1) | Signal transduction | Low to medium | Fludioxonil |
| FRAC 17   | Sterol biosynthesis inhibitors class III | 3-Keto reductase in C4 de-methylation | Inhibition of sterol biosynthesis in membrane | Low to medium | Fenhexamid |
| FRAC M03  | Dithiocarbamates and relatives | Multi-site mode of action | Low | Thiram |
| FRAC M04  | Phthalimides | Multi-Site Mode of Action | Low | Captan |
relies on a variety of mechanisms, the most significant effects are observed when different organisms are applied in combination (Sylla et al., 2015; Xu and Jeger, 2013). As alternative to applying living microbes, use of extracts or volatiles derived from biocontrol microbes has been suggested (Huang et al., 2011). Use of non-synthetic antifungal substances, like phenol-rich olive oil mill wastewater, has also been reported to control B. cinerea growth in vitro and on strawberries (Vagelas et al., 2009). However, these approaches are not implemented on a commercial scale due to high costs compared to the conventional B. cinerea control

Postharvest treatments

It is common practice to handpick strawberries and place them into clamshells in the field, in order to reduce wounding and bruising of the fruit. Rapid and constant cooling of strawberries at temperatures below 2.5 °C is another critical strategy to reduce or inhibit reactivation of B. cinerea quiescent infections (Nunes et al., 1995). Often, strawberries are also stored in modified atmospheres, which are generally low in oxygen and high in carbon dioxide to slow down metabolic processes, senescence and fungal decay (Feliziani and Romanazzi, 2016). Relative humidity during storage is usually kept around 85%–90% to prevent dehydration of fruit, but limit fungal growth (Almeida et al., 2015).

Novel postharvest treatments of strawberries have been suggested to prevent B. cinerea infections during storage. Examples are edible fruit coatings of chitosan, silk fibroin or methylcellulose that prevent water loss and can include antifungal compounds (Marelli et al., 2016; Nadim et al., 2015; Romanazzi et al., 2017). MeJA treatment to induce fruit defence mechanisms (Zhang et al., 2006), ultraviolet and visual light treatment (Saks et al., 1996), enrichment of storage atmosphere with chlorine or ozone (Avis et al., 2006; Nadas et al., 2003), and soft mechanical stimulation (Tomas-Grau et al., 2017) have also been tested as alternative treatments. Most of these approaches are still in an experimental stage or not yet adaptable to commercial settings.

LOOKING INTO THE FUTURE: IMPROVING STRAWBERRY RESISTANCE TO BOTRYTIS CINEREA

Several aspects of the genetics of resistance to B. cinerea are unclear in strawberry. Significant phenotypic variation of incidence or severity of grey mould has been reported; however, F. x ananassa genotypes appear to be universally susceptible and complete resistance has not been observed (Bestfleisch et al., 2015). Substantial genotypic variation has not been documented and the heritability of resistance to B. cinerea is unknown. Mild phenotypic differences in fruit resistance levels reported in various postharvest studies (Bestfleisch et al., 2015; Lewers et al., 2012) indicate that genetic variation for resistance may be limited and that its heritability is low. A contributing factor is the intrinsic characteristics of the pathogen, its broad host range, diverse ways of infection and necrotrophic lifestyle, which explain the absence of a gene-for-gene resistance of strawberry to B. cinerea (Amil-Ruiz et al., 2011). Therefore, breeding for escape and tolerance, which includes physiological and biochemical traits, is a more practical option (Elad and Evensen, 1995). While limited in scale and scope, earlier studies strongly suggest that the incidence and progression of B. cinerea infections differ between cultivars with soft fruit and those with firm fruit (Barritt, 1980; Gooding, 1976). Hence, previously reported differences amongst cultivars could be the result of the pleiotropic effects of selection for increased fruit firmness and shelf life and the associated developmental and ripening changes, as opposed to direct genetic gains in innate resistance to the pathogen.

As discussed, fruit firmness is an important trait associated with resistance to B. cinerea (Hancock et al., 2008; Terry et al., 2004). The strawberry germplasm displays natural variation for fruit firmness and developing cultivars with firmer fruit is an important aim in breeding programmes (Hummer et al., 2011; Salentijn et al., 2003). Changes in flower morphology could also enhance tolerance to B. cinerea. In strawberry, most B. cinerea infections in fruit appear to originate from primary infections of flowers or secondary infections caused by direct contact with infected flower parts (Bristow et al., 1986; Jarvis, 1962; Powelson, 1960). It was reported that removal of stamen and petals result in lower grey mould incidence (Jersch et al., 1989; Powelson, 1960). Faster abscission of flower parts, especially petals, has the potential to aid the escape of strawberries from B. cinerea infections (Elad and Evensen, 1995). Similarly, plants with pistillate flowers (i.e. flowers with pistils but no stamen) have a lower grey mould incidence in fruit (Bristow et al., 1986; Elad and Evensen, 1995). B. cinerea growth inhibition in stamens is reported to vary within the strawberry germplasm, potentially due to differences in their biochemical composition (Bristow et al., 1986). Similarly, antifungal compounds in fruit can prevent or limit B. cinerea infections. Several reports indicate that anthocyanin accumulation contributes to tolerance of strawberries to B. cinerea (Jersch et al., 1989; Saks et al., 1996). Anthocyanins do not just improve tolerance to grey mould but also provide health benefits (Terry et al., 2004). Breeding for higher anthocyanin content in strawberries is possible and facilitated by existing variation in the germplasm (Fredericks et al., 2013; Jing, 2012). Inducing anthocyanin accumulation in flowers could also help to limit flower infections.

As breeding for higher B. cinerea tolerance will be tedious and likely will not result in complete resistance, complementary approaches should be considered. Currently, no genetically modified strawberry cultivars are commercially grown; however,
several reports show great potential to improve tolerance to grey mould via trans- or cis-genesis. For example, the expression of chitinases or PGIPs from other organisms in strawberries can prevent or slow down fungal infections (Powell et al., 2000). Another potential transgenic approach is to increase fruit firmness by altering the expression or activity of pectin degrading enzymes, such as PL or PG (Jimenez-Bermudez et al., 2002). The existing natural variation of PL expression levels and activity in the cultivated strawberry germplasm could be used for cis- or transgenic approaches. Increasing phenolic levels in strawberries by genetic modifications can also be explored as the transcription factor MYB10 was identified as a regulator of anthocyanin levels in strawberries (Lin-Wang et al., 2010; Lin-Wang et al. 2014); Medina-Puche et al., 2014). Transgenic plants (both F. x ananassa and F. vesca) with ectopic overexpression of MYB10 show elevated anthocyanin levels throughout the entire plant (Lin-Wang et al., 2010); however, the resistance of these plants against B. cinerea have not been tested. In summary, these novel breeding approaches should be supported by integrative management strategies including horticultural and agronomic practices, and potentially biocontrol, to achieve maximum control of the disease.

CONCLUSION

Many details about grey mould of strawberries are still poorly understood. Future research is necessary to characterize the genetic pathways and biochemical components that are involved in strawberry-B. cinerea interactions. Molecular analyses of the infection process and the physiological causes for the failure of host defences should provide a basis to develop robust solutions against the disease, or at least provide information for control strategies that are likely to fail and therefore be discouraged. Furthermore, current disease management needs to be re-evaluated to cope with increasing restrictions and lack of efficacy of fungicides. Investigations on biocontrol approaches and pre- and postharvest treatments are necessary to manage grey mould. On the other hand, breeding for escape and tolerance against B. cinerea can be a feasible approach for commercial varieties. Research on genetic modifications of strawberry that restrict B. cinerea infections could also be used for guiding conventional breeding efforts or developing new varieties once the market is ready for their acceptance.

ACKNOWLEDGEMENTS

We would like to acknowledge Casper van den Abeele and Pedro Bello for contributions with the images in Figures 1 and 3. We thank Jaclyn A. Adaskaveg and Nancy Nou Her for their review of the manuscript’s narrative.

REFERENCES

Adikaram, N.K.B., Joyce, D.C. and Terry, L.A. (2002) Biocontrol activity and induced resistance as a possible mode of action for Aureobasidium pullulans against grey mould of strawberry fruit. Australas. Plant Pathol. 31(3), 223–229.

Almeida, M.L.B., Herber Moura, C.F., Innecco, R., dos Santo, A. and Rodrigues de Miranda, F. (2015) Postharvest shelf-life and fruit quality of strawberry grown in different cropping systems. Afr. J. Agric. Res. 10(43), 4053–4061. https://doi.org/10.5897/AJAR2015.10239.

Amil-Ruiz, F., Blanco-Portales, R., Muñoz-Blanco, J. and Caballero, J.L. (2011) The strawberry plant defense mechanism: A molecular review. Plant Cell Physiol. 52(11), 1873–1903. https://doi.org/10.1093/pcc/pcr136.

Amselem, J., Cuomo, C.A., van Kan, J.A.L., Vlaud, M., Benito, E.P., Couloux, A., Coutinho, P.M., de Vries, R.P., Dyer, P.S., Filling, S., Fournier, E., Gout, L., Hahn, M., Kohn, L., Lapalu, N., Plummer, K.M., Pradier, J.-M., Quevillon, E., Sharon, A., Simon, A., ten Have, A., Tudzynski, B., Tudzynski, P., Wincker, P., Andrew, M., Anthouard, V., Beever, R.E., Beffa, R., Benoit, I., Bouidet, B., Chen, Z., Choquere, M., Collémare, J., Cotton, P., Danchin, E.G., Da Silva, C., Gaultier, A., Giraud, C., Giraud, T., Gonzalez, C., Grossetete, S., Gündüner, U., Henriassat, B., Howlett, B.J., Kodira, C., Kreutschmer, M., Lappartient, A., Lerose, M., Lewis, C., Mauceli, E., Neveuville, C., Oeser, B., Pearson, M., Poulain, J., Pousserseau, N., Quesneville, H., Rascle, C., Schumacher, J., Segurens, B., Sexton, A., Silva, E., Sirven, C., Soanes, D.M., Talbot, N.J., Templeton, M., Yandava, C., Yarden, O., Zeng, Q., Rollins, J.A., Lebrun, M.-H. and Dickman, M. (2011) Genomic analysis of the necrotrophic fungal pathogens Sclerotinia sclerotiorum and Botrytis cinerea. PLoS Genet. 7(8), e1002230. https://doi.org/10.1371/journal.pgen.1002230.

Avis, T.J., Martinez, C. and Tweddell, R.J. (2006) Effect of chlorine atmospheres on the development of rhizopus rot [Rhizopus stolonifer] and gray mold [Botrytis cinerea] on stored strawberry fruits. Can. J. Plant Pathol. 28(4), 526–532. https://doi.org/10.1080/07060660609507330.

Ayub, R.A., Reis, L., Bosetto, L., Lopes, P.Z., Galvão, C.W. and Etto, R.M. (2018) Brassinosteroid plays a role on pink stage for receptor and transcription factors involved in strawberry fruit ripening. Plant Growth Regul. 84(1), 159–167. https://doi.org/10.1007/s10725-017-0329-5.

van Baarlen, P., Legendre, L. and van Kan, J.A.L. (2007) Plant defence compounds against botrytis infection. In: Botrytis: Biology, Pathology and Control (Elad, Y., Williamson, B., Tudzynski, P. and Delen, N., eds), pp. 143–161. Dordrecht: Springer, Netherlands. https://doi. org/10.1007/978-1-4020-2626-3_9.

Babalar, M., Arshari, M., Talaei, A. and Khosroshahi, A. (2007) Effect of post- and preharvest salicylic acid treatment on ethylene production, fungal decay and overall quality of selva strawberry fruit. Food Chem. 105(2), 449–453. https://doi.org/10.1016/j.foodchem.2007.03.021.

Barnes, S.E. and 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.

Barritt, B.H. (1980) Resistance of strawberry clones to Botrytis fruit rot. J. Amer. Soc. Hort. Sci. 105, 160–164.

Bassolino, L., Zhang, Y., Schoonebeeck, H.-J., Kiferle, C., Perata, P. and Martin, C. (2013) Accumulation of anthocyanins in tomato skin extends shelf life. New Phytol. 200(3), 650–655. https://doi.org/10.1111/nph.12524.

Basu, A., Nguyen, A., Betts, N.M. and Lyons, T.J. (2014) Strawberry as a functional food: An evidence-based review. Crit. Rev. Food Sci. Nutr. 54(6), 790–806. https://doi.org/10.1080/01448192.2011.608174.

Bestfleisch, M., Luderer-Pflimfl, M., Höver, M., Schulte, E., Wünsche, J.N., Hanke, M.V. and Flachowsky, H. (2015) Evaluation of strawberry...
in tomato ripening mutants. *Physiol. Plant.* 139(1), 107–117. https://doi.org/10.1111/j.1399-3054.2009.01342.x.

Lee, I.-C., Hong, S.-W., Whang, S.-S., Lim, P.-O., Nam, H.-G. and Koo, J.-C. (2011) Age-dependent action of an ABA-inducible receptor kinase, RPK1, as a positive regulator of senescence in Arabidopsis leaves. *Plant Cell Physiol.* 52(4), 651–662. https://doi.org/10.1093/pcp/pcr026.

Legard, D.E., MacKenzie, S.J., Mertely, J.C., Chandler, C.K. and Peres, N.A. (2005) Development of a reduced use fungicide program for control of *Botrytis* fruit rot on annual winter strawberry. *Plant Dis.* 89(12), 1353–1358.

Leroch, M., Plesken, C., Weber, R.W.S., Kauff, F., Scalliet, G. and 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*. *Appl. Environ. Microbiol.* 79(1), 159–167. https://doi.org/10.1128/AEM.02655-12.

Lewers, K.S., Luo, Y. and Vinyard, B.T. (2012) Evaluating strawberry breeding selections for postharvest fruit decay. *Euphytica* 186(2), 539–555. https://doi.org/10.1007/s10681-012-0654-8.

Li, C., Jia, H., Chai, Y. and Shen, Y. (2011) Abscisic acid perception and signaling transduction in strawberry: A model for non-climacteric fruit ripening. *Plant Signal. Behav.* 6(12), 1950–1953. https://doi.org/10.4161/psb.6.12.18024.

Li, Q., Ji, K., Sun, Y., Luo, H., Wang, H. and Leng, P. (2013) The role of FaBG3 in fruit ripening and *B. cinerea* fungal infection of strawberry. *Plant J.* 76, 24–35. https://doi.org/10.1111/tjp2.12272.

Liang, Y., Guan, Y., Wang, S., Li, Y., Zhang, Z. and Li, H. (2018) Identification and characterization of known and novel microRNAs in strawberry fruits induced by *Botrytis cinerea*. *Sci. Rep.* 8(1). https://doi.org/10.1038/s41598-018-2929-7.

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

Lin-Wang, K., Bolitho, K., Grafton, K., Kortstee, A., Karunairatnam, S., McGhie, T.K., Espley, R.V., Hellens, R.P. and Allan, A.C. (2010) An R2R3 MYB transcription factor associated with regulation of the anthocyanin biosynthetic pathway in roseaeae. *BMC Plant Biol.* 10(1), 50.

Lin-Wang, K., McGhie, T.K., Wang, M., Liu, Y., Warren, B., Storey, R., Espley, R.V. and Allan, A.C. (2014) Engineering the anthocyanin regulatory complex of strawberry (*Fragaria vesca*). *Front. Plant Sci.* 5 (November). https://doi.org/10.3389/fpls.2014.00651.

Manteau, S., Abouna, S., Lambert, B. and Legendre, L. (2003) Differential regulation by ambient pH of putative virulence factor secretion by the phytopathogenic fungus *Botrytis cinerea*. *FEMS Microbiol. Ecol.* 43(3), 359–366. https://doi.org/10.1111/j.1574-6941.2003.tb01076.x.

Marelli, B., Brencle, M.A., Kaplan, D.L. and Omenetto, F.G. (2016) Silk fibroin as edible coating for perishable food preservation. *Sci. Rep.* 6, 25263. https://doi.org/10.1038/srep25263.

McCormack, P.J., Wildman, H.G. and Jeffries, P. (1994) Production of antibacterial compounds by phytoplane-inhabiting yeasts and yeast-like fungi. *Appl. Environ. Microbiol.* 60(3), 927–931.

Medina-Puche, L., Cumplido-Laso, G., Amil-Ruiz, F., Hoffmann, T., Ring, L., Rodriguez-Francisco, A., Caballero, J.L., Schwab, W., Muñoz-Blanco, J. and Blanco-Portales, R. (2014) MYB10 plays a major role in the regulation of flavonoid/phenylpropanoid metabolism during ripening of *Fragaria × ananassa* fruits. *J. Exp. Bot.* 65(2), 401–417. https://doi.org/10.1093/jxb/ert377.

Mehl, L., Schaar, J.G., Kjelsen, T.D., Tran, D.H., Salentijn, E.M.J., Schouten, H.J. and Iversen, T.H. (2004) A gene encoding a polygalacturonase-inhibiting protein (PGIP) shows developmental regulation and pathogen-induced expression in strawberry. *New Phytol.* 163(1), 99–110. https://doi.org/10.1111/j.1469-8137.2004.01088.x.

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

Merchant, C., Vallarino, J.G., Osorio, S., Aragüez, I., Villarreal, N., Ariza, M.T., Martinez, G.A., Medina-Escobar, N., Civello, M.P., Fernie, A.R., Botella, M.A. and Valpuesta, V. (2013) Ethylene is involved in strawberry fruit ripening in an organ-specific manner. *J. Exp. Bot.* 64(14), 4421–4431. https://doi.org/10.1093/jxb/ert257.

Mertely, J.C., MacKenzie, S.J. and Legard, D.E. (2002) Timing of fungicide applications for *Botrytis cinerea* based on development stage of strawberry flowers and fruit. *Plant Dis.* 86(9), 1019–1024.

Mukkun, L. and Singh, Z. (2009) Methyl jasmonate plays a role in fruit ripening of ‘pajaro’ strawberry through stimulation of ethylene biosynthesis. *Sci. Hortic.* 123(1), 5–10. https://doi.org/10.1016/j.scienta.2009.07.006.

Nadas, A., Olmo, M. and Garcia, J.M. (2003) Growth of *Botrytis cinerea* and strawberry quality in ozone-enriched atmospheres. *J. Food Sci.* 68(5), 1798–1802.

Nadim, Z., Ahmad, E., Sarikhanhi, H. and Chayjan, R.A. (2015) Effect of methylcellulose-based edible coating on strawberry fruit’s quality maintenance during storage: Quality of strawberry. *J. Food Process. Preserv.* 39(1), 80–90. https://doi.org/10.1111/jfpp.12227.

Nagpala, E.G., Guidarelli, M., Gasperotti, M., Masuero, D., Bertolini, P., Vrhovsek, U. and Baraldi, E. (2016) Polyphenols variation in fruits of the susceptible strawberry cultivar Alba during ripening and upon fungal pathogen interaction and possible involvement in unripe fruit tolerance. *J. Agric. Food Chem.* 64(9), 1869–1878. https://doi.org/10.1021/acscrc.5b06005.

Nakajima, M. and Akutsu, K. (2014) Virulence factors of *Botrytis cinerea*. *J. Gen. Plant Pathol.* 80(1), 15–23. https://doi.org/10.1016/j.jpp.2013.04.092-090.

Neri, F., Cappellin, L., Spadoni, A., Cameledi, I., Algarra Alarcon, A., Aprea, E., Romano, A., Gasperi, F. and Biasiolli, F. (2015). Role of strawberry volatile organic compounds in the development of *Botrytis cinerea* infection. *Plant Pathol.* 64(3), 709–717. https://doi.org/10.1111/ppa.12287.

Nunes, M.C.N., Brecht, J.K., Morais, A.M.M.B. and Sargent, S.A. (1995) Physical and chemical quality characteristics of strawberries after storage are reduced by a short delay to cooling. *Postharvest Biol. Technol.* 6(1–2), 17–28.

Osorio, S., Alba, R., Damasceno, C.M.B., Lopez-Casado, G., Lohse, M., Zanor, M.I., Tohge, T., Ulate, B., Ulate, B., Visser, L., Greve, C., Bennet, A.B. and Labavitch, J.M. (2000) Transgenic expression of pear turonase-inhibiting protein (PGIP) shows developmental regulation and pathogen-induced expression in strawberry. *New Phytol.* 163(1), 99–110. https://doi.org/10.1111/j.1469-8137.2004.01088.x.
PGf in tomato limits fungal colonization. *Mol. Plant-Microbe Interact.* 13(9), 942–950.

Powelson, E.L. (1960) Initiation of strawberry fruit rot caused by *Botrytis cinerea*. *Phytopathology* 50(7), 491–494.

Preuß, A., Augustin, C., Figueroa, C.R., Hoffmann, T., Valpuesta, V., Sevilla, J.F. and Schwab, W. (2014) Expression of a functional jasmonic acid carboxyl methyltransferase is negatively correlated with strawberry fruit development. *J. Plant Physiol.* 171(15), 1315–1324. https://doi.org/10.1016/j.jplph.2014.06.004.

Prusky, D. and Lichter, A. (2007) Activation of quiescent infections by postharvest pathogens during transition from the biotrophic to the necrotrophic stage. *FEMS Microbiol. Lett.* 268(1), 1–8. https://doi.org/10.1111/j.1574-6968.2006.00603.x.

Puhl, I. and Treutter, D. (2008) Ontogenetic variation of catechin biosynthesis as basis for infection and quiescence of *Botrytis cinerea* in developing strawberry fruits. *J. Plant Dis. Prot.* 115(6), 247–251. https://doi.org/10.1007/BF03356272.

Rhaïnds, M., Kovač, J. and English-Loeb, G. (2002) Impact of strawberry cultivar and incidence of pests on yield and profitability of strawberries under conventional and organic management systems. *Biol. Agric. Hortic.* 19(4), 333–353. https://doi.org/10.1111/j.1574-6968.2002.9754937.

Ries, S.M. (1995) RPD No. 704 - Gray Mold of Strawberry. Available at http://imp.illinois.edu/diseases/series700?rdp704/ accessed on Jan 4, 2019

Romanazzi, G., Feliziani, E., Bautista-Baños, S. and Sivakumar, D. (2017) Shelf life extension of fresh fruit and vegetables by chitosan treatment. *Rev. Food Sci. Nutr.* 15(4), 562–571.

Schoonbeek, H., Del Sorbo, G. and De Waard, M.A. (2001) The ABC transporter CbctB affects the sensitivity of *Botrytis cinerea* to the phytalexin resveratrol and the fungicide fenpiclonil. *Mol. Plant-Microbe Interact.* 14(4), 1–7.

Sevilla, J.F. and Schwab, W. (2014) Expression of a functional jasmonic acid carboxyl methyltransferase as basis for infection and quiescence of *Botrytis cinerea* in developing strawberry fruits. *J. Plant Physiol.* 171(15), 1315–1324. https://doi.org/10.1016/j.jplph.2014.06.004.

Sylla, J., Alsanius, B.W., Krüger, E. and Wohanka, W. (2011) Initiation of strawberry fruit rot caused by *Botrytis cinerea* – strawberry fruit pathosystem. In: Botrytis : Biology, Pathology and Control. Int. J. Mol. Sci. 70(7), 491–494. https://doi.org/10.1038/s41598-017-14239-6.

Sutton, J.C. (1990) Epidemiology and management of *Botrytis* leaf blight of onion and gray mold of strawberry: A comparative analysis. *Can. J. Plant Pathol.* 12(1), 101–110. https://doi.org/10.1080/070606909501048.

Swartzberg, D., Kirshner, B., Rav-David, D., Elad, Y. and Granot, D. (2008) *Botrytis cinerea* induces senescence and is inhibited by autoregulated expression of the IPT gene. *Eur. J. Plant Pathol.* 120(3), 289–297. https://doi.org/10.1007/s10658-007-9217-6.

Sylla, J., Alsanius, B.W., Krüger, E. and Wohanka, W. (2015) Control of *Botrytis cinerea* in strawberries by biological control agents applied as single or combined treatments. *Eur. J. Plant Pathol.* 143(3), 461–471. https://doi.org/10.1007/s10658-015-0698-4.
Terry, L.A., Joyce, D.C., Adikaram, N.K.B. and Khambay, B.P.S. (2004) Preformed antifungal compounds in strawberry fruit and flower tissues. Postharvest Biol. Technol. 31(2), 201–212. https://doi.org/10.1016/j.postharvbio.2003.08.003.

Terry, L.A., Chope, G.A. and Bordonaba, J.G. (2007) Effect of water deficit irrigation and inoculation with Botrytis cinerea on strawberry (Fragaria x ananassa) fruit quality. J. Agric. Food Chem. 55(26), 10812–10819. https://doi.org/10.1021/jf072101n.

Tomas-Grau, R.H., Requena-Serra, F.J., Hael-Conrad, V., Martínez-Zamora, M.G., Guerrero-Molina, M.F. and Díaz-Ricci, J.C. (2017) Soft mechanical stimulation induces a defense response against Botrytis cinerea in strawberry. Plant Cell Rep. 37(2), 239–250. https://doi.org/10.1007/s00299-017-2226-9.

USDA (2016) Noncitrus Fruits and Nuts 2016 Summary. Available from http://usda.mannlib.cornell.edu/usda/current/noncfruinu/noncfruinu-06-27-2017.pdf accessed Oct 9, 2018.

Vagelas, I., Papachatzis, A., Kalorizou, H. and Wogiatzi, E. (2009) Biological control of Botrytis fruit rot (Gray Mold) on strawberry and red pepper fruits by olive oil mill wastewater. Biotechnol. Biotechnol. Equip. 23(4), 1489–1491. https://doi.org/10.2478/V10133-009-0017-3.

Van Kan, J.A.L., Stassen, J.H.M., Mosbach, A., Van Der Lee, T.A.J., Faino, L., Farmer, A.D., Papasotiriou, D.G., Zhou, S., Seidl, M.F. and Cottam, E.L., Farmer, A.D., Papasotiriou, D.G., Zhou, S., Seidl, M.F. and Cottam, E. (2017) A gapless genome sequence of the fungus Botrytis cinerea. Mol. Plant Pathol. 18(1), 75–89.

Vellicce, G.R., Díaz, J.C., Ricci, L.H. and Castagnaro, A.P. (2006) Enhanced resistance to Botrytis cinerea mediated by the transgenic expression of the chitinase gene Ch5B in strawberry. Transgenic Res. 15(1), 57–68. https://doi.org/10.1007/s11248-005-2543-6.

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

Wang, S.Y. and Lin, H.-S. (2000) Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. J. Agric. Food Chem. 48(2), 140–146. https://doi.org/10.1021/jf9908345.

Wang, M., Weiberg, A., Lin, F.-M., Thomma, B.P.H.J., Huang, H.-D. and Jin, H. (2016) Bidirectional cross-kingdom RNAi and fungal uptake of external RNAs confer plant protection. Nat. Plants. 2, 16151. https://doi.org/10.1038/nplants.2016.151.

Wang, Q.-H., Zhao, C., Zhang, M., Li, Y.-Z., Shen, Y.-Y. and Guo, J.-X. (2017a) Transcriptome analysis around the onset of strawberry fruit ripening uncovers an important role of oxidative phosphorylation in ripening. Sci. Rep. 7(February), 41477. https://doi.org/10.1038/srep41477.

Wang, M., Weiberg, A., Dellota, E., Yamane, D. and Jin, H. (2017b) Botrytis small RNA Bc-SiR37 suppresses plant defense genes by cross-kingdom RNAi. RNA Biol. 14(4), 421–428. https://doi.org/10.1080/15476286.2017.1291112.

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

Wedge, D.E., Curry, K.J., Kreiser, B., Curry, A., Abril, M. and Smith, B.J. (2013) Fungicide resistance profiles for 13 Botrytis cinerea isolates from strawberry in southeastern Louisiana. Int. J. Fruit Sci. 13(4), 413–429. https://doi.org/10.1080/15538362.2013.789253.

Weiberg, A., Wang, M., Lin, F.-M., Zhao, H., Zhang, Z., Kaloshian, I., Huang, H.-D. and Jin, H. (2013) Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways. Science, 342(6154), 118–123. https://doi.org/10.1126/science.1239705.

Xiao, C.L., Chandler, C.K., Price, J.F., Duval, J.R., Mertely, J.C. and Legard, D.E. (2001) Comparison of epidemics of Botrytis fruit rot and powdery mildew of strawberry in large plastic tunnel and field production systems. Plant Dis. 85(8), 901–909.

Xu, X.-M. and Jeger, M.J. (2013) Theoretical modeling suggests that synergy may result from combined use of two biocontrol agents for controlling foliar pathogens under spatial heterogeneous conditions. Phytopathology, 103(8), 768–775.

Zhang, L. and van Kan, J.A.L. (2013) Botrytis cinerea mutants deficient in D-galacturonic acid catabolism have a perturbed virulence on Nicotiana benthamiana and Arabidopsis, but not on tomato: Botrytis ga - lacturonic acid catabolism. Mol. Plant Pathol. 14(1), 19–29. https://doi.org/10.1111/j.1364-3703.2012.00825.x.

Zhang, F.S., Wang, X.Q., Ma, S.J., Cao, S.F., Li, N., Wang, X.X. and Zheng, Y.H. (2006) Effects of methyl jasmonate on postharvest decay in strawberry fruit and the possible mechanisms involved. IV International Conference on Managing Quality in Chains-The Integrated View on Fruits and Vegetables Quality, 712, 693–698.

Zhang, Y., Butelli, E., De Stefano, R., Schoonbeek, H.-J., Magusin, A., Pagliarani, C., Wellner, N., Hill, L., Orzaez, D., Granell, A., Jones, J.D.G and Martin, C. (2013) Anthocyanins double the shelf life of tomatoes by delaying overripening and reducing susceptibility to gray mold. Curr. Biol. 23(12), 1094–1100. https://doi.org/10.1016/j.cub.2013.04.072.

MOLECULAR PLANT PATHOLOGY (2019) © 2019 THE AUTHORS. MOLECULAR PLANT PATHOLOGY PUBLISHED BY BRITISH SOCIETY FOR PLANT PATHOLOGY AND JOHN WILEY & SONS LTD