Universitat de Lleida

Effect of light on nectarine-*Monilinia* spp. interaction and analysis of fungal and fruit responses during *M. laxa* infection

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Ph.D. THESIS RESEARCH

Effect of light on nectarine-*Monilinia* spp. interaction and analysis of fungal and fruit responses during *M. laxa* infection

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Most of the experiments of this Ph.D. research have been carried out in the laboratories of the Postharvest program of the Institute of AgriFood Research and Technology (IRTA)-Fruitcentre, located in Lleida (Catalonia, Spain).

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Intentarem posar llum a la foscor.

Carles Porta

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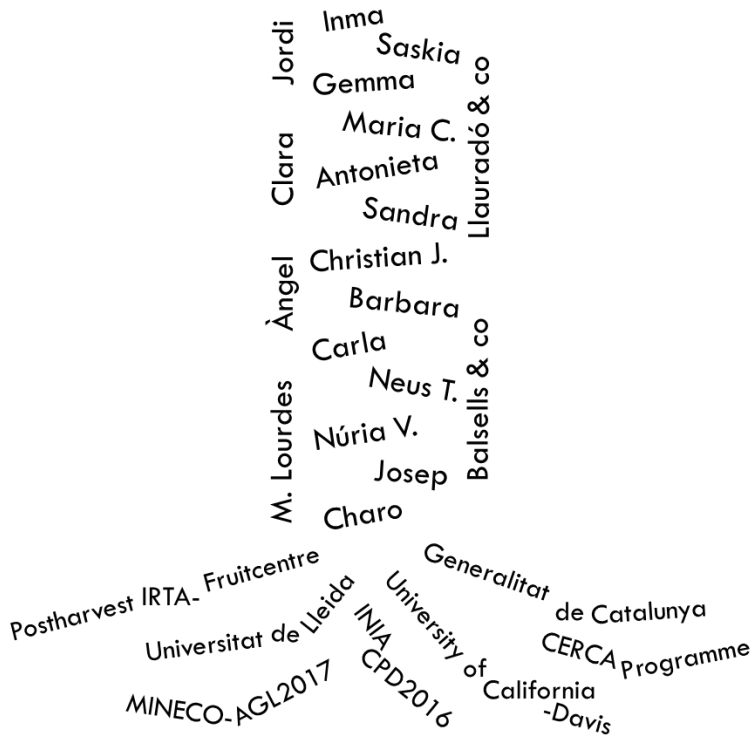

Vosaltres heu estat les arrels, la saba, el tronc i les branques que heu donat els fruits d'aquesta tesi.

Thank you.

Gracias.

GRÀCIES.

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Abbreviations

B	Bagged
CAZy	Carbohydrate-active enzyme
CD	Cheek diameter
CPMC6	*M. fructicola* single-spore strain 6
CWDE	Cell wall degrading enzymes
DA Index	Single index of absorbance difference
DA-meter	Difference in absorbance or delta absorbance (DA) meter
DE	Differential expression
DEG(s)	Differentially expressed gene(s)
dpi	Days post-inoculation
EPPO	European and Mediterranean Plant Protection Organization
ET	Ethylene
FF	Flesh firmness
fPox	Fungal peroxidases
GC-MS	Gas chromatograph-mass spectrometry
GO	Gene ontology
HCA	Hierarchical cluster analysis
hpi	Hours post-inoculation
HS-SPME	Headspace solid-phase microextraction
JA	Jasmonic acid
KEGG	Kyoto encyclopedia of genes and genomes
MEP	Methylerythritol phosphate
ML8L	*M. laxa* single-spore strain 8L
MVA	Mevalonic acid
PCA	Principal component analysis
PDA	Potato dextrose agar
PDA-T	PDA supplemented with 25% tomato pulp
Pfam	Protein family database
PHI	Pathogen-host interaction
PLS	Partial least square
PpFOLK	Farnesol kinase/ probable phytol kinase 3
PpFPS2	Farnesyl diphosphate synthase/ farnesyl pyrophosphate synthase 2 (FPS2 / FPPS2)
PpHMGR1	Hydroxymethylglutaryl-CoA reductase (NADPH)
PpHMGS	Hydroxymethylglutaryl-CoA synthase

PpIDI	Isopentenyl-diphosphate delta-isomerase (IDI or IPI)
PpLIS1	(3S)-Linalool synthase 1
PpLIS2	(3S)-Linalool synthase 2
PpPFT1	Protein farnesyltransferase subunit beta
PpSIMT	Protein-S-isoprenylcysteine O-methyltransferase
PpSM	Squalene monooxygenase
PpSQS	Squalene/phytoene synthase/ Farnesyl-diphosphate farnesyltransferase
PpTEF2	Elongation factor 2
RT-qPCR	Real-time quantitative polymerase chain reaction
SignalP	Presence of secretion signal peptides
SSC	Soluble solids content
TA	Titratable acidity
TCDB	Transporter classification database
UB	Unbagged
VIP	Variable importance plot
VOC(s)	Volatile organic compound(s)

Summary/ Resum/ Resumen

Brown rot is a significant disease in stone fruit caused by *Monilinia* spp. These pathogens can infect fruit during its growth, when most control strategies are applied, but the main fruit losses occur at the postharvest period. This thesis encompasses the study of i) the effect of light as a factor affecting brown rot development (from preharvest to postharvest), and ii) fungal virulence factors and fruit defense mechanisms in response to brown rot (host-pathogen interaction studies). The first objective of this thesis was to evaluate the effect of light (i.e., three lighting treatments) along the postharvest storage on bagged (fruit that was bagged during preharvest) and unbagged fruit which were artificially inoculated with two *Monilinia* species (*M. laxa* and *M. fructicola*). These evaluations suggested that the bagging effect together with storing the fruit under lighting postharvest treatments influence fruit responses to *Monilinia* spp. Besides, the effect of these lighting treatments was also evaluated on the *in vitro* behavior of *Monilinia* spp. and on their further capacity to infect nectarines. Results showed that *M. laxa* has a broader morphogenic response to light than *M. fructicola*, altering their ability to infect nectarines (chapter 1). Next, the effect of lighting treatments on fruit quality and natural fungal disease incidence (fruit decay) of bagged and unbagged nectarines was also addressed. Fruit bagging slightly altered fruit quality on harvest day, but its effect was almost subsided after lighting treatment storage. Besides, it reduced fruit decay after postharvest storage (chapter 2). About host-pathogen studies, a dual RNA-Sequencing analysis was performed in two nectarine developmental stages that showed different susceptibility to *M. laxa*. Results highlighted the global strategies deployed by both the fruit (e.g., uniquely, or highly induced responses in the resistant tissue) and the pathogen (e.g., key strategies for colonizing or establishing on fruit) during their interaction (chapter 3). As highlighted by this study, the role of some secondary metabolites (i.e., terpenoid metabolism and volatile organic compounds (VOCs)) was also assessed in fruit tissues that presented different susceptibility to *M. laxa* through gene expression analyses and VOCs profile analyses, respectively. The first study elucidated some biosynthetic pathways that may play essential roles in mediating the resistance to *M. laxa* (chapter 4). The second one provided potential VOCs that favor the disease development, but also VOCs that may have antifungal properties against *M. laxa* (chapter 5).

La podridura marró és una important malaltia de la fruita de pinyol causada per diferents espècies de *Monilinia*. Aquests patògens poden infectar la fruita durant el seu creixement, moment en el qual s'apliquen la majoria d'estratègies de control, però les principals pèrdues de fruita es produeixen durant el període de postcollita. Aquesta tesi engloba l'estudi de i) l'efecte de la llum com a factor que afecta al desenvolupament de la podridura marró (des de la precollita fins a la postcollita), i ii) els factors de virulència fúngics i els mecanismes de defensa de la fruita en resposta a la podridura marró (estudis d'interacció hoste-patogen). El primer objectiu d'aquesta tesi va ser avaluar l'efecte de la llum (en concret, tres tractaments d'il·luminació) durant el període d'emmagatzematge de postcollita en fruita embossada (fruita que s'havia embossat durant el període de creixement a camp) i fruita sense embossar, inoculades artificialment amb dues espècies de *Monilinia* (*M. laxa* i *M. fructicola*). Aquestes avaluacions van suggerir que l'embossat realitzat a camp juntament amb l'emmagatzematge sota diferents tractaments d'il·luminació influïa en les respostes de la fruita davant de les *Monilinia* spp. A més, també es va avaluar l'efecte d'aquests tractaments d'il·luminació sobre el comportament *in vitro* de *Monilinia* spp. i sobre la seves capacitats per infectar nectarines. Els resultats van mostrar que *M. laxa* presenta una resposta morfogènica més àmplia a la llum que *M. fructicola*, alterant la seva capacitat per infectar nectarines (capítol 1). A continuació, també es va abordar l'efecte dels tractaments d'il·luminació sobre la qualitat global de la fruita i sobre la incidència d'infeccions naturals en nectarines embossades i sense embossar. L'embossat de la fruita va alterar lleugerament la qualitat de la fruita el dia de la collita, però el seu efecte gairebé es va reduir després d'un emmagatzematge sota condicions d'il·luminació. A més, l'embossat va reduir la incidència d'infeccions naturals després de l'emmagatzematge de postcollita (capítol 2). En relació als estudis d'interacció hoste-patogen, es va realitzar una anàlisi dual de seqüenciació d'ARN en dos estadis de desenvolupament de la nectarina que mostraven una susceptibilitat diferent a *M. laxa*. Els resultats van destacar les estratègies globals desplegades tant pel fruit (p. ex., respostes úniques o altament induïdes en el teixit resistent), com pel patogen (p. ex., estratègies clau per colonitzar o establir-se a la fruita) durant la seva interacció (capítol 3). Com destaca aquest estudi, el paper d'alguns metabòlits secundaris (en concret, el metabolisme dels terpenoides i els compostos orgànics volàtils (COVs)) també es va avaluar en teixits de nectarina que presentaven una susceptibilitat diferent a *M. laxa* mitjançant anàlisis d'expressió gènica de gens de biosíntesi de terpendoies i anàlisi del perfil de COVs, respectivament. El primer estudi va dilucidar algunes vies biosintètiques que poden tenir un paper important en la mediació de la resistència a *M. laxa* (capítol 4). El segon va proporcionar no només COVs potencials que afavoreixen el desenvolupament de la malaltia, sinó també COVs que poden presentar propietats antifúngiques en contra de *M. laxa* (capítol 5).

La podredumbre parda es una enfermedad importante en fruta de hueso que está causada por *Monilinia* spp. Estos patógenos pueden infectar a la fruta durante todo su crecimiento, momento en el cual se aplican la mayoría de las estrategias de control, aunque las principales pérdidas ocurren durante el periodo de postcosecha. Esta tesis abarca los estudios de i) el efecto de la luz como factor que afecta al desarrollo de la podredumbre parda (desde la precosecha hasta la postcosecha), y ii) factores de virulencia fúngicos y mecanismos de defensa de la fruta durante el desarrollo de la podredumbre parda (estudios de interacción huésped-patógeno). El primer objetivo de esta tesis fue evaluar el efecto de la luz (en concreto, tres tratamientos de iluminación) durante el almacenamiento de postcosecha de nectarinas embolsadas y no embolsadas en precosecha, y artificialmente inoculadas con dos especies de *Monilinia* (*M. laxa* y *M. fructicola*). Estas evaluaciones sugirieron que la combinación de embolsado en campo con el almacenamiento de postcosecha en condiciones de diferentes tratamientos de iluminación influye en las respuestas de la fruta frente a *Monilinia* spp. Además, también se evaluó el efecto de estos tratamientos sobre el comportamiento *in vitro* de *Monilinia* spp. y sobre su posterior capacidad para infectar nectarinas. Los resultados mostraron que *M. laxa* presenta cambios morfológicos en respuesta a la luz más amplios que *M. fructicola,* y ven alterada su capacidad para infectar nectarinas (capítulo 1). A continuación, se abordó el efecto de los tratamientos de iluminación sobre la calidad global de la fruta y la incidencia de podredumbres naturales en las nectarinas embolsadas y no embolsadas. El embolsado de la fruta alteró ligeramente la calidad de la fruta en el momento de la cosecha, pero este efecto prácticamente desapareció después del almacenamiento en condiciones de iluminación. Además, el embolsado redujo la incidencia de podredumbres naturales en postcosecha (capítulo 2). Con relación a los estudios de interacción huésped-patógeno, se realizó un análisis dual de secuenciación masiva del ARN en dos estadios del desarrollo de la nectarina con diferente susceptibilidad a *M. laxa*. Los resultados resaltaron las estrategias globales desplegadas tanto por el fruto (por ejemplo, respuestas únicas o altamente inducidas en el tejido resistente), como por el patógeno (por ejemplo, estrategias clave para colonizar o establecerse en la fruta) durante su interacción (capítulo 3). Como se destacó en este estudio, el papel de algunos metabolitos secundarios (por ejemplo, los terpenoides y los compuestos orgánicos volátiles (COVs)) también se evaluó en tejidos de fruta con una susceptibilidad diferente a *M. laxa*, mediante análisis de expresión génica de la ruta de biosíntesis de los terpenoides y análisis de los perfiles de COVs, respectivamente. El primer estudio reveló algunas vías biosintéticas que pueden desempeñar un papel importante en la mediación de la resistencia a *M. laxa* (capítulo 4). El segundo, proporcionó no solo COVs potenciales que favorecen el desarrollo de la enfermedad, sino también COVs que pueden tener propiedades antifúngicas frente a *M. laxa* (capítulo 5).

1. INTRODUCTION

This introduction aims to provide the importance of the nectarine and its losses caused by brown rot (*Monilinia* spp.), detailing those factors and mechanisms participating in the interaction nectarine-*Monilinia* spp. Relevant topics are highlighted in bold, while missing information related to specific points of the interaction are underlined.

1.1. Stone fruit

1.1.1. Production and exportation

Stone fruit or drupes comprises species of the ***Prunus* genus**, which belongs to the Rosaceae family. This genus has hundreds of species, and the most economically important cultivated members are peach, nectarine, plum, apricot, cherry, and almond (Lino et al., 2016; Mari et al., 2019). The stone fruits are cultivated for their edible flesh with delicious flavors.

Stone fruit is the 10th worldwide most-produced crop (Shahbandeh, 2019), being **peach** (*Prunus persica* (L.) Batch) and **nectarine** (*P. persica* var. *nucipersica* (Borkh.) Schneider) the most produced species (***Table* 1**) (FAO, 2021). Asia is the most producer continent, making almost three-quarters of worldwide production, followed by Europe and the Americas (**Figure 1**).

Table 1. *Total world production and harvested area of the main stone fruit species in 2019 (FAO, 2021).*

	Production (Mt)	**Harvested area (Ha)**
Peaches and nectarines	25.7	1,527,052
Plums and sloes	12.6	2,727,745
Apricots	4.1	561,750
Cherries (sweet and sour)	4.0	668,008

Since 1961, China has been the leader in peach and nectarine **production**, followed by Italy, the USA, Spain, and Greece. Spain experienced a progressive increase of 13-times more production from 1961 (0.12 Mt) until 2019 (1.55 Mt), although the harvested area started to decrease in 2015 (FAO, 2021). Since 2017, the year of the highest production of peaches and nectarines in Spain, this country became the second worldwide producer and the first in Europe, followed by Italy until the last registered data in 2019 (FAO, 2021). In Spain, among the non-citrus fruit, stone fruit represented the largest produced group in 2018 (43%), being Aragón the first producer (29.7%), closely followed by Catalunya (28.2%) and Región de Murcia (19.8%) (Ministerio de Agricultura, 2019). In Catalunya, peach and nectarines are the most produced fruit trees (47% of total fruit trees non-citrus), generating a total of 408,650 tons in 2018, being Lleida the principal producer (92.6%) (Ministerio de Agricultura, 2019).

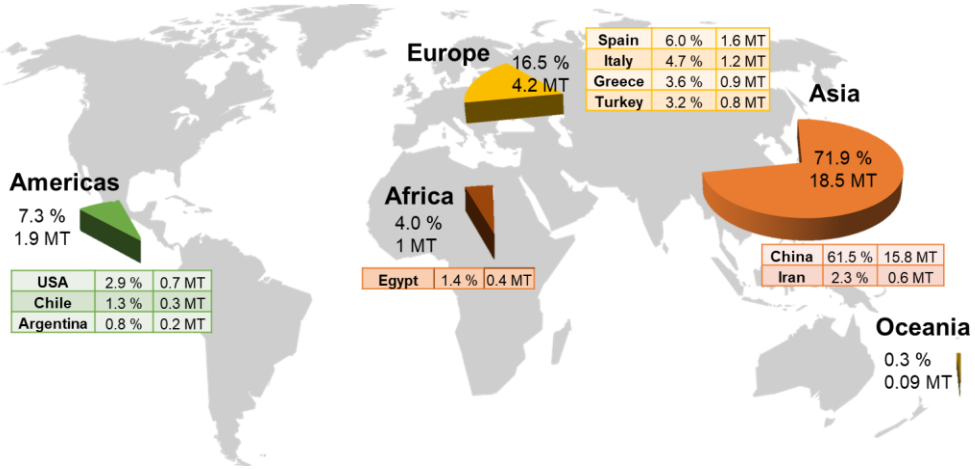

Figure 1. *Production share of peaches and nectarines by region in 2019 with the top 10 country producers (FAO, 2021).*

Spain was the European leader for nectarine **exportation** and the second for peaches in 2019, followed by Greece (Eurostat, 2021). Spain exported 60% (107 Kt) of the total peach and nectarine exports from the European Union (179 Kt) in 2019, which represented 7% of its production that year. Brazil and Norway were the leading importers of peaches and nectarines from Spain, followed by the Arabic United Emirates and France for peach and South Africa and Colombia for nectarine (MAPA, 2020a). Contrary to expected, Spain still imported 1.44 Kt of peaches and nectarines in 2019, mainly from Chile and Morocco (Eurostat, 2021).

Out of the excellent position of production and exportation of peach and nectarine in Spain, some **concerns** should be considered. The average price per kg in markets constantly increases (average of 2.10 € kg^{-1} in 2020) (MAPA, 2020c). Besides, the consumption of peach in 2018 experienced a 24% reduction for the past five years. However, it reverted and remarkably increased 16% from 2018 to 2020 (MAPA, 2018, 2020a), which can be attributed to the increasing prices and the competence with other summer fruits. One of the worst scenarios that fruit and vegetables have worldwide is the excess of production and the food loss and waste as a consequence, in which about half of all fruit and vegetables are lost and not consumed (Porat et al., 2018). In Spain, in 2019, 8 Kt of peach and 10 Kt of nectarine were retired and distributed to other utilities such as animal food and free distribution (MAPA, 2020a, 2020b). Hence, efforts should be driven to increase the good perception of stone fruit

for people through improving health concerns, farmer's conditions, and the fruit quality.

1.1.2. Peach and nectarine

1.1.2.1. Morphological and growth features

Stone fruit is a **drupe** comprised of an inner stone, a soft flesh (or pulp), and a thin outer skin (or peel). The skin is composed of a cuticle (coating of wax), which prevents water loss, mechanical injury, and pathogen attack, and the epidermis (heavy-walled cells), which provides most of the skin's mechanical strength (Crisosto et al., 2020). Skin can vary among species and cultivars, from light yellow to red. On the fruit surface, peach has trichomes (hairs or 'fuzzy' phenotype), which are extensions of some epidermal cells, whereas nectarine has glabrous skin (Bassi and Monet, 2008). In fact, the nectarine is a phenotype of a unique mutation originated from peach in the gene codifying for the hairy vs. glabrous skin trait (Vendramin et al., 2014). Bassi and Monet (2008) also suggested that the fuzzy skin could help to protect the fruit from pathogen attacks. However, the uniform skin of nectarines makes them susceptible to other external damages (e.g., russeting or injuries). Regarding flesh, it can be yellow or white, and its consistency determines melting, non-melting, or stony hard nectarine and peach cultivars (Bassi and Monet, 2008). Nectarine fruit can have freestone or clingstone flesh adherence to the stone (Crisosto et al., 2020).

To reach the full fruit size, the fruit undergoes four **growth stages** that depend on the cultivar, climatic conditions, and even some tree management practices (e.g., thinning or crop load per tree) (Crisosto and Day, 2012). Nectarines are **climacteric fruit** that follow a growth stage different from non-climacteric fruit. Nectarine growth stages comprise a stage of cell division (stage I), followed by the hardening of the stone (stage II), cell enlargement and elongation (stage III), and maturity phase (stage IV) (Bassi and Monet, 2008). At this last stage, the fruit has reached full maturity and can be harvested, naming that stage as "physiological maturity" or **maturity**. In particular, the fruit has evolved enough to have the minimal accepted quality for consumers after some postharvest shelf life (Crisosto et al., 1995). After harvest or during postharvest shelf life, fruit keeps changing through fruit softening, a process called **ripening**, until the optimal maturity for consumer acceptance, called "maturity at consumption" or "ready to eat". At that point, the fruit has reached the appropriate organoleptic and texture (fruit quality) for direct consumption (Crisosto et al., 2020). Hence, the harvest maturity day affects the visual quality, ultimate flavor, market life, as well as the

susceptibility to physiological and mechanical disorders and susceptibility to invasion by rot organisms (Crisosto and Day, 2012). Overall, the developmental process from immature to mature stage and finally, ripeness includes several **physical, chemical, and physiological** modifications. The knowledge of changes occurring at all mentioned levels will improve the shelf life of the fruit, reduce any mechanical or biological damage, and accomplish the retail's and consumer's requirements.

1.1.2.2. Physiological events

Peaches and nectarines can ripen on the tree or after harvest (if picked mature) because they are climacteric fruit (Ramina et al., 2008). Climacteric fruit is characterized by a dramatic increase in the respiration rate during ripening. **Respiration** consists of the oxidation of complex sugars to produce carbon dioxide, water, and energy. Respiration rate is high during stage I of fruit development; it then decreases through stage II and part of stage III, it rises gradually at the end of stage III, and it finally increases again, reaching the climacteric peak at stage IV of maturity, followed by ripening (Ramina et al., 2008). In parallel, the transition from maturation to ripening can depend on the hormone **ethylene** (Tadiello et al., 2016), the main regulator of ripening in climacteric fruits. Fruit produces low and steady levels of ethylene during immature stages and then high increased levels in mature fruit until ripe fruit (Baró-Montel et al., 2020), and decreases once fully ripe. In particular, immature fruit belongs to system 1, which is associated with fruit development and produces basal ethylene levels. In contrast, mature fruit belongs to system 2, which is involved in ripening, which increases over time after harvest (Paul et al., 2012). System 1 is regulated in an auto-inhibitory manner, whereas system 2 is autocatalytic (i.e., the production is ethylene-induced) (Oetiker and Yang, 1995). Hence, both respiration and ethylene rates reach the climacteric peak simultaneously or soon afterward. This peak coincides with or follows eating ripeness (Ramina et al., 2008).

In the ripening process of climacteric fruit, ethylene coordinates interactive numerous **signaling and metabolic pathways** for the progress of ripening through a complex transcriptional network (reviewed in Liu et al., 2015; Paul et al., 2012). Ethylene is biosynthesized through methionine by 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) and ACC oxidase (ACO) and perceived through endoplasmic reticulum receptors of plant tissues. In addition to its effect on plant growth and development, ethylene has numerous effects on the senescence, storage life of fruits and is also **involved in responses** to biotic and abiotic stresses.

1.1.2.3. Physical and chemical changes

Associated with fruit development until ripe fruit, first visual changes are loss of green color and development of yellow, red, or other colors intrinsic to cultivar. The **color change** is associated with chlorophyll degradation, and other pigments such as carotenoids are uncovered in the yellow-fleshed cultivars (Ramina et al., 2008). This process is also accompanied by the biosynthesis of anthocyanins (responsible for all colors from blue to red) in either the epidermis or the flesh (Bassi and Monet, 2008). Such alteration of pigmentation content can be used to monitor fruit maturity (Spadoni et al., 2016).

Throughout the development process, the fruit undergoes a continuous process of fruit softening. This decreases **fruit firmness**, which is accelerated as the fruit reaches the harvest date. Fruit softening requires ethylene, which regulates the rate of softening and at the transcriptional level of cell wall metabolism-related enzymes (Hayama et al., 2006). Cell wall modifications involve depolymerization and alterations to various polymers (e.g., matrix glycans and pectin) during the different periods of fruit maturation (Brummell et al., 2004). Enzymes such as cell wall degrading enzymes (CWDE) (e.g., polygalacturonase and pectate lyase) play key roles in maintaining texture and causing softening (Jiang et al., 2020).

Another process involved in fruit development until ripe fruit is the alteration of sugars and organic acids. Along this process, the starch of fruit is converted to sugars, and its acidity declines (Crisosto and Day, 2012). During fruit development, among the total **soluble sugars** in fruit, sucrose increases with time, glucose remains steadily, fructose decreases first and then increases, and sorbitol decreases at the last stages but with low amounts (Desnoues et al., 2014). Hence, the final proportion of the main sugars is about 3:1:1 for sucrose, glucose, and fructose, respectively (Colarič et al., 2004). In parallel, **organic acids** (e.g., malate and citrate), which account for most of the titratable acidity, are impaired during fruit development. In particular, throughout peach development, citric acid increases across time and peaks at the 3rd stage approximately and then starts to decrease, whereas malic acid first decreases and then increases at the last stage (Bae et al., 2014; Baró-Montel et al., 2020).

The composition of **antioxidants compounds** (e.g., phenolic compounds and ascorbic acid) also changes along fruit development. Fruit antioxidant capacity and total phenolic content (although to a lesser extent) peak at the hardening phase and then decrease, whereas ascorbic acid increases along with fruit development (Baró-Montel et al., 2020). As fruit evolves, it also produces **volatile compounds** that

characterize fruit aroma, arising from substrates such as fatty acids, amino acids, phenolics, and terpenoids (Lara et al., 2020). Compounds that confer the green aroma are aldehydes and C6 alcohols (e.g., hexanal, trans-2-hexanal, hexanol, trans-2-hexanol). At the same time, the main components of mature and ripe fruit are lactones (e.g., γ-decalactone and δ-decalactone) (Wang et al., 2009). Other compounds such as benzaldehyde and linalool increase over time. Finally, once the fruit has reached full ripeness, it begins senescence, which includes further softening, loss of desirable flavor, and complete breakdown and death of the tissues (Crisosto and Day, 2012). Hence, all physiological, physical, and chemical changes described herein influence fruit quality from harvest day to consumption day.

1.1.3. Fruit quality

The term "**fruit quality**" englobes mechanical (e.g., mass, volume, and firmness) and sensorial properties (texture, taste, flavor, and aroma), appearance, nutritional value, fruit safety, and defects (i.e., the absence of physiological and pathological disorders) (Crisosto and Costa, 2008). However, the specific meaning of fruit quality (i.e., minim and maxim parameters desired) depends on each step of the fruit chain, from growers to packing houses (e.g., packers, shippers, distributors, wholesalers) to retailers and consumers (Crisosto et al., 2020). That is probably why some of these parameters are established for marketing standards by official regulations (e.g., European Union Commission). Still, some other traits are only recommendations elaborated by researchers to growers and distributors.

Fruit size is determined by the diameter of the equatorial section (cheek diameter, CD), weight, or number of units. Minimum values for **weight** (> 65 g) and **diameter** (> 51 mm) are established depending on the category, but tolerances up to 10% for each measurement are allowed (European Commission, 2019b). Regarding **fruit flesh firmness** (FF), it used to be standardized to 63.7 N of maximum (European Commission, 2004), but the last regulation published in 2019 does not include specifications for this parameter (European Commission, 2019b). In this case, researchers and producers have conducted their studies to establish FF values for both the selling and consumption time. For instance, Crisosto (2002) recommends that fruit should be transferred to the retail store before it reaches 26 – 35 N, but consumers are satisfied when FF is between 3 to 10 N (Bonany et al., 2014).

Several compounds including sugars, organic acids, aromatics (volatiles), and phenolics as well as changes in color, texture, and flavor are among the most known

parameters contributing to the overall organoleptic quality of fruit (Bassi and Monet, 2008; Bae et al., 2014). The sugar content is based on **soluble solids content** (SSC), assessed by a refractometer. High SSC has been the most influencing factor for consumer acceptance of peaches and nectarines. Still, other factors such as **titratable acid** (TA), volatile compounds and the sugar-to-acid ratio (SSC:TA), and even flavor and texture are also perceived by consumers and should be considered (Crisosto and Day, 2012; Iglesias and Echeverría, 2009). It seems that within an acceptable TA range, consumers are more sensitive to the SSC:TA, than SSC alone. The recommended value for SSC of the flesh should be greater than or equal to 8% (or ºBrix) minimum (OECD, 2010; European Commission, 2019b). However, studies demonstrate that consumers prefer grades equal or superior to 10 or even 12% (Crisosto and Crisosto, 2005; Ramina et al., 2008). Regarding TA, which is usually expressed in relation to malic acid concentration (g malic acid L^{-1}), Iglesias and Echeverría (2009) proposed that, in particular for nectarine cultivars, they could be classified as sub-acid (< 3.4) or sweet/equilibrated/acid (3.4 – 6 / 6 – 8 / 8 – 10, respectively) cultivars.

The described fruit quality parameters depend not only on the changes that fruit has taken throughout fruit development until eating ripeness but also on other **external components**. Some of these factors include cultivar type (Iglesias and Echeverría, 2009), environmental conditions (Lopresti et al., 2014), tree management (Mataffo et al., 2020), canopy position (Minas et al., 2018), mineral nutrition (Crisosto and Costa, 2008), harvest date (Reig et al., 2012), harvesting handling and transportation (Crisosto et al., 2020) and storage conditions (Veloso et al., 2021). Hence, since there are a wide variety of external stimuli influencing fruit characteristics and properties that may affect the capacity of fruit to face harmful inputs (i.e., abiotic and biotic stresses), the environment that englobes the fruit throughout its life should be revised and further studied.

1.1.4. Host-environment interaction

Plants are continuously exposed to a variety of environmental stresses. These factors include **abiotic stresses** such as extremes of temperature, high light intensity, drought, air pollutants, salinity, mechanical damage (Vickers et al., 2009), and **biotic stresses** such as pathogen infections and herbivore attacks (Li et al., 2019) (**Figure 2**). To some extent, all these biotic and abiotic stresses cause, among others, an oxidative stress, metabolic imbalances, alteration of hormone responsive pathways and programmed cell death (Alkan and Fortes, 2015; Vickers et al., 2009).

In general, fruit, particularly stone fruit, are affected by those stresses causing physiological and pathological diseases that can occur both at **preharvest** and during the **postharvest chain** (Eckert and Ratnayake, 1983), which could lead to considerable **fruit losses**. The effect of preharvest factors including environmental conditions (e.g., temperature, solar radiation, and precipitation) in the field (reviewed in Crisosto and Costa, 2008; Minas et al., 2018) and during postharvest, especially during storage (e.g., storage atmosphere and temperature) (reviewed in Manganaris and Crisosto, 2020) on peach and nectarine are well reported. However, other factors such as **light** incidence along the postharvest chain (e.g., from packinghouses to market) (**Figure 2**) are less elucidated.

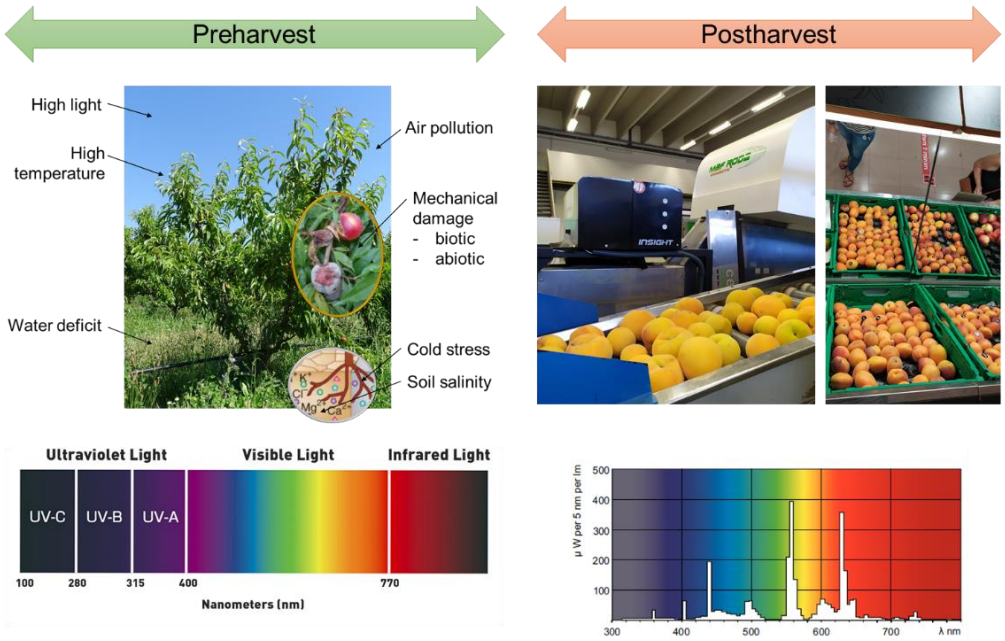

Figure 2. *Environmental stresses that sedentary plants can be submitted in the orchard (preharvest) to throughout the postharvest chain of fruit (i.e., packinghouse and supermarket). Left image displays a nectarine tree with detail of fruit affected by brown rot and roots (image of roots and text adapted from Vickers et al. (2009)). Sunlight spectrum is detailed on the left downside (preharvest) (Lumigrow, 2021), and an example of the light spectrum of fluorescents used in supermarkets is detailed on the right downside (postharvest) (Philips, Spain).*

1.1.4.1. Light

Light regulates many aspects of plant growth and development (Folta and Carvalho, 2015). Plants sense the quantity (intensity), quality (wavelength of electromagnetic spectrum, i.e., color), direction, and duration (photoperiod) of light through chloroplasts and photoreceptors, which depend on the composition of the electromagnetic spectrum (i.e., ultraviolet-B, ultraviolet-A, blue, red, and far-red light) and overall determine its effect on plants (reviewed in Ouzounis et al., 2015; Roeber et al., 2020). In particular, the light that fruit perceives can be altered from factors present in the orchard (e.g., thinning method, fruit position in the canopy, canopy architecture, or even photo-selective nets) but also present along the postharvest chain (e.g., artificial lighting). They can alter physicochemical fruit properties at harvest and postharvest (Ilić and Fallik, 2017), reviewed below.

The first light that fruit receives in the field is **solar radiation**. As a consequence of the global climate change, future episodes will comprise high temperatures but also increases in the UV-B radiation (Hashimoto et al., 1990) (a small fraction of the solar spectrum), the highest-energetic radiation on the earth's surface (Santin et al., 2021). Altitude and harvest time can also influence the source of light. Altitude increases solar radiation, and hence, UV radiation as well. In this line, late-season cultivars are more exposed to sunlight than early-season ones.

There are some **cultural practices** that can alter the solar radiation that fruit perceives in the field. Among them, photo-selective nets are used to manipulate the light quality (i.e., electromagnetic spectrum) to improve several fruit characteristics (e.g., yield and phytochemical composition) but also to protect crops from adverse climate and pests (reviewed in Ilić and Fallik, 2017). Another cultural practice is bagging the fruit (**fruit bagging**), which alters the light transmittance on fruit (Xu et al., 2010). Bags are made of various materials (Ali et al., 2021), and are used to improve the external appearance and reduce pests and fungal diseases in the field (Sharma et al., 2014; Campbell et al., 2021).

All aspects of tree management in the orchard also influence the light incidence on fruit. Pruning and thinning influence the crop load and global canopy architecture, which determines the **fruit position in the canopy** that affects light interception for fruit (Minas et al., 2018). For instance, sunlight can increase fruit shelf-life, as observed in fruit grown outside canopy compared to those produced inside the canopy (Crisosto and Costa, 2008). This fruit, which develops in the lighting positions, is more resistant to internal breakdown. Besides, exterior fruit within the canopy position has

higher weight and SSC and lower flesh firmness than inner peaches (Lewallen and Marini, 2003).

After harvest, the fruit undergoes several environments where light can also affect the fruit. As mentioned, artificial lighting is present along the postharvest chain, from packinghouses to market and consumer houses. This type of light can be found in a photoperiod regime or as postharvest treatments, and studies conducted on stone fruit should be considered. The **use of light** to improve stone fruit crop productivity has been widely studied because solar radiation affects photosynthesis and, in turn, the plant's energy balance (Flore, 1994; Folta and Carvalho, 2015). For instance, blue light strongly influences color change and increases anthocyanin content in sweet cherries (Kokalj et al., 2019). Due to the antioxidant activity of color pigments, the use of light-emitting diodes (LEDs) has been tested on fruit to increase their natural antioxidants for human health benefits. Blue light treatment enhances antioxidant activities (e.g., catalase and ascorbate peroxidase), antioxidant compounds (ascorbic acid), radical-scavenging activity, and fruit quality (increase of total sugar content and titratable acidity) in strawberries during storage (Xu et al., 2014). In stone fruit, several authors have reported the effect of concrete sections of the visible spectrum on stone fruit, such as blue light on the enhancement of the fruit quality of peaches (Gong et al., 2015), and UV-B light's effect on the loss of firmness but without affecting the SSC and the titratable acidity (Santin et al., 2019).

Light can also modulate **plant responses to stress**, both abiotic (e.g., enhance thermotolerance and improve drought tolerance) and biotic (e.g., reducing growth-defense trade-offs) (Roeber et al., 2020). For instance, solar UV-B radiation affects plant defense signaling through jasmonic acid-mediated responses (Ballaré, 2014) and the phenolics response to the pathogen *Monilinia fructicola* (Santin et al., 2018).

1.1.4.2. Biotic stresses: fungal diseases

Many pathogenic agents such as fungi, bacteria, viruses, viroids, insects, mites, and nematodes can cause diseases in all parts of trees (e.g., blossoms, foliage, branches, trunks, fruit) and all stages of the development of stone fruit crops.

Phytopathogenic fungi are the most common causing-disease agent, provoking diseases such as brown rot (*Monilinia* spp.), peach leaf curl (*Taphrina deformans*), powdery mildew (*Podosphaera pannosa*), rust (*Tranzschelia* spp.), peach scab (*Fusicladosporium* spp.), among others, in **blossoms, foliage, and fruit** (Adaskaveg et al., 2008). After harvest, some of these fungi are also responsible for the greatest

deterioration problem along the postharvest chain (Crisosto and Day, 2012). The main **postharvest diseases** are brown rot, Rhizopus Rot (*Rhizopus* spp.), grey mould (*Botrytis cinerea*), and *Mucor* decay (*Muccor piriformis*) (Mari et al., 2019). Other diseases such as sour rot (*Geotrichum candidum*), blue mold (*Penicillium* spp.), Alternaria rot (*Alternaria* spp.), and Cladosporium Rot (*Cladosporium* spp.), can also occur to a lesser extent (Mari et al., 2019). Among them, **brown rot** caused by *Monilinia* spp. is the main stone fruit disease that appears in the field and during postharvest storage and chain. Considerable efforts are being conducted to minimize this disease worldwide.

1.2. Brown rot

Brown rot is caused by fungi of the ***Monilinia* genus**, which belongs to the *Sclerotiniaceae* family. This disease affects stone fruit trees of the *Rosaceae* family, mainly to *Prunus* genus, such as peaches, nectarines, cherries, apricots, and almonds. It can also affect pome fruits (*Malus* and *Pyrus* genus) (Byrde and Willetts, 1977).

Fruit losses caused by *Monilinia* spp. and their **economic impact** are considerable, although they are difficult to predict precisely. The incidence of brown rot in peach and nectarine orchards can reach up to 7% on the harvest day, but if the incidence of brown rot on harvest day is 30%, all production would be lost after one week of their harvest (Villarino et al., 2012). In line with this, in highly favorable conditions, the incidence of brown rot from untreated trees can reach up to 80%, more than double of treated trees, seen in Spanish and Italian peach orchards after one week at shelf-life conditions (Larena et al., 2005). Thus, fruit losses in postharvest are greater than orchard losses in Europe, also seen in California nectarine orchards (Hong et al., 1997). However, usually, **annual crop losses** due to brown rot are lower. Thus, by calculating an average percentage of losses (10%), the losses are estimated to be 1.7 M euros for peach and nectarine worldwide (Martini and Mari, 2014).

1.2.1. *Monilinia* spp.

Out of the 39 species (including subspecies and formae speciales) described for the *Monilinia* genus (Mycobank, 2021), the main three species that cause brown rot are ***M. laxa*, *M. fructicola***, and ***M. fructigena***. Their behavior in fruit characterizes them, in which *M. laxa* usually forms a cottony layer of greenish-gray color, *M. fructicola* produces a very dusty brownish sporulation, and M. *fructigena* grows in concentric circles of white-beige color (**Figure 3**). As hosts, *M. laxa* and *M. fructicola* are more

common in stone fruit, whereas *M. fructigena* is more recurrent in pome fruits like apples and pears (Byrde and Willetts, 1977).

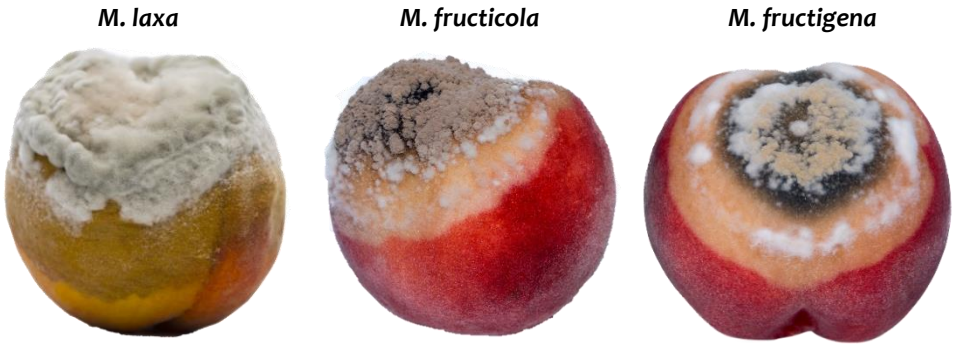

Figure 3. *Brown rot in fruit caused by* Monilinia *spp. Images are courtesy of the Postharvest Program of IRTA.*

Brown rot is present in all **stone fruit areas worldwide** and is epidemic in many countries. *Monilinia fructicola* was first detected in Europe in 2001 (Lichou et al., 2002), and it was not detected in Spain until 2006 (De Cal et al., 2009). The frequency of this species has increased over the years in Spanish orchards (Villarino et al., 2013). *Monilinia laxa* is the main causal agent of brown rot in Europe (Rungjindamai et al., 2014), and nowadays, it is also found worldwide (Obi et al., 2018). Currently, both species coexist in European orchards, although *M. fructicola* is more virulent than *M. laxa* (Kreidl et al., 2015; Villarino et al., 2016).

Monilinia spp. are **necrotrophic fungi**, i.e., they can colonize fruit tissues causing cellular death and feeding on cell remains to grow and reproduce. In **the field**, the pathogen can rot all parts of the tree, including buds, sprouts, branches, flowers, and fruit (Villarino et al., 2010). The species of *Monilinia* are polycyclic pathogens since they reproduce through numerous secondary cycles following the growth cycle of the crop (Obi et al., 2018). The fungus can survive winter in several tissues, such as mummified fruit (Casals et al., 2015) and canker on twigs (Kreidl et al., 2015), resulting in a primary source of inoculum in the spring (Villarino et al., 2010). When favoring conditions allow the infection in any tissue, infected tissue (e.g., buds, blossoms, fruit) can serve as a secondary source of inoculum for infecting other tree parts (Villarino et al., 2012). In **postharvest**, where greater losses than in the orchard occur (Hong et al., 1997; Villarino et al., 2012), it is believed that the infections occur in the field and appear along the postharvest chain rather than infections at postharvest (Casals et al.,

2021). Hence, a vast range of factors influences the brown rot manifestation through the development and shelf-life of fruit.

1.2.2. Favoring factors

Plant disease epidemics occur because of the timely combination of up to five elements: a plant (e.g., susceptible hosts) and a virulent pathogen must contact and interact in a proper environment over some time and also be influenced by human actions, which can accelerate or delay the disease (Agrios, 2005a, b). In line with this, several factors such as **virulence factors of fungi** (e.g., cell-wall degrading enzymes), **environment** (e.g., humectation period and light), favorable **host factors** (e.g., fruit developmental stage), **characteristics** intrinsic to the stone fruit cultivar (e.g., cuticular composition), and **control strategies** (e.g., preventive and curative), are crucial to trigger the cycle of brown rot (Mustafa et al., 2021).

1.2.2.1. Environment

Climatic conditions are crucial in all stages of *Monilinia* spp. infection process. Temperature and wetness period influence penetration and spread of both *M. laxa* (Gell et al., 2008) and *M. fructicola* (Luo and Michailides, 2001). Villarino et al. (2012) found that temperature can explain up to 82% of the incidence of brown rot after harvest. The optimal **temperature** to reach the highest rot development rate for both *Monilinia* spp. is 25 ºC on peaches and nectarines (Bernat et al., 2017). In particular, the optimal range of *M. fructicola* to cause decay and mycelia is higher (20-33 °C) than that for *M. laxa* (15-30 °C), although *M. laxa* only develops mycelia in a narrower and lower range of temperature (20-25 ºC) (Bernat et al., 2017). However, both *Monilinia* spp. can germinate out of those ranges (0 to 35 ºC) (Casals et al., 2010).

The germination of spores of *Monilinia* spp. also depends on the **water availability**, which needs to be above 0.87 of water activity (Casals et al., 2010). In this line, the incidence of latent infections of *M. laxa* and *M. fructicola* increases with a more extended **wetness period** (Gell et al., 2008; Luo and Michailides, 2001; respectively). Other factors such as **solar radiation**, **wind speed**, and **rainfall** along the crop season influence the dynamics of spore density of *M. laxa* and *M. fructicola* (Gell et al., 2009). Hence, climatic conditions of high temperature and some periods of humectation (e.g., late-season cultivars) will favor the development of brown rot in the orchard. Contrary, fruit storage between −1 °C to 0°C will retard the appearance of disease symptoms.

1.2.2.2. Light: effects on fungal behavior

Like plants, fungi sense light and use it as an **input of information** to induce adaptative responses to regulate fungal behavior and development (Tisch and Schmoll, 2010; Corrochano, 2019). Hence, light can be perceived as both necessary and as abiotic stress. Fungi can sense the quantity, quality, direction, and duration of light through a complex of photoreceptors (Idnurm and Crosson, 2009; Tisch and Schmoll, 2010; Schumacher and Gorbushina, 2020). Fungi like *Botrytis cinerea*, a closely related specie to *Monilinia* spp., have a broad action spectrum by responding to the **entire visible spectrum and beyond** (Schumacher, 2017). Recently, photoreceptors for each monochromatic section of the spectrum and photoresponse-related regulatory family of velvet proteins have been described for *M. laxa* (Rodríguez-Pires et al., 2021). Like fruit, fungi can perceive light from similar **sources** to the ones described for fruit (reviewed in 1.1.4.1). The effect of light at the gene expression level has been elucidated for several biological functions in the main three *Monilinia* spp. (De Miccolis Angelini et al., 2018). Hence, within its composition, light can alter fungal biology in all conditions in which fungi can remain (e.g., environment, on fruit, *in vitro*).

The effect of light on fungal biological responses such as **conidiation** (i.e., the transition from sexual to asexual reproduction) (Corrochano, 2019) can be *Monilinia* spp. dependent. Whereas *M. fructicola* can produce conidia both under complete darkness (Tran et al., 2020) and under photoperiod (Baró-Montel et al., 2019a) in *in vitro* conditions, *M. laxa* only produces conidia under photoperiod and remain in fluffy vegetative mycelium under complete darkness (Rodríguez-Pires et al., 2021). Light can also induce **pigmentation** in several fungi, such as carotenoids and melanin (Fuller et al., 2015; Corrochano, 2019). In fact, *M. laxa* grown under dark conditions reveals an off-white mycelium (Rodríguez-Pires et al., 2021). Regarding **colony growth** in *in vitro* conditions, red light accelerates the growth rate of *M. laxa* compared to daylight, black and green light, all under a photoperiod regime (Rodríguez-Pires et al., 2021). Light can also alter **cell viability**, although it has not been described for either *M. fructicola* or *M. laxa*. In other species such as *Penicillium digitatum*, continuous blue light, and complete darkness increase not only *in vitro* cell viability but also its **capability to infect** oranges compared to non-continuous light (Lafuente et al., 2018). Along this line, the infection of *B. cinerea* proceeds better when the hyphae are protected from direct light, i.e., under darkness or once hyphae have penetrated the host (Schumacher, 2017). Hence, in addition to the light effect on fungal behavior, light also influences the interaction with its hosts. Thus, the **pathogen-host-**

environment system becomes a complex interaction to study (Carvalho and Castillo, 2018).

1.2.2.3. Developmental stage and intrinsic fruit characteristics

Monilinia spp. can infect fruit at any **growth stage**, although fruit susceptibility increases with maturation. Usually, fruit is susceptible at initial stages (SI and SII, cell division and pit hardening), then it becomes less susceptible at stage SIII (growth restarts: cell enlargement and elongation), which is about one month before harvest, and susceptibility increases as the harvest day approaches (stage SIII). However, the **degree of susceptibility** may also depend on *Monilinia* spp. For instance, ***M. fructicola*** presents the afore-mentioned behavior on prune (Luo and Michailides, 2001) but can cause disease in all four stages of peach (Baró-Montel et al., 2020). Regarding ***M. laxa***, it follows the mentioned pattern in peaches and apricots (Mari et al., 2003; Guidarelli et al., 2014), but aggressive *M. laxa* strains can cause a similar degree of disease in all four stages of peach (Baró-Montel et al., 2020).

The fact that susceptibility to *Monilinia* spp. increases with maturation can be explained for several reasons related to the physical, chemical, and physiological **modifications** ongoing during fruit development. Continuing changes related to cell enlargement affecting surface integrity may also be part of such susceptibility. The expansion rate causes cuticular cracking, favorable for *Monilinia* spp. infections, and thus, bigger fruits can be more susceptible (Bellingeri et al., 2018). The chemical composition is also relevant herein. The increasing content of sucrose (Baró-Montel et al., 2020) and SSC (Gradziel, 1994) in fruit development also favors the availability of carbon sources and energy for fungi. In parallel, the decrease of surface conductance and changes in cuticular wax composition (e.g., alkanes increase) at the end of fruit growth stages also increases susceptibility to *M. laxa* but in a cultivar-dependent manner (Lino et al., 2020). More details are addressed in section 3.1.

1.2.3. Infection development and virulence factors

The **infection process** starts when the pathogen and the host come into contact. Once conidia of *Monilinia* spp. are adhered to the cuticle of the fruit surface, the process begins when it germinates to produce a germ tube (Lino et al., 2016). At that stage, if the combination of factors (e.g., environment and intrinsic fruit properties) is not favorable for the development of the disease, the pathogen can remain **quiescent** on plant tissue surfaces (Luo et al., 2005) or cause **latent infections** (Garcia-Benitez et al., 2020) in which favorable conditions will trigger the disease cycle. When conditions are

favorable, germ tube develops and can penetrate directly through skin cracks, wounds, or indirectly through stomata. Besides, *Monilinia* spp. can penetrate the cuticle by developing appressoria (**Figure 4**) (Lino et al., 2016).

At that stage, *Monilinia* spp. increase its **infection machinery**, deploying an extensive collection of proteins (Rodríguez-Pires et al., 2020b). The availability of *Monilinia* spp. genome sequences have provided new sources to increase the knowledge of the infective processes, like deciphering the different behavior among *Monilinia* spp. (Marcet-Houben et al., 2021). As a necrotrophic pathogen, it possesses a large family of cell wall degrading enzymes (CWDEs) (Agrios, 2005c), such as endopolygalacturonases, pectin methyl esterases, and rhamnogalacturonan hydrolases described for the main three *Monilinia* spp. (Chou et al., 2015; De Miccolis Angelini et al., 2018; Baró-Montel et al., 2019b; Rodríguez-Pires et al., 2020a). *Monilinia* spp. can also induce phytotoxic activity (Garcia-Benitez et al., 2019) and the synthesis of melanin (described in *M. fructicola*) (Yu et al., 2020). To favor the environment for the proper activity and production of enzymes (Prusky et al., 2020), both *M. laxa* and *M. fructicola* can acidify the tissue around the infection site (De Cal et al., 2013; Baró-Montel et al., 2019b). Necrotrophic pathogens can also detoxify plant defense compounds that are present or produced by plants (Westrick et al., 2021). Recently, several effectors (proteins secreted to manipulate the defense responses of host cells) have been identified in *M. fructicola,* suggesting their role in inducing cell death in stone fruit (Vilanova et al., 2021).

Once the infection is established, the pathogen starts the **colonization** of the epidermis and mesocarp, causing exocarp necrosis and rupture of the epidermis, total cell degradation of the cuticle and the epidermis (reviewed in Lino et al., 2016).

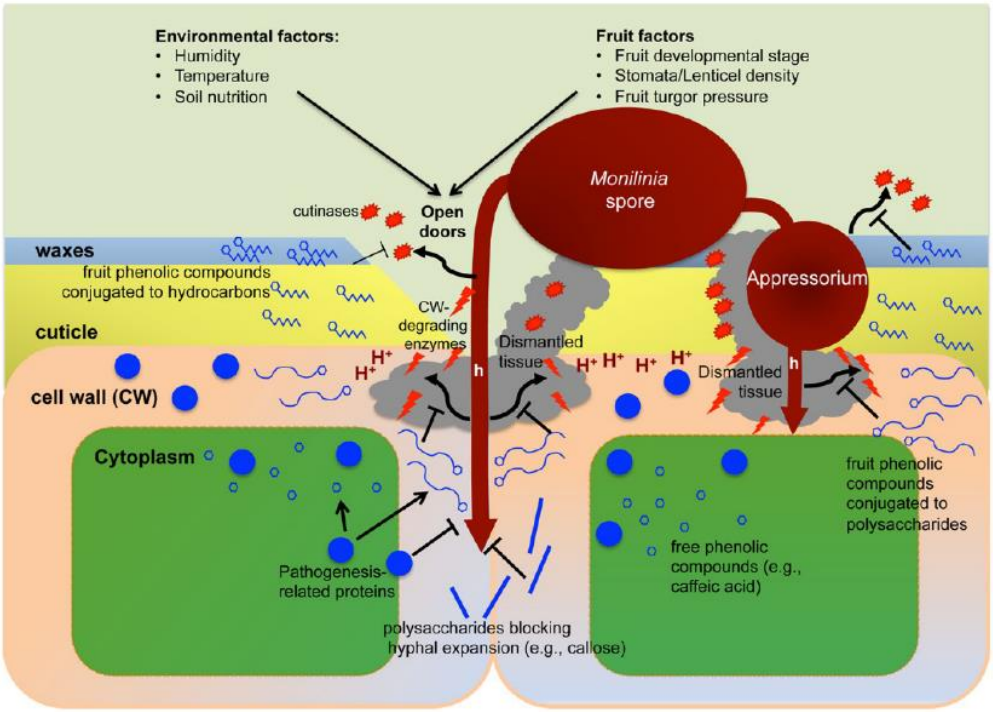

Figure 4. *Main components of the biochemical warfare between* Monilinia *spp. fungi and* Prunus *fruits (Lino et al., 2016).*

1.2.4. Control strategies

Brown rot is controlled with a combination of cultural practices, chemical fungicides applied both in the orchard and postharvest, and controlled postharvest storage, all defined under **integrated disease management** (Rungjindamai et al., 2014). **Cultural practices** include tree management such as training and pruning (Bussi et al., 2015; Bellingeri et al., 2018) and removing natural inoculum sources such as primary (e.g., from mummified fruit) or secondary ones (e.g., from infected fruit) (Villarino et al., 2012; Casals et al., 2015). During harvest and packing operations, careful handling is also crucial to avoid or reduce mechanical injuries, which would make the fruit more susceptible to plant pathogens (Crisosto and Day, 2012). **Storage conditions** are crucial to minimize the losses associated with rotting organisms and reduce deterioration in susceptible cultivars to physiological disorders (Crisosto and Day, 2012). Peaches and nectarines are stored at −1 °C to 0°C, in which stone fruit can last around 2-4 weeks (Crisosto et al., 1995).

Chemical fungicides are applied in the field using an established program (Mari et al., 2019), acting both preventively and curatively, depending on when infections occur

(Casals et al., 2021). For instance, some of the registered fungicides in Spain are Luna® Privilege and Folicur® of Bayer, based on fluopyram and tebuconazole, respectively (MAPA, 2022). However, there have been reported, for instance, the appearance of mutations in *M. laxa* that confer conventional fungicide resistance in California and Spanish orchards (Ma et al., 2005; Egüen et al., 2016). Besides, social pressure has increased the demand for chemical-free fresh fruit due to the increasing health concerns related to the environmental print, leading to the implementation of **alternative technologies** (Obi et al., 2018). Actually, the European Green Deal, which aims to overcome the challenges caused by climate change and environmental degradation, covers, among others, the use of sustainable pesticides and developing more organic farming systems (European Commission, 2019a). Several strategies have been proposed to control brown rot in the field and postharvest. Only a few are currently applied under commercial conditions (Usall et al., 2015), described below.

Biocontrol products and integrated approaches based on the combination and/or the alternation of biocontrol agents (BCA) and fungicides have been proven effective in controlling brown rot in stone fruit in the field (De Curtis et al., 2019; Casals et al., 2021). Currently, only three BCA products based on *Bacillus subtilis* (Serenade®), *Bacillus amyloliquefaciens* (Amylo-X®), or *Saccharomyces cerevisiae* (Julietta®) are authorized for organic cultivars to control brown rot in Spain (MAPA, 2022). Other authorized products in Spain are ARMICARB® based on potassium bicarbonate (MAPA, 2022). In line with these strategies, another one is the application of **treatments** such as physical means, natural and synthetic chemicals, biocontrol agents, and plant volatiles organic compounds (among others) to increase both the activation of the natural defense system of plants (**induction resistance**) (Romanazzi et al., 2016) and give direct effects on the pathogens (Tian et al., 2016). Other ongoing control strategies are cultivar **breeding strategies** for biotic resistance (Obi et al., 2019), which consist of researching resistance regions in the genome. Some quantitative traits have already been described in peach against *Monilinia* spp. (reviewed in Marimon de María, 2020).

Chemical fungicides can also be applied during postharvest to control brown rot. For instance, an authorized product in Spain is Scholar®, based on Fluidoxonil (MAPA, 2022). When postharvest chemical treatments are insufficient, the current strategy is to apply synthetic chemical products in the packinghouse (Mari et al., 2019). In this line, sanitization practices in the packinghouse are crucial to reduce the inoculum of pathogens present, such as spores of *Penicillium* spp. (Mari et al., 2019).

As described, there is considerable research for improving the preharvest and postharvest treatments to control brown rot, most of them in the line of minimizing the use of chemicals. The interest towards **sustainable and organic agriculture** has increased over the years.

1.2.3.1. Fruit bagging

Fruit bagging is a **mechanical technique** that consists of introducing the fruit into a bag during the stone hardening phase and after thinning, between 2 to 4 months before harvest (growth stage SII approximately), depending on the cultivar. The bags remain on fruit during all maturity stages and are removed after harvest. Bags can be of many materials (e.g., paraffin, nylon, plastic, paper) and different colors (e.g., white, yellow, brown) (Ali et al., 2021). Paper bags are usually impregnated with wax to resist water and are tightened to branches with a staple with the fruit inside (**Figure 5**).

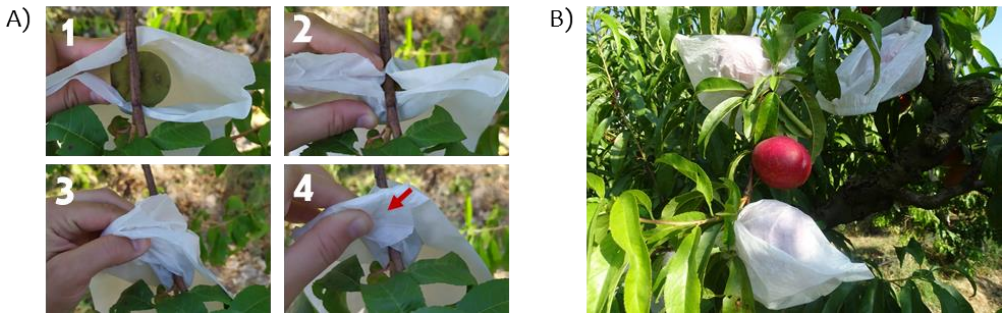

Figure 5. *Fruit bagging with white paper bags. A) Process of bagging consists of 4 steps. The red arrow indicates the position of the staple. B) Bagged and unbagged nectarines in the field before harvest.*

The pioneer of the bagging technique was Japan for its use in pears and grapes, but it is now widely applied for several crops (tropical fruit, apples, peaches, etc.) in Asia, Australia, and the USA (Ali et al., 2021), and later to Europe (Hudina and Stampar, 2011). The bagging technique can be employed both in **traditional and organic orchards**. In Spain, bagging is widely used for "Calanda" peaches, first used to protect the fruit against the Mediterranean fly, contact with pesticides, and climatic incidences (Faci et al., 2014). After, growers realized that fruit peel acquired a more homogeneous yellow color than before, which the consumers highly appreciated. In organic orchards, its use has increased in late cultivars, in which the pressure of flies and fungal diseases, such as brown rot, is considerably high. Some authors have already reported that fruit

bagging reduces the incidence of brown rot in non-treated plums (Keske et al., 2014) and organic peaches (Campbell et al., 2021). The price of papers bags can be, e.g., 0.008 € each in Spain, and bagging one ha of peaches or nectarines (e.g., 625 trees) can cost up to 2.800 € for the grower. However, the benefits of fruit bagging for reducing fruit losses are much higher than such costs, although economic analysis should be performed to estimate better rentability.

As mentioned above (section 1.1.4.1), bags alter the sunlight that fruit perceives (Xu et al., 2010) and so can change many **physicochemical properties and appearance qualities**. Among them, bagging can increase size and weight, leading to a marketable yield increase (Allran, 2017); it can reduce cracking and russeting incidence (Campbell et al., 2021), improve color development (e.g., decrease in carotenoids content) (Liu et al., 2015b; Zhou et al., 2019; Zhu et al., 2020) and increases volatile aroma content (Jia et al., 2005). The type of bag also influences appearance qualities and properties, e.g., yellow paper bags decrease the red color and anthocyanin content in peach skin compared to white polypropylene bags (Liu et al., 2015b). However, all effects on fruit, including responses to pathogens, can depend on the type of bag used, the harvest time, and even the intrinsic properties of cultivar, and current information related to that in stone fruit is scarce.

1.2.5. Host-pathogen and host-pathogen-environment interaction

The study of the **host-pathogen interaction** is one of the most promising tools to improve the current strategies for brown rot control and even look for new ones. Thanks to the availability of genomes of both players, *Monilinia* spp. (Landi et al., 2018, 2020; Naranjo-Ortíz et al., 2018; Rivera et al., 2018; De Miccolis Angelini et al., 2019; Vilanova et al., 2021) and *Prunus persica* (Verde et al., 2013, 2017), information regarding virulence factors and plant defense mechanisms that, together with transcriptomics and proteomics studies, contribute to the current understanding of *Monilinia* spp.-stone fruit interactions. Studies at genomic, transcriptomic or proteomic level relay on research conducted on *Monilinia* spp. for one side, (Rodríguez-Pires et al., 2020b; Marcet-Houben et al., 2021; Vilanova et al., 2021) and on stone fruit in the other side (Guidarelli et al., 2014; Papavasileiou et al., 2020). Hence, such as observed in other pathosystems like Arabidopsis-*B. cinerea* (Zhang et al., 2019), omics studies aiming to provide simultaneous responses to this interaction are of potential interest.

As hosts and pathogens are not alone in this game, the **environment** should also be considered. Daily, crops face a complex environment with multiple stressors acting simultaneously (Santin et al., 2018). Hence, to study the postharvest fruit behavior toward a combined biotic and abiotic factor such **light** is crucial. The role of light is more studied in the interaction of *B. cinerea* and its hosts than in *Monilinia* spp.-stone fruit. For instance, white and green light decreases lesion diameter in *B. cinerea*-inoculated grapes (Zhu et al., 2013). Few studies regarding the interaction of *Monilinia* spp. and its hosts have been conducted. One of them has shown that a photoperiod with high intensity of white light increases the diameter length of lesions caused by *M. laxa* in nectarine fruit, compared to a photoperiod with low intensity or complete darkness. However, the incidence of *M. laxa* on fruit was not affected across conditions (Rodríguez-Pires et al., 2020). However, no studies aimed to decipher this abiotic factor in *M. fructicola*-stone fruit interaction.

Hence, because light can affect brown rot development on stone fruit, it is crucial to understand the mechanism that plants pose to face *Monilinia* spp. and the responses in front of these pathogens.

1.3. Fruit responses to *Monilinia* spp.

The arrival of spores of *Monilinia* spp. on the fruit surface triggers complex multifactorial responses and actions to face the pathogen attack. In addition to the mechanisms generated by both the fungus and the fruit (seen in Figure 4), together with all factors favoring *Monilinia* spp. infection, the fruit actions that undergo and invoke can be classified in multiple levels. The combination of all elements will determine the outcome of the disease (**Figure 6**).

1.3.1. Constitutive and inducible defenses

Plants possess constitutive and inducible defenses to face pathogens. **Constitutive defenses** (or passive defenses) include inherent properties that are in plants before the pathogen's arrival, which can be physical and chemical barriers present in the fruit surface (Lino et al., 2016). The first **physical level** of defense is the epicuticular wax layer that covers the cuticle, comprised of a mixture of hydrocarbons and alkenes. These may form crystals that prevent the film of water formation for the germination of the spore. The second barrier is the cuticle, which consists of a structure of hydrocarbon polymers and cutin. It may display attributes such as trichomes or natural openings such as stomata. The cuticle is also considered a **chemical barrier** because of various biochemical compounds (i.e., multicomponent barrier).

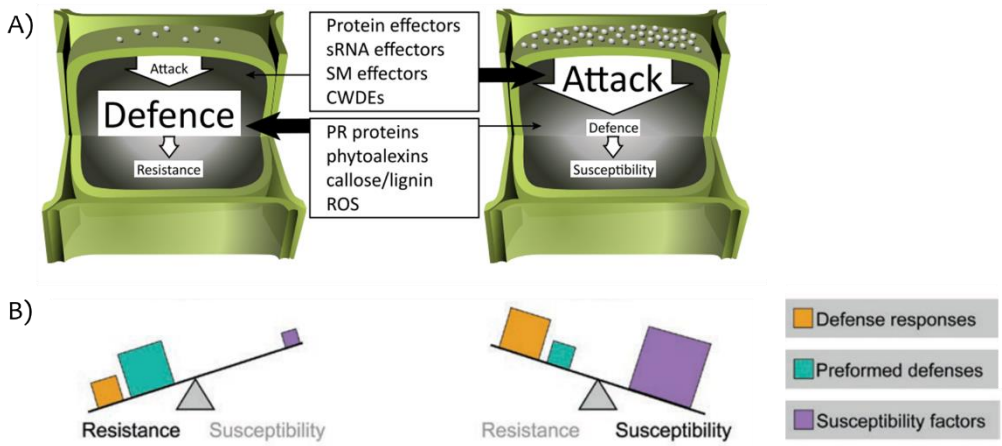

Figure 6. *Balance of events and factors that leads to plant resistance or susceptibility to pathogens.* ***A)*** *Favoring factor (e.g., spore density) regulates the amplitude of attack and defense in* Botrytis*-plant, which can be attributed to any favoring factor in* Monilinia *spp.-stone fruit. Boxes list important fungal attack components (upper box) and plant defense components (lower box), respectively (adapted from Veloso and van Kan, 2018).* ***B)*** *Contributing factors to resistance or susceptibility in fruit, in which sizes of squares indicate the relative magnitude of that feature (adapted from Silva et al., 2021).*

The epidermis cell wall is the last physical barrier, mainly reinforced with lignin, that varies in composition and thickness (Lino et al., 2016). Phenolic acids are one of the most studied compounds of the chemical barrier for the resistance to brown rot (Lino et al., 2016) since they can act as antioxidants against the pathogen attack (preformed antifungal compounds). Phenolic compounds (e.g., chlorogenic acid) can inhibit the cutinase activity in *M. fructicola* (Lee and Bostock, 2007). In addition, high phenolics content, such as the high content of chlorogenic and neochlorogenic acids present in immature fruit, can inhibit *M. laxa* melanin biosynthesis (Villarino et al., 2011), which is a determinant factor for peach infection (De Cal and Melgarejo, 1993). Along this line, some peach cultivars with high content phenolic compounds have shown to be less susceptible to *M. laxa* (Gununu et al., 2019).

Inducible defenses are triggered in response to the detection of the pathogen (Pandey et al., 2016) and involucrate several metabolic pathways and metabolisms. The interaction between the pathogen and the host takes place when the plant's pattern recognition receptors (PRRs) identify the pathogen-associated molecular patterns (PAMP) (Zipfel, 2014). This interaction induces a response called PAMP-triggered immunity (PTI) (first layer of innate immunity) which triggers signaling

cascades and transcriptome reprogramming. However, when pathogens produce virulence factors or effectors, and these are recognized by resistance (R) proteins receptors, these receptors induce a response called effector-triggered immunity (ETI) (the second layer of innate immunity), suppressing the PTI to facilitate pathogenesis. This recognition is faster and more robust than PTI respons and often culminates in hypersensitive cell death response (HR) (reviewed in Jones and Dangl, 2006; Pandey et al., 2016), limiting nutrients and water to restrain pathogen growth.

One of the earliest cellular responses is the **oxidative burst**, generated from reactive oxygen species (ROS) production via oxygen consumption. ROS is mainly produced in NADPH oxidases and cell wall peroxidases. It may have multiple functions, such as strengthening host cell walls and signaling molecules for defense gene activation (Torres et al., 2006). Another defense mechanism is the induction of defense proteins such as defensins (i.e., small antimicrobial peptides), hydrolytic enzymes (e.g., chitinases), and pathogenesis-related proteins (PR) (Lino et al., 2016).

Furthermore, both immunity responses can induce the host **hormone signaling** transduction pathway (**Figure 7**). Jasmonic acid and ethylene are actively involved in defense against necrotrophic pathogens, but the salicylic acid pathway has also been shown to be involved in such response (reviewed AbuQamar et al., 2017). All these hormones are connected in a vast and complex network and participate in other stresses (e.g., drought and heat tolerance; reviewed in Müller and Munné-Bosch (2015). In particular, Jasmonic and ethylene mediate the host's response against necrotrophs (Glazebrook, 2005), acting as signaling molecules. Besides, as mentioned before, ethylene is involved in the ripening and senescence process, which are conductive to disease susceptibility (Van Der Ent and Pieterse, 2012; Blanco-Ulate et al., 2013). Hence, ethylene can have a dual role in plant defense responses. Finally, other responses include the production of **secondary metabolites** (**Figure 7**), also involved in many other abiotic stresses (Bartwal et al., 2013).

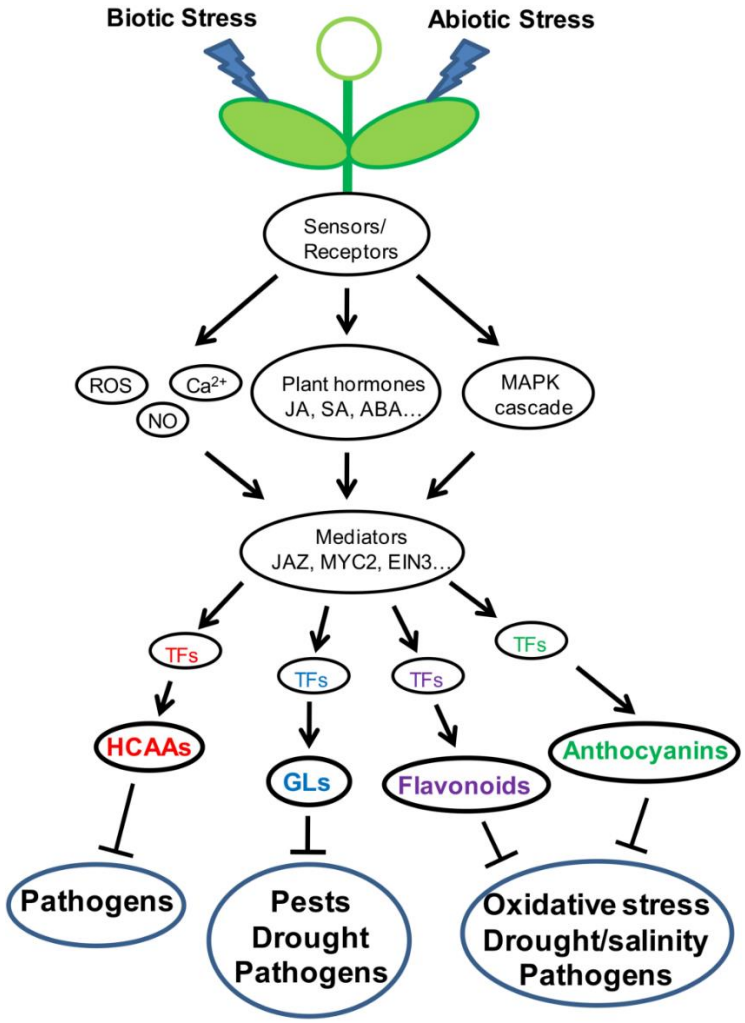

Figure 7*. Signal transduction and regulation of secondary metabolism in response to biotic/abiotic stresses in* Arabidopsis*. Upon a stress, the plant induces many molecules and processes such as plant hormones (Jasmonic acid (JA), salicylic acid (SA), abscisic acid (ABA)), ROS, Ca^{2+}, and MAPK cascade. Signals are transduced by mediators such as JAZ, MYC2, EIN3 to the downstream transcription factors (TFs), which regulate gene expression and subsequent biosynthesis of secondary metabolites (e.g., hydroxycinnamic acid amide (HCAA), glucosinolates (GLs), flavonoids, and anthocyanins) that are involved in different resistances (blue circles) (Meraj et al., 2020).*

1.3.2. Secondary metabolism

Secondary metabolites are a diverse group of plant **organic compounds** widely distributed in plants. These compounds are of low molecular weight and are synthesized in low concentrations. These compounds are not directly involved in growth, development, or reproduction of plants, but they do have a crucial role in protective functions under **biotic and abiotic stresses**, such as antimicrobial, photoprotective, structure-stabilizing, and signaling functions (Bartwal et al., 2013; Liu et al., 2017; Khare et al., 2020) (**Figure 7**).

Secondary metabolites can be classified into three major groups: **Terpenoids** (terpenes or isoprenoids), **phenolics,** and **nitrogen or sulfur-containing** compounds (Lara et al., 2020). These compounds are derived from main primary pathways, including glycolysis, the tricarboxylic citric acid cycle, the pentose phosphate pathway, aliphatic and aromatic amino acids, and shikimic acid pathway (Aharoni et al., 2005; Khare et al., 2020) (**Figure 8**).

Terpenoids represent the largest and most diverse class of secondary metabolites and contribute fruity characteristics to peach (Abbas et al., 2017; Lara et al., 2020). All terpenoids are derived from the five-carbon (C5) precursor isopentenyl diphosphate (IPP) and its double-bond isomer dimethylallyl diphosphate (DMAPP) (Tholl, 2015), considered "isoprene units" (Lara et al., 2020). Their biosynthesis comes from two pathways, the cytosolic mevalonic acid (MVA) pathway, which predominantly provides the precursors for sesquiterpenoids, brassinosteroids, and triterpenoids, and the plastidial methylerythritol phosphate (MEP/DOXP) pathway, which supplies precursors for hemiterpenoids, monoterpenoids, diterpenoids and carotenoids (Tholl, 2015). Terpenoids can be classified according to their number of units (reviewed in Bartwal et al., 2013):

- Monoterpenes, 2 isoprene units (C10 terpenes)
- Sesquiterpenes, 3 isoprene units (C15 terpenes)
- Diterpenes, 4 isoprene units (C20 terpenes)
- Triterpenes, 6 isoprene units (C30 terpenes)
- Tetraterpenes, 8 isoprene units (C40 terpenes)
- Polyterpenoids, 8 isoprene units (> C40 terpenes)

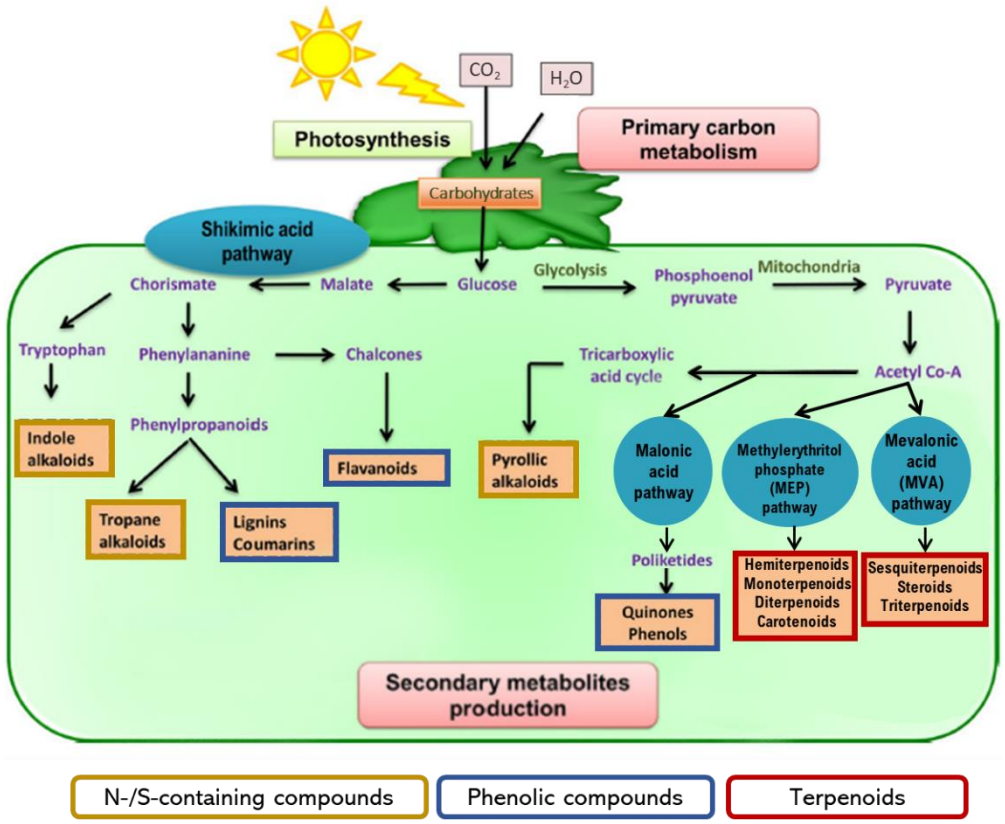

Figure 8. *Biochemical pathways conducting the synthesis of secondary metabolites in plants, classified as nitrogen or sulfur-containing compounds, phenolic compounds, and terpenoids. Adapted from Khare et al. (2020) and Lara et al. (2020).*

Phenolic compounds cover a large group of monomeric and polymeric phenols and polyphenols. Phenylpropanoids, whose pathway initiates with phenylalanine, are simple phenolic compounds that serve as precursors of compounds such as benzoic acid derivatives, flavonoids (flavones, flavanones, isoflavones, flavonols, 3-deoxy flavonoids, and anthocyanins), coumarins, stilbenes, lignans and lignins, and condensed tannins. Phenolic acids also include derivatives of benzoic acid such as hydroxybenzoic acids and derivatives of cinnamic acid, called "hydroxycinnamic acids" (e.g., caffeic, p-coumaric, and chlorogenic acids) (Lara et al., 2020).

The **nitrogen and/or sulfur-containing compounds** group is large and diverse, which includes alkaloids (derived from amino acids), glucosinolates (nitrogen- and sulfur-containing metabolites), cyanogenic glucosides, glutathione, glucosinolates, phytoalexins, thionins, defensins, and lectins (Bartwal et al., 2013; Lara et al., 2020).

Some secondary metabolites can be emitted from plant cells and termed "**volatile organic compounds"** (VOCs). In particular, volatile terpenoids constitute the largest class of plant volatile compounds and are predominantly isoprenes, monoterpenes, and sesquiterpenes (Abbas et al., 2017; Lara et al., 2020). **Peach volatiles** can be classified in many ways. The most common categories are esters, alcohols, aldehydes, C6 compounds, C9 compounds, lactones, ketones, terpenoids, C13 norisoprenoid, and phenylalanine derived compounds (Wang et al., 2009; Montero-Prado et al., 2013; Xi et al., 2017).

The role of VOCs has been described in several environments. For instance, peach volatiles can be naturally emitted in response to **biotic stresses**. Peaches inoculated with *M. fructicola* emitted a different volatile profile pattern, such as increasing or inducing the production of compounds such aldehydes (e.g., benzaldehyde) and esters (e.g., heptyl acetate and ethyl benzoate) or decreasing or inhibiting the production of other aldehydes (e.g., hexanal), esters (e.g., hexyl acetate) and alcohols (e.g., E-2-hexen-1-ol) (Liu et al., 2018). Besides, peach volatiles have an antifungal activity tested in *in vitro* conditions, and several chemical VOCs are effective in controlling postharvest decay under laboratory conditions (Mari et al., 2016). However, VOCs with antimicrobial activity such as alcohols, aldehydes, essential oils, and isothiocyanates, that have been shown to be effective, their application in fruit shows a critical challenge due to several issues such as those related to large-scale application, registration processes, the degradation of VOCs and the organoleptic impact, among others (Mari et al., 2016). Hence, looking for naturally emitted peach volatiles that can be enhanced in the biotic resistance is of potential interest. The research of the biosynthetic genes regulating terpenoid synthesis and looking for methodologies that reveal volatiles in non-susceptible tissues to brown rot can elucidate new sources of knowledge for the further enhancement of stone fruit metabolites to control brown rot.

1.4. References

Abbas, F., Ke, Y., Yu, R., Yue, Y., Amanullah, S., Jahangir, M. M., et al. (2017). Volatile terpenoids: multiple functions, biosynthesis, modulation and manipulation by genetic engineering. *Planta* 246, 803–816. doi:10.1007/s00425-017-2749-x.

AbuQamar, S., Moustafa, K., and Tran, L. S. P. (2017). Mechanisms and strategies of plant defense against *Botrytis cinerea*. *Crit. Rev. Biotechnol.* 37, 262–274. doi:10.1080/07388551.2016.1271767.

Adaskaveg, J. E., Schnabel, G., and Förster, H. (2008). "Diseases of peach caused by fungi and fungal-like organisms: Biology, epidemiology and management," in *The Peach: Botany, Production and Uses*, eds. D. R. Layne and D. Bassi, 352–406. doi:10.1079/9781845933869.0352.

Agrios, G. N. (2005a). "Parasitism and disease development," in *Plant Pathology*, ed. G. N. Agrios (Elsevier Academic Press), 77–104.

Agrios, G. N. (2005b). "Plant disease epidemiology," in *Plant Pathology*, ed. G. N. Agrios (Elsevier), 265–291.

Agrios, G. N. (2005c). *Plant Pathology*. 5th ed. Elsevier Academic Press doi:10.1111/j.1365-3059.1952.tb00010.x.

Aharoni, A., Jongsma, M. A., and Bouwmeester, H. J. (2005). Volatile science? Metabolic engineering of terpenoids in plants. *Trends Plant Sci.* 10, 594–602. doi:10.1016/j.tplants.2005.10.005.

Ali, M. M., Anwar, R., Yousef, A. F., Li, B., Luvisi, A., De Bellis, L., et al. (2021). Influence of bagging on the development and quality of fruits. *Plants* 10, 1–17. doi:10.3390/plants10020358.

Alkan, N., and Fortes, A. M. (2015). Insights into molecular and metabolic events associated with fruit response to post-harvest fungal pathogens. *Front. Plant Sci.* 6, 889. doi:10.3389/fpls.2015.00889.

Allran, J. (2017). Investigation of peach fruit bagging to produce high quality fruit and to manage pests and diseases. Available at: https://tigerprints.clemson.edu/all_theses/2695/.

Bae, H., Yun, S. K., Jun, J. H., Yoon, I. K., Nam, E. Y., and Kwon, J. H. (2014). Assessment of organic acid and sugar composition in apricot, plumcot, plum, and peach during fruit development. *J. Appl. Bot. Food Qual.* 87, 24–29. doi:10.5073/JABFQ.2014.087.004.

Ballaré, C. L. (2014). Light regulation of plant defense. *Annu. Rev. Plant Biol.* 65, 335–363. doi:10.1146/annurev-arplant-050213-040145.

Baró-Montel, N., Giné-Bordonaba, J., Torres, R., Vall-llaura, N., Teixidó, N., and Usall, J. (2020). Scrutinising the relationship between major physiological and compositional changes during 'Merrill O'Henry' peach growth with brown rot susceptibility. *Food Sci. Technol. Int.* 27, 366–379. doi:10.1177/1082013220959988.

Baró-Montel, N., Vall-llaura, N., Giné-Bordonaba, J., Usall, J., Serrano-Prieto, S., Teixidó, N., et al. (2019a). Double-sided battle: The role of ethylene during *Monilinia* spp. infection in peach at different phenological stages. *Plant Physiol. Biochem.* 144, 324–333. doi:10.1016/j.plaphy.2019.09.048.

Baró-Montel, N., Vall-llaura, N., Usall, J., Teixidó, N., Naranjo-Ortíz, M. A., Gabaldón, T., et al.

(2019b). Pectin methyl esterases and rhamnogalacturonan hydrolases: weapons for successful *Monilinia laxa* infection in stone fruit? *Plant Pathol.* 68, 1381–93. doi:10.1111/ppa.13039.

Bartwal, A., Mall, R., Lohani, P., Guru, S. K., and Arora, S. (2013). Role of secondary metabolites and brassinosteroids in plant defense against environmental stresses. *J. Plant Growth Regul.* 32, 216–232. doi:10.1007/s00344-012-9272-x.

Bassi, D., and Monet, R. (2008). "Botany and taxonomy," in *The Peach: Botany, Production and Uses*, eds. D. R. Layne and D. Bassi, 1–36. doi:10.1079/9781845933869.0001.

Bellingeri, M., Quilot-Turion, B., Lino, L. O., and Bevacqua, D. (2018). The crop load affects brown rot progression in fruit orchards: High fruit densities facilitate fruit exposure to spores but reduce the infection rate by decreasing fruit growth and cuticle cracking. *Front. Ecol. Evol.* 5, 1–7. doi:10.3389/fevo.2017.00170.

Bernat, M., Segarra, J., Xu, X. M., Casals, C., and Usall, J. (2017). Influence of temperature on decay, mycelium development and sporodochia production caused by *Monilinia fructicola* and *M. laxa* on stone fruits. *Food Microbiol.* 64, 112–118. doi:10.1016/j.fm.2016.12.016.

Blanco-Ulate, B., Vincenti, E., Powell, A. L. T., and Cantu, D. (2013). Tomato transcriptome and mutant analyses suggest a role for plant stress hormones in the interaction between fruit and *Botrytis cinerea*. *Front. Plant Sci.* 4, 1–16. doi:10.3389/fpls.2013.00142.

Bonany, J., Carbó, J., Echeverria, G., Hilaire, C., Cottet, V., Iglesias, I., et al. (2014). Eating quality and European consumer acceptance of different peach (*Prunus persica* (L.) Batsch) varieties. *J. Food, Agric. Environ.* 12, 67–72.

Brummell, D. A., Dal Cin, V., Crisosto, C. H., and Labavitch, J. M. (2004). Cell wall metabolism during maturation, ripening and senescence of peach fruit. *J. Exp. Bot.* 55, 2029–2039. doi:10.1093/jxb/erh227.

Bussi, C., Plenet, D., Merlin, F., Guillermin, A., and Mercier, V. (2015). Limiting brown rot incidence in peach with tree training and pruning. *Fruits* 70, 303–309. doi:10.1051/fruits/2015030.

Byrde, R. J. W., and Willetts, H. J. (1977). The brown rot fungi of fruit: their biology and control.

Campbell, D., Sarkhosh, A., Brecht, J. K., Gillett-Kaufman, J. L., Liburd, O., Melgar, J. C., et al. (2021). Bagging organic peaches reduces physical injuries and storage decay with minimal effects on fruit quality. *HortScience* 56, 52–58. doi:10.21273/HORTSCI15391-20.

Carvalho, S. D., and Castillo, J. A. (2018). Influence of light on plant–phyllosphere interaction. *Front. Plant Sci.* 9, 1–16. doi:10.3389/fpls.2018.01482.

Casals, C., Guijarro, B., De Cal, A., Torres, R., Usall, J., Perdrix, V., et al. (2021). Field validation of biocontrol strategies to control brown rot on stone fruit in several European countries. *Pest Manag. Sci.* doi:10.1002/ps.6281.

Casals, C., Segarra, J., De Cal, A., Lamarca, N., and Usall, J. (2015). Overwintering of *Monilinia* spp. on mummified stone fruit. *J. Phytopathol.* 163, 160–167. doi:10.1111/jph.12298.

Casals, C., Vinas, I., Torres, R., Griera, C., and Usall, J. (2010). Effect of temperature and water activity on *in vitro* germination of *Monilinia* spp. *J. Appl. Microbiol.* 108, 47–54. doi:10.1111/j.1365-2672.2009.04402.x.

Chou, C. M., Yu, F. Y., Yu, P. L., Ho, J. F., Bostock, R. M., Chung, K. R., et al. (2015). Expression of five endopolygalacturonase genes and demonstration that *MfPG1* overexpression diminishes virulence in the brown rot pathogen *Monilinia fructicola*. *PLoS One* 10, 1–17. doi:10.1371/journal.pone.0132012.

Colarič, M., Štampar, F., and Hudina, M. (2004). Contents of sugars and organic acids in the cultivars of peach (*Prunus persica* L.) and nectarine (*Prunus persica* var. *nucipersica* Schneid.). *Acta Agric. Slov.* 1, 53–61.

Corrochano, L. M. (2019). Light in the fungal world: From photoreception to gene transcription and beyond. *Annu. Rev. Genet.* 53, 149–170. doi:10.1146/annurev-genet-120417-031415.

Crisosto, C. H. (2002). How do we increase peach consumption? in *Acta Horticulturae*, 601–605. doi:10.17660/ActaHortic.2002.592.82.

Crisosto, C. H., and Costa, G. (2008). "Preharvest factors affecting peach quality," in *The Peach: Botany, Production and Uses*, eds. D. R. Layne and D. Bassi (CAB International), 536–549. doi:10.1079/9781845933869.0536.

Crisosto, C. H., and Crisosto, G. M. (2005). Relationship between ripe soluble solids concentration (RSSC) and consumer acceptance of high and low acid melting flesh peach and nectarine (*Prunus persica* (L.) Batsch) cultivars. *Postharvest Biol. Technol.* 38, 239–246. doi:10.1016/j.postharvbio.2005.07.007.

Crisosto, C. H., and Day, K. R. (2012). "Stone Fruit," in *Crop Post-Harvest: Science and Technology*, eds. D. Rees, G. Farrell, and J. Orchard (Blackwell Publishing Ltd), 212–225. doi:10.1002/9781444354652.ch10.

Crisosto, C. H., Echeverría, G., and Manganaris, G. A. (2020). "Peach and Nectarine," in *Manual on postharvest handling of Mediterranean tree fruits and nuts*, eds. C. H. Crisosto and G. M. Crisosto, 53–87.

Crisosto, C. H., Mitchell, F. G., and Johnson, S. (1995). Factors in fresh market stone fruit quality. *Postharvest News Inf.* 6, 17–21.

De Cal, A., Gell, I., Usall, J., Viñas, I., and Melgarejo, P. (2009). First report of brown rot caused by *Monilinia fructicola* in peach orchards in Ebro Valley, Spain. *Plant Dis.* 93, 763. doi:10.1094/PDIS-93-7-0763A.

De Cal, A., and Melgarejo, P. (1993). Effects of pyroquilon on the infection process of *Monilinia laxa* causing peach twig blight. *Pestic. Sci.* 39, 267–269. doi:10.1002/ps.2780390403.

De Cal, A., Sandín-España, P., Martinez, F., Egüen, B., Chien-Ming, C., Lee, M. H., et al. (2013). Role of gluconic acid and pH modulation in virulence of *Monilinia fructicola* on peach fruit. *Postharvest Biol. Technol.* 86, 418–423. doi:10.1016/j.postharvbio.2013.07.012.

De Curtis, F., Ianiri, G., Raiola, A., Ritieni, A., Succi, M., Tremonte, P., et al. (2019). Integration of biological and chemical control of brown rot of stone fruits to reduce disease incidence on fruits and minimize fungicide residues in juice. *Crop Prot.* 119, 158–165. doi:10.1016/j.cropro.2019.01.020.

De Miccolis Angelini, R. M., Abate, D., Rotolo, C., Gerin, D., Pollastro, S., and Faretra, F. (2018). De novo assembly and comparative transcriptome analysis of *Monilinia fructicola*, *Monilinia laxa* and *Monilinia fructigena*, the causal agents of brown rot on stone fruits. 19, 1–21. doi:10.1186/s12864-018-4817-4.

De Miccolis Angelini, R. M., Romanazzi, G., Pollastro, S., Rotolo, C., Faretra, F., and Landi, L. (2019). New high-quality draft genome of the brown rot fungal pathogen *Monilinia fructicola*. *Genome Biol. Evol.* 11, 2850–2855. doi:10.1093/gbe/evz207.

Desnoues, E., Gibon, Y., Baldazzi, V., Signoret, V., Génard, M., and Quilot-Turion, B. (2014). Profiling sugar metabolism during fruit development in a peach progeny with different fructose-to-glucose ratios. *BMC Plant Biol.* 14, 1–13. doi:10.1186/s12870-014-0336-x.

Eckert, J. W., and Ratnayake, M. (1983). "Host-pathogen interactions in postharvest diseases," in *Post-Harvest Physiology and Crop Preservation*, ed. M. Lieberman (Plenum Press), 247–264. doi:10.1007/978-1-4757-0094-7.

Egüen, B., Melgarejo, P., and De Cal, A. (2016). The effect of fungicide resistance on the structure of *Monilinia laxa* populations in Spanish peach and nectarine orchards. *Eur. J. Plant Pathol.* 145, 815–827. doi:10.1007/s10658-016-0871-4.

European Commission (2004). COMMISSION REGULATION (EC) 1861/2004.

European Commission (2019a). Agriculture and the Green Deal. Available at: https://ec.europa.eu/info/strategy/priorities-2019-2024/european-green-deal/agriculture-and-green-deal_en [Accessed October 30, 2021].

European Commission (2019b). COMMISSION DELEGATED REGULATION (EU) 2019/428.

Eurostat, E. statistics (2021). Fruit and vegetables. Available at: https://agridata.ec.europa.eu/extensions/DataPortal/fruit-and-vegetables.html [Accessed July 20, 2021].

Faci, J. M., Medina, E. T., Martínez-Cob, A., and Alonso, J. M. (2014). Fruit yield and quality response of a late season peach orchard to different irrigation regimes in a semi-arid environment. *Agric. Water Manag.* 143, 102–112. doi:10.1016/j.agwat.2014.07.004.

FAO (2021). Database of Food and Agriculture Organization of the United Nations. Available at: http://www.fao.org/faostat/en/#data/QC/visualize [Accessed January 13, 2022].

Flore, J. A. (1994). "Stone Fruit," in *Handbook of Environmental Physiology of Fruit Crops. Vol 1 Temperate Crops*, eds. B. Schaffer and P. C. Andersen, 233–270.

Folta, K. M., and Carvalho, S. D. (2015). Photoreceptors and control of horticultural plant traits. *HortScience* 50, 1274–1280. doi:10.21273/hortsci.50.9.1274.

Fuller, K. K., Loros, J. J., and Dunlap, J. C. (2015). Fungal photobiology: visible light as a signal for stress, space and time. *Curr. Genet.* 61, 275–288. doi:10.1007/s00294-014-0451-0.

Garcia-Benitez, C., Casals, C., Usall, J., Sánchez-Ramos, I., Melgarejo, P., and De Cal, A. (2020). Impact of postharvest handling on preharvest latent infections caused by *Monilinia* spp. in nectarines. *J. Fungi* 6, 1–14. doi:10.3390/jof6040266.

Garcia-Benitez, C., Melgarejo, P., Sandin-España, P., Sevilla-Morán, B., and De Cal, A. (2019). Degrading enzymes and phytotoxins in *Monilinia* spp. *Eur. J. Plant Pathol.* 154, 305–318. doi:10.1007/s10658-018-01657-z.

Gell, I., De Cal, A., Torres, R., Usall, J., and Melgarejo, P. (2008). Relationship between the incidence of latent infections caused by *Monilinia* spp. and the incidence of brown rot of peach fruit: factors affecting latent infection. *Eur. J. Plant Pathol.* 121, 487–498. doi:10.1007/s10658-008-9268-3.

Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annu. Rev. Phytopathol.* 43, 205–227. doi:10.1146/annurev.phyto.43.040204.135923.

Gong, D., Cao, S., Sheng, T., Shao, J., Song, C., Wo, F., et al. (2015). Effect of blue light on ethylene biosynthesis, signalling and fruit ripening in postharvest peaches. *Sci. Hortic. (Amsterdam).* 197, 657–664. doi:10.1016/j.scienta.2015.10.034.

Gradziel, T. M. (1994). Changes in susceptibility to brown rot with ripening in three clingstone peach genotypes. *J. Amer. Soc. Hort. Sci* 119, 101–105. doi:10.21273/JASHS.119.1.101.

Guidarelli, M., Zubini, P., Nanni, V., Bonghi, C., Rasori, A., Bertolini, P., et al. (2014). Gene expression analysis of peach fruit at different growth stages and with different susceptibility to *Monilinia laxa*. *Eur. J. Plant Pathol.* 140, 503–513. doi:10.1007/s10658-014-0484-8.

Gununu, P. R., Munhuweyi, K., Obianom, P. C., and Sivakumar, D. (2019). Assessment of eleven South African peach cultivars for susceptibility to brown rot and blue mould. *Sci. Hortic. (Amsterdam).* 254, 1–6. doi:10.1016/j.scienta.2019.04.067.

Hashimoto, M., Styrikovich, M., Nishioka, S., Rouviere, C., Williams, T., Ball, R., et al. (1990). "Human settlement; the energy, transport and industrial sectors; human health; air quality; and changes in ultraviolet-B radiation," in *Climate Change. The IPCC Impacts Assessment*, eds. W. M. Tegart, G. W. Sheldon, and D. C. Griffiths Available at: https://www.ipcc.ch/report/ar1/wg2/.

Hayama, H., Shimada, T., Fujii, H., Ito, A., and Kashimura, Y. (2006). Ethylene-regulation of fruit softening and softening-related genes in peach. *J. Exp. Bot.* 57, 4071–4077. doi:10.1093/jxb/erl178.

Hong, C., Holtz, B. A., Morgan, D. P., and Michailides, T. J. (1997). Significance of thinned fruit as a source of the secondary inoculum of *Monilinia fructicola* in California nectarine orchards. *Plant Dis.* 81, 519–524. doi:10.1094/PDIS.1997.81.5.519.

Hudina, M., and Stampar, F. (2011). Effect of fruit bagging on quality of "Conference" pear (*Pyrus communis* L.). *Eur. J. Hortic. Sci.* 76, 176–181.

Idnurm, A., and Crosson, S. (2009). The photobiology of microbial pathogenesis. *PLoS Pathog.* 5, 11–13. doi:10.1371/journal.ppat.1000470.

Iglesias, I., and Echeverría, G. (2009). Differential effect of cultivar and harvest date on nectarine colour, quality and consumer acceptance. *Sci. Hortic. (Amsterdam).* 120, 41–50. doi:10.1016/j.scienta.2008.09.011.

Ilić, Z. S., and Fallik, E. (2017). Light quality manipulation improves vegetable quality at harvest and postharvest: A review. *Environ. Exp. Bot.* 139, 79–90. doi:10.1016/j.envexpbot.2017.04.006.

Jiang, L., Feng, L., Zhang, F., Luo, H., and Yu, Z. (2020). Peach fruit ripening: Proteomic comparative analyses of two cultivars with different flesh texture phenotypes at two ripening stages. *Sci. Hortic. (Amsterdam).* 260, 108610. doi:10.1016/j.scienta.2019.108610.

Jones, J. D. G., and Dangl, J. L. (2006). The plant immune system. *Nature* 444, 323–329. doi:10.1038/nature05286.

Keske, C., Treutter, D., and Neumüller, M. (2014). Effect of bagging on brown rot incidence in European Plum. *Ecofruit. 16th Int. Conf. Org. Grow. Proceedings, 17-19 Febr. 2014, Hohenheim, Ger. 2014 pp.228-231 ref.7*, 228–231. doi:9783000450716.

Khare, S., Singh, N. B., Singh, A., Hussain, I., Niharika, K., Yadav, V., et al. (2020). Plant secondary metabolites synthesis and their regulations under biotic and abiotic constraints. *J. Plant Biol.* 63, 203–216. doi:10.1007/s12374-020-09245-7.

Kokalj, D., Zlatić, E., Cigić, B., and Vidrih, R. (2019). Postharvest light-emitting diode irradiation of sweet cherries (*Prunus avium* L.) promotes accumulation of anthocyanins. *Postharvest Biol. Technol.* 148, 192–199. doi:10.1016/j.postharvbio.2018.11.011.

Kreidl, S., Edwards, J., and Villalta, O. N. (2015). Assessment of pathogenicity and infection requirements of *Monilinia* species causing brown rot of stone fruit in Australian orchards. *Australas. Plant Pathol.* 44, 419–430. doi:10.1007/s13313-015-0362-7.

Lafuente, M. T., Alférez, F., and González-Candelas, L. (2018). Light-emitting diode blue light alters the ability of *Penicillium digitatum* to infect citrus fruits. *Photochem. Photobiol.* 94, 1003–1009. doi:10.1111/php.12929.

Landi, L., De Miccolis Angelini, R. M., Pollastro, S., Abate, D., Faretra, F., and Romanazzi, G. (2018). Genome sequence of the brown rot fungal pathogen *Monilinia fructigena*. *BMC Res. Notes* 11, 10–12. doi:10.1186/s13104-018-3854-z.

Landi, L., Pollastro, S., Rotolo, C., Romanazzi, G., Faretra, F., and De Miccolis Angelini, R. M. (2020). Draft genomic resources for the brown rot fungal pathogen *Monilinia laxa*. *Mol. Plant-Microbe Interact.* 33, 145–148. doi:10.1094/MPMI-08-19-0225-A.

Lara, M. V., Bonghi, C., Famiani, F., Vizzotto, G., Walker, R. P., and Drincovich, M. F. (2020). Stone fruit as biofactories of phytochemicals with potential roles in human nutrition and health. *Front. Plant Sci.* 11, 1–21. doi:10.3389/fpls.2020.562252.

Larena, I., Torres, R., De Cal, A., Liñán, M., Melgarejo, P., Domenichini, P., et al. (2005). Biological control of postharvest brown rot (*Monilinia* spp.) of peaches by field applications of *Epicoccum nigrum*. *Biol. Control* 32, 305–310. doi:10.1016/j.biocontrol.2004.10.010.

Lee, M. H., and Bostock, R. M. (2007). Fruit exocarp phenols in relation to quiescence and development of *Monilinia fructicola* infections in *Prunus* spp.: A role for cellular redox? *Phytopathology* 97, 269–277. doi:10.1094/PHYTO-97-3-0269.

Lewallen, K. S., and Marini, R. P. (2003). Relationship between flesh firmness and ground color in peach as influenced by light and canopy position. *J. Am. Soc. Hortic. Sci.* 128, 163–170. doi:10.21273/jashs.128.2.0163.

Li, T., Wang, Y. H., Liu, J. X., Feng, K., Xu, Z. S., and Xiong, A. S. (2019). Advances in genomic, transcriptomic, proteomic, and metabolomic approaches to study biotic stress in fruit crops. *Crit. Rev. Biotechnol.* 39, 680–692. doi:10.1080/07388551.2019.1608153.

Lichou, J., Mandrin, J. F., Breniaux, D., Mercier, V., Giauque, P., Desbrus, D., et al. (2002). A new, powerful monilia: *Monilia fructicola* chooses stone-fruit trees for its attacks. *Phytoma* 547, 22–25. Available at: https://www.cabdirect.org/cabdirect/abstract/20023049075.

Lino, L. O., Pacheco, I., Mercier, V., Faoro, F., Bassi, D., Bornard, I., et al. (2016). Brown rot strikes *Prunus* fruit: An ancient fight almost always lost. *J. Agric. Food Chem.* 64, 4029–4047. doi:10.1021/acs.jafc.6b00104.

Lino, L. O., Quilot-Turion, B., Dufour, C., Corre, M. N., Lessire, R., Génard, M., et al. (2020). Cuticular waxes of nectarines during fruit development in relation to surface conductance and susceptibility to *Monilinia laxa*. *J. Exp. Bot.* 71, 5521–5537. doi:10.1093/jxb/eraa284.

Liu, H., Cao, X., Liu, X., Xin, R., Wang, J., Gao, J., et al. (2017). UV-B irradiation differentially regulates terpene synthases and terpene content of peach. *Plant Cell Environ.* 40, 2261–2275. doi:10.1111/pce.13029.

Liu, M., Pirrello, J., Chervin, C., Roustan, J. P., and Bouzayen, M. (2015a). Ethylene control of fruit ripening: Revisiting the complex network of transcriptional regulation. *Plant Physiol.* 169, 2380–2390. doi:10.1104/pp.15.01361.

Liu, Q., Zhao, N., Zhou, D., Sun, Y., Sun, K., Pan, L., et al. (2018). Discrimination and growth tracking of fungi contamination in peaches using electronic nose. *Food Chem.* 262, 226–234. doi:10.1016/j.foodchem.2018.04.100.

Liu, T., Song, S., Yuan, Y., Wu, D., Chen, M., Sun, Q., et al. (2015b). Improved peach peel color development by fruit bagging. Enhanced expression of anthocyanin biosynthetic and regulatory genes using white non-woven polypropylene as replacement for yellow paper. *Sci. Hortic. (Amsterdam).* 184, 142–148.

Lopresti, J., Goodwin, I., McGlasson, B., Holford, P., and Golding, J. (2014). Variability in size and soluble solids concentration in peaches and nectarines. *Hortic. Rev. (Am. Soc. Hortic. Sci).* 42, 253–311. doi:10.1002/9781118916827.ch05.

Lumigrow Available at: https://www.lumigrow.com/learning-center/blogs/full-spectrum-led-grow-lights [Accessed September 9, 2021].

Luo, Y., and Michailides, T. J. (2001). Factors affecting latent infection of prune fruit by *Monilinia fructicola*. *Phytopathology* 91, 864–872.

Luo, Y., Michailides, T. J., Morgan, D. P., Krueger, W. H., and Buchner, R. P. (2005). Inoculum dynamics, fruit infection, and development of brown rot in prune orchards in California. *Phytopathology* 95, 1132–1136. doi:10.1094/PHYTO-95-1132.

Ma, Z., Yoshimura, M. A., Holtz, B. A., and Michailides, T. J. (2005). Characterization and PCR-based detection of benzimidazole-resistant isolates of *Monilinia laxa* in California. *Pest Manag. Sci.* 61, 449–457. doi:10.1002/ps.982.

Manganaris, G. A., and Crisosto, C. H. (2020). *Stone fruits: Peaches, nectarines, plums, apricots*. Elsevier Inc. doi:10.1016/b978-0-12-804599-2.00017-x.

MAPA (2018). Fruta de Hueso: Análisis Campaña 2018. 1–12.

MAPA (2020a). Boletín de fruta de hueso. Campaña 2020. Available at: https://www.mapa.gob.es/es/agricultura/temas/producciones-agricolas/boletinfrutadehueso2020n15-2020_tcm30-543165.pdf.

MAPA (2020b). Informe de retiradas en el marco de los programas operativos de las OPFH. 1–22.

MAPA (2020c). Informe del cosumo alimentario en España 2020.

MAPA (2022). Registro de Productos Fitosanitarios. *Minist. Agric. Pesca y Aliment.* Available at: https://www.mapa.gob.es/es/agricultura/temas/sanidad-vegetal/productos-fitosanitarios/registro/menu.asp [Accessed January 13, 2022].

Marcet-Houben, M., Villarino, M., Vilanova, L., De Cal, A., van Kan, J. A. L., Usall, J., et al. (2021). Comparative genomics used to predict virulence factors and metabolic genes among *Monilinia* species. *J. Fungi* 7. doi:10.3390/jof7060464.

Mari, M., Bautista-Baños, S., and Sivakumar, D. (2016). Decay control in the postharvest system: Role of microbial and plant volatile organic compounds. *Postharvest Biol. Technol.* 122, 70–81. doi:10.1016/j.postharvbio.2016.04.014.

Mari, M., Casalini, L., Baraldi, E., Bertolini, P., and Pratella, G. C. (2003). Susceptibility of apricot and peach fruit to *Monilinia laxa* during phenological stages. *Postharvest Biol. Technol.* 30, 105–109. doi:10.1016/S0925-5214(03)00138-8.

Mari, M., Spadaro, D., Casals, C., Collina, M., De Cal, A., and Usall, J. (2019). "Stone Fruits," in *Postharvest Diseases of Fresh Horticultural Produce*, eds. L. Palou and J. L. Smilanick (CRC Press), 111–140.

Marimon de María, N. (2020). Towards an integrated control of peach powdery mildew (*Podosphaera pannosa*) through the application of molecular tools in epidemiological and genetic resistance studies. Thesis.

Martini, C., and Mari, M. (2014). "*Monilinia fructicola*, *Monilinia laxa* (Monilinia Rot, Brown Rot)," in *Postharvest Decay: Control Strategies*, ed. Silvia Bautista-Baños (Elsevier), 233–265. doi:10.1016/B978-0-12-411552-1.00007-7.

Mataffo, A., Scognamiglio, P., Basile, B., Lisanti, M. T., Tenore, G. C., Graziani, G., et al. (2020). Crop load affects the nutritional quality of flat peaches (*Prunus persica* L. var. *platycarpa* (Decne.) L.H. Bailey). *Italus Hortus* 27, 41–54. doi:10.26353/j.itahort/2020.2.4154.

Meraj, T. A., Fu, J., Raza, M. A., Zhu, C., Shen, Q., Xu, D., et al. (2020). Transcriptional factors regulate plant stress responses through mediating secondary metabolism. *Genes (Basel).* 11, 346. doi:10.3390/genes11040346.

Minas, I. S., Tanou, G., and Molassiotis, A. (2018). Environmental and orchard bases of peach fruit quality. *Sci. Hortic. (Amsterdam).* 235, 307–322. doi:10.1016/j.scienta.2018.01.028.

Ministerio de Agricultura, P. y alimentación (2019). Anuario de Estadística. Available at: https://www.mapa.gob.es/estadistica/pags/anuario/2019/anuario/AE19.pdf.

Montero-Prado, P., Bentayeb, K., and Nerín, C. (2013). Pattern recognition of peach cultivars (*Prunus persica* L.) from their volatile components. *Food Chem.* 138, 724–731. doi:10.1016/j.foodchem.2012.10.145.

Müller, M., and Munné-Bosch, S. (2015). Ethylene response factors: A key regulatory hub in hormone and stress signaling. *Plant Physiol.* 169, 32–41. doi:10.1104/pp.15.00677.

Mustafa, M. H., Bassi, D., Corre, M.-N., Lino, L. O., Signoret, V., Quilot-Turion, B., et al. (2021). Phenotyping brown rot susceptibility in stone fruit: A literature review with emphasis on peach. *Horticulturae* 7, 115. doi:10.3390/horticulturae7050115.

Mycobank (2021). Available at: https://www.mycobank.org/ [Accessed August 11, 2021].

Naranjo-Ortíz, M. A., Rodríguez-Píres, S., Torres, R., Cal, A. De, Usall, J., and Gabaldón, T. (2018). Genome sequence of the brown rot fungal pathogen *Monilinia laxa*. *BMC Res. Notes* 11, 1–2. doi:10.1186/s13104-018-3854-z.

Obi, V. I., Barriuso, J. J., and Gogorcena, Y. (2018). Peach brown rot: Still in search of an ideal

management option. *Agriculture* 8, 1–34. doi:10.3390/agriculture8080125.

Obi, V. I., Barriuso, J. J., Usall, J., and Gogorcena, Y. (2019). Breeding strategies for identifying superior peach genotypes resistant to brown rot. *Sci. Hortic. (Amsterdam).* 246, 1028–1036. doi:10.1016/j.scienta.2018.10.027.

OECD (2010). Peaches and nectarines, International Standards for Fruit and Vegetables. OECD Publishing, Paris doi:https://doi.org/10.1787/9789264084926-en-fr.

Oetiker, J. H., and Yang, S. F. (1995). The role of ethylene in fruit ripening. *Acta Hortic.* 398, 167–178. doi:10.17660/actahortic.1995.398.17.

Ouzounis, T., Rosenqvist, E., and Ottosen, C. O. (2015). Spectral effects of artificial light on plant physiology and secondary metabolism: A review. *HortScience* 50, 1128–1135. doi:10.21273/hortsci.50.8.1128.

Pandey, D., Rajendran, S. R. C. K., Gaur, M., Sajeesh, P. K., and Kumar, A. (2016). Plant defense signaling and responses against necrotrophic fungal pathogens. *J. Plant Growth Regul.* 35, 1159–1174. doi:10.1007/s00344-016-9600-7.

Papavasileiou, A., Tanou, G., Samaras, A., Samiotaki, M., Molassiotis, A., and Karaoglanidis, G. (2020). Proteomic analysis upon peach fruit infection with *Monilinia fructicola* and *M. laxa* identify responses contributing to brown rot resistance. *Sci. Rep.* 10, 7807. doi:10.1038/s41598-020-64864-x.

Paul, V., Pandey, R., and Srivastava, G. C. (2012). The fading distinctions between classical patterns of ripening in climacteric and non-climacteric fruit and the ubiquity of ethylene-An overview. *J. Food Sci. Technol.* 49, 1–21. doi:10.1007/s13197-011-0293-4.

Philips Available at: https://www.lighting.philips.es/welcome [Accessed November 16, 2020].

Porat, R., Lichter, A., Terry, L. A., Harker, R., and Buzby, J. (2018). Postharvest losses of fruit and vegetables during retail and in consumers' homes: Quantifications, causes, and means of prevention. *Postharvest Biol. Technol.* 139, 135–149. doi:10.1016/j.postharvbio.2017.11.019.

Prusky, D., de Assis, L. J., Baroncelli, R., Benito, E. P., del Castillo, V. C., Chaya, T., et al. (2020). Nutritional factors modulating plant and fruit susceptibility to pathogens: BARD workshop, Haifa, Israel, February 25–26, 2018. *Phytoparasitica* 48, 317–333. doi:10.1007/s12600-020-00803-w.

Ramina, A., Tonutti, P., and Mcglasson, W. (2008). "Ripening, Nutrition and Postharvest Physiology," in *The Peach: Botany, Production and Uses*, eds. D. R. Layne and D. Bassi, 550–574.

Reig, G., Alegre, S., Iglesias, I., Echeverría, G., and Gatius, F. (2012). Fruit quality, colour development and index of absorbance difference (I_{AD}) of different nectarine cultivars at different harvest dates. *Acta Hortic.* 934, 1117–1126. doi:10.17660/ActaHortic.2012.934.150.

Rivera, Y., Zeller, K., Srivastava, S., Sutherland, J., Galvez, M., Nakhla, M., et al. (2018). Draft genome resources for the phytopathogenic fungi *Monilinia fructicola*, *M. fructigena*, *M. polystroma*, and *M. laxa*, the causal agents of brown rot. *Phytopathology* 108, 1141–1142. doi:10.1094/phyto-12-17-0418-a.

Rodríguez-Pires, S., De Cal, A., Espeso, E. A., Rasiukeviciut, N., and Melgarejo, P. (2021). Light-

photoreceptors and proteins related to *Monilinia laxa*. *J. Fungi* 7. doi:10.3390/jof7010032.

Rodríguez-Pires, S., Melgarejo, P., De Cal, A., and Espeso, E. A. (2020a). Pectin as carbon source for *Monilinia laxa* exoproteome and expression profiles of related genes. *Mol. Plant-Microbe Interact.* 33, 1116–1128. doi:10.1094/MPMI-01-20-0019-R.

Rodríguez-Pires, S., Melgarejo, P., De Cal, A., and Espeso, E. A. (2020b). Proteomic studies to understand the mechanisms of peach tissue degradation by *Monilinia laxa*. *Front. Plant Sci.* 11, 1–12. doi:10.3389/fpls.2020.01286.

Rodríguez-Pires, S., Garcia-Companys, M., Espeso, E. A., Melgarejo, P., and De Cal, A. (2020). Influence of light on the *Monilinia laxa* stone fruit interaction. *Plant Pathol.*, 1–10. doi:10.1111/ppa.13294.

Roeber, V. M., Bajaj, I., Rohde, M., Schmülling, T., and Cortleven, A. (2020). Light acts as a stressor and influences abiotic and biotic stress responses in plants. *Plant Cell Environ.*, 1–20. doi:10.1111/pce.13948.

Romanazzi, G., Sanzani, S. M., Bi, Y., Tian, S., Gutiérrez Martínez, P., and Alkan, N. (2016). Induced resistance to control postharvest decay of fruit and vegetables. *Postharvest Biol. Technol.* 122, 82–94. doi:10.1016/j.postharvbio.2016.08.003.

Rungjindamai, N., Jeffries, P., and Xu, X.-M. (2014). Epidemiology and management of brown rot on stone fruit caused by *Monilinia laxa*. *Eur. J. Plant Pathol.* 140, 1–17. doi:10.1007/s10658-014-0452-3.

Santin, M., Giordani, T., Cavallini, A., Bernardi, R., Castagna, A., Hauser, M. T., et al. (2019). UV-B exposure reduces the activity of several cell wall-dismantling enzymes and affects the expression of their biosynthetic genes in peach fruit (*Prunus persica* L., cv. Fairtime, melting phenotype). *Photochem. Photobiol. Sci.* 18, 1280–1289. doi:10.1039/c8pp00505b.

Santin, M., Neugart, S., Castagna, A., Barilari, M., Sarrocco, S., Vannacci, G., et al. (2018). UV-B Pre-treatment alters phenolics response to *Monilinia fructicola* infection in a structure-dependent way in peach skin. *Front. Plant Sci.* 9. doi:10.3389/fpls.2018.01747.

Santin, M., Ranieri, A., Hauser, M. T., Miras-Moreno, B., Rocchetti, G., Lucini, L., et al. (2021). The outer influences the inner: Postharvest UV-B irradiation modulates peach flesh metabolome although shielded by the skin. *Food Chem.* 338. doi:10.1016/j.foodchem.2020.127782.

Schumacher, J. (2017). How light affects the life of *Botrytis*. *Fungal Genet. Biol.* 106, 26–41. doi:10.1016/j.fgb.2017.06.002.

Schumacher, J., and Gorbushina, A. A. (2020). Light sensing in plant- and rock-associated black fungi. *Fungal Biol.* 124, 407–417. doi:10.1016/j.funbio.2020.01.004.

Shahbandeh, M. (2019). Global fruit production in 2017, by variety. Available at: https://www.statista.com/statistics/264001/worldwide-production-of-fruit-by-variety/ [Accessed December 16, 2019].

Sharma, R. R., Reddy, S. V. R., and Jhalegar, M. J. (2014). Pre-harvest fruit bagging: A useful approach for plant protection and improved post-harvest fruit quality - A review. *J. Hortic. Sci. Biotechnol.* 89, 101–113. doi:10.1080/14620316.2014.11513055.

Silva, C. J., van den Abeele, C., Ortega-Salazar, I., Papin, V., Adaskaveg, J. A., Wang, D., et al. (2021). Host susceptibility factors render ripe tomato fruit vulnerable to fungal disease

despite active immune responses. *J. Exp. Bot.* 72, 2696–2709. doi:10.1093/jxb/eraa601.

Spadoni, A., Cameldi, I., Noferini, M., Bonora, E., Costa, G., and Mari, M. (2016). An innovative use of DA-meter for peach fruit postharvest management. *Sci. Hortic. (Amsterdam).* 201, 140–144. doi:10.1016/j.scienta.2016.01.041.

Tadiello, A., Ziosi, V., Negri, A. S., Noferini, M., Fiori, G., Busatto, N., et al. (2016). On the role of ethylene, auxin and a GOLVEN-like peptide hormone in the regulation of peach ripening. *BMC Plant Biol.* 16, 1–17. doi:10.1186/s12870-016-0730-7.

Tholl, D. (2015). Biosynthesis and biological functions of terpenoids in plants. *Adv. Biochem. Eng. Biotechnol.* 148, 63–106. doi:10.1007/10_2014_295.

Tian, S., Torres, R., Ballester, A. R., Li, B., Vilanova, L., and González-Candelas, L. (2016). Molecular aspects in pathogen-fruit interactions: Virulence and resistance. *Postharvest Biol. Technol.* 122, 11–21. doi:10.1016/j.postharvbio.2016.04.018.

Tisch, D., and Schmoll, M. (2010). Light regulation of metabolic pathways in fungi. *Appl. Microbiol. Biotechnol.* 85, 1259–1277. doi:10.1007/s00253-009-2320-1.

Torres, M. A., Jones, J. D. G., and Dangl, J. L. (2006). Reactive oxygen species signaling in response to pathogens. 141, 373–378. doi:10.1104/pp.106.079467.

Tran, T. T., Li, H., Nguyen, D. Q., Sivasithamparam, K., Jones, M. G. K., and Wylie, S. J. (2020). Comparisons between genetic diversity, virulence and colony morphology of *Monilinia fructicola* and *Monilinia laxa* isolates. *J. Plant Pathol.* 102, 743–751. doi:10.1007/s42161-020-00498-2.

Usall, J., Casals, C., Sisquella, M., Palou, L., and De Cal, A. (2015). Alternative technologies to control postharvest diseases of stone fruits. *Stewart Postharvest Rev.* 11, 1–6. doi:10.2212/spr.2015.4.2.

Van Der Ent, S., and Pieterse, C. M. J. (2012). "Ethylene: multi-tasker in plant-attacker interactions," in *Annual Plant Reviews*, ed. Michael T. McManus (Blackwell Publishing Ltd), 343–377. doi:10.1002/9781118223086.ch13.

Veloso, A., Ferreira, D., Gaspar, P. D., Andrade, L. P., Espírito-Santo, C., Silva, P. D., et al. (2021). Influence of storage conditions on fruit quality of "Royal Time" and "Royal Summer" peach cultivars. *Rev. Ciências Agrárias* 2021, 82–90. doi:10.19084/rca.21781.

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

Vendramin, E., Pea, G., Dondini, L., Pacheco, I., Dettori, M. T., Gazza, L., et al. (2014). A unique mutation in a MYB gene cosegregates with the nectarine phenotype in peach. *PLoS One* 9. doi:10.1371/journal.pone.0090574.

Verde, I., Abbott, A. G., Scalabrin, S., Jung, S., Shu, S., Marroni, F., et al. (2013). The high-quality draft genome of peach (*Prunus persica*) identifies unique patterns of genetic diversity, domestication and genome evolution. *Nat. Genet.* 45, 487–494. doi:10.1038/ng.2586.

Verde, I., Jenkins, J., Dondini, L., Micali, S., Pagliarani, G., Vendramin, E., et al. (2017). The Peach v2.0 release: High-resolution linkage mapping and deep resequencing improve chromosome-scale assembly and contiguity. *BMC Genomics* 18, 1–18. doi:10.1186/s12864-017-3606-9.

Vickers, C. E., Gershenzon, J., Lerdau, M. T., and Loreto, F. (2009). A unified mechanism of action for volatile isoprenoids in plant abiotic stress. *Nat. Chem. Biol.* 5, 283–291. doi:10.1038/nchembio.158.

Vilanova, L., Valero-Jiménez, C. A., and van Kan, J. A. L. (2021). Deciphering the *Monilinia fructicola* genome to discover effector genes possibly involved in virulence. *Genes (Basel).* 12, 1–15. doi:10.3390/genes12040568.

Villarino, M., Eguen, B., Lamarca, N., Segarra, J., Usall, J., Melgarejo, P., et al. (2013). Occurrence of *Monilinia laxa* and *M. fructigena* after introduction of *M. fructicola* in peach orchards in Spain. *Eur. J. Plant Pathol.* 137, 835–845. doi:10.1007/s10658-013-0292-6.

Villarino, M., Melgarejo, P., and De Cal, A. (2016). Growth and aggressiveness factors affecting *Monilinia* spp. survival peaches. *Int. J. Food Microbiol.* 227, 6–12. doi:10.1016/j.ijfoodmicro.2016.01.023.

Villarino, M., Melgarejo, P., Usall, J., Segarra, J., and De Cal, A. (2010). Primary inoculum sources of *Monilinia* spp. in Spanish peach orchards and their relative importance in brown rot. *Plant Dis.* 94, 1048–1054. doi:10.1094/pdis-94-8-1048.

Villarino, M., Melgarejo, P., Usall, J., Segarra, J., Lamarca, N., and De Cal, A. (2012). Secondary inoculum dynamics of *Monilinia* spp. and relationship to the incidence of postharvest brown rot in peaches and the weather conditions during the growing season. *Eur. J. Plant Pathol.* 133, 585–598. doi:10.1007/s10658-011-9931-y.

Villarino, M., Sandín-España, P., Melgarejo, P., and De Cal, A. (2011). High chlorogenic and neochlorogenic acid levels in immature peaches reduce *Monilinia laxa* infection by interfering with fungal melanin biosynthesis. *J. Agric. Food Chem.* 59, 3205–3213. doi:10.1021/jf104251z.

Wang, Y. J., Yang, C. X., Li, S. H., Yang, L., Wang, Y. N., Zhao, J. B., et al. (2009). Volatile characteristics of 50 peaches and nectarines evaluated by HP-SPME with GC-MS. *Food Chem.* 116, 356–364. doi:10.1016/j.foodchem.2009.02.004.

Westrick, N. M., Smith, D. L., and Kabbage, M. (2021). Disarming the host: Detoxification of plant defense compounds during fungal necrotrophy. *Front. Plant Sci.* 12, 1–18. doi:10.3389/fpls.2021.651716.

Xi, W., Zheng, Q., Lu, J., and Quan, J. (2017). Comparative analysis of three types of peaches: Identification of the key individual characteristic flavor compounds by integrating consumers' acceptability with flavor quality. *Hortic. Plant J.* 3, 1–12. doi:10.1016/j.hpj.2017.01.012.

Xu, F., Shi, L., Chen, W., Cao, S., Su, X., and Yang, Z. (2014). Effect of blue light treatment on fruit quality, antioxidant enzymes and radical-scavenging activity in strawberry fruit. *Sci. Hortic. (Amsterdam).* 175, 181–186. doi:10.1016/j.scienta.2014.06.012.

Xu, H. X., Chen, J. W., and Xie, M. (2010). Effect of different light transmittance paper bags on fruit quality and antioxidant capacity in loquat. *J. Sci. Food Agric.* 90, 1783–1788. doi:10.1002/jsfa.4012.

Yu, F.-Y., Chiu, C.-M., Lee, Y.-Z., Lee, S.-J., Chou, C.-M., You, B.-J., et al. (2020). Polyketide synthase gene expression in relation to chloromonilicin and melanin production in *Monilinia fructicola*. *Phytopathology*, 1–64. doi:10.1094/phyto-02-20-0059-r.

Zhang, W., Corwin, J. A., Copeland, D. H., Feusier, J., Eshbaugh, R., Cook, D. E., et al. (2019). Plant-necrotroph co-transcriptome networks illuminate a metabolic battlefield. *Elife* 8, 1–32. doi:10.7554/elife.44279.

Zhou, H., Yu, Z., and Ye, Z. (2019). Effect of bagging duration on peach fruit peel color and key protein changes based on iTRAQ quantitation. *Sci. Hortic. (Amsterdam).* 246, 217–226. doi:10.1016/j.scienta.2018.10.072.

Zhu, M., Fang, W., Chen, C., Wang, L., and Cao, K. (2020). Effects of shading by bagging on carotenoid accumulation in peach fruit flesh. *J. Plant Growth Regul.* doi:10.1007/s00344-020-10227-9.

Zhu, P., Zhang, C., Xiao, H., Wang, Y., Toyoda, H., and Xu, L. (2013). Exploitable regulatory effects of light on growth and development of *Botrytis cinerea*. *J. Plant Pathol.* 95, 509–517. doi:10.4454/JPP.V95I3.038.

Zipfel, C. (2014). Plant pattern-recognition receptors. *Trends Immunol.* 35, 345–351. doi:10.1016/j.it.2014.05.004.

2. OBJECTIVES

The aim of the present thesis was to decipher components of the host-pathogen-environment interaction by studying the effect of **light as an abiotic factor** on *Monilinia* spp.-nectarine interaction and elucidating **fungal virulence factors** and **fruit defense mechanisms** in response to ***Monilinia laxa* infection**. To achieve this main goal, the following specific objectives were evaluated.

Regarding light:

Objective 1. To determinate the effect of lighting treatments and darkness on:

1.1. the behavior of *M. laxa* and *M. fructicola* in *in vitro* conditions (ecophysiology) and whether these treatments affect to their capacity to infect fruit (chapter 1)
1.2. brown rot development and ethylene production in artificially inoculated nectarines during postharvest storage (chapter 1)
1.3. the prevention of natural fungal disease incidence (fruit decay) and fruit quality during postharvest storage (chapter 2)

Objective 2. To evaluate the effect of fruit bagging as a light modulator during preharvest on:

2.1. fruit quality parameters on harvest day and after postharvest storage (chapter 2)
2.2. natural fungal disease incidence (fruit decay) after postharvest storage (chapter 2)
2.3. fruit susceptibility to *M. laxa* and *M. fructicola* during postharvest storage (chapter 1)

Regarding host-pathogen interaction:

Objective 3. To unravel strategies deployed by nectarine and *M. laxa* during their interaction through:

3.1. identifying host defense responses involved in resistance or susceptibility to brown rot (chapter 3)
3.2. determining relevant strategies employed by *M. laxa* to cause disease (chapter 3)

Objective 4. To evaluate the putative role of nectarine terpenoid metabolism and volatiles in response to *M. laxa* infection:

4.1. assessing the expression of terpenoid biosynthesis genes of nectarine in response to *M. laxa* (chapter 4)
4.2. determining the most relevant volatile organic compounds associated with the interaction with *M. laxa* (chapter 5)

3. METHODOLOGY

The results of this thesis have been grouped into five chapters, each of them corresponding to a scientific article. In this section, a scheme of the methodologies conducted in this thesis are summarized in Figures 1 to 5, and further details are collected in each chapter of the Results section.

Chapter 1

Light intensity alters the behavior of *Monilinia* spp. *in vitro* and the disease development on stone fruit-pathogen interaction

Firstly, to assess the effect of light in the behavior of *M. laxa* and *M. fructicola* in *in vitro* conditions (ecophysiology), cultures of *M. laxa* and *M. fructicola,* initiated with 10 µL of 10^5 conidia mL^{-1} on Petri dishes containing PDA and/or PDA plates supplemented with 25% tomato pulp (PDA-T), were incubated for one week under different postharvest treatments consisting of two lighting treatments (T1 and T2) and darkness (control) (**Figure 1**). After the incubation under each treatment, several ecophysiological parameters for both species were evaluated (**Figure 2**), comprising growth parameters (colony morphology, conidiation, conidia morphology, and growth rate), and conidial viability. Germination was assessed for 6 h (each 30 - 60 min).

Next, to evaluate the effect on the capacity of *M. laxa* and *M. fructicola* to infect fruit after both pathogens were exposed to the lighting treatments, fungal suspensions of each species were prepared from 7-day-old cultures on PDA-T grown under each treatment. These suspensions were used to artificially inoculate (10 µL of 10^5 conidia mL^{-1}) two organically grown cultivars of nectarines ('Fantasia' and 'Venus'), previously homogenized using a portable DA-Meter (immature and mature fruit extremes were discarded) (**Figure 1**). Fruit was incubated under darkness and high humidity. Along 7 days, disease symptoms were examined to evaluate the aggressiveness parameters, including incidence, severity, incubation periods, and latency (**Figure 2**). In fruit inoculated with *M. fructicola*, conidiation (concentration of conidia) was also determined after 7 days of incubation.

Finally, the study of both the effects of postharvest lighting treatments and fruit bagging on the nectarine-*Monilinia* spp. interaction was conducted in the same experimental design. For that, unbagged and bagged (bagged at least one month before harvest) fruit of four organically grown nectarine cultivars ('Fantasia', 'Venus', 'Nectatinto', and 'Albared') were harvested at commercial maturity (**Figure 1**). After removing the bags, the fruit was homogenized using a portable DA-meter (immature and mature fruit extremes were discarded). Fruit was inoculated with 50 µL of 10^5 conidia mL^{-1} of either *M. laxa* or *M. fructicola* and a mock treatment (sterile water with 0.01% Tween-80). Fruit was first incubated at high humidity conditions for conidia's establishment for 24h and then stored under the lighting treatments (T1 and T2) and darkness. Along 7 days, diseases symptoms were examined to evaluate the aggressiveness parameters (incidence, severity, and incubation period) (**Figure 2**). Ethylene measurements at 4 time points along the infection period were also conducted on *M. laxa, M. fructicola*, and mock-inoculated fruit.

Chapter 2

Impact of fruit bagging and postharvest storage conditions on quality and decay of organic nectarines

After assessing the effect of lighting treatments and fruit bagging on nectarine-*Monilinia* spp. interaction, similar studies were conducted on non-artificially inoculated fruit (naturally infected fruit). The same experimental design used in chapter 1 was conducted for non-inoculated fruit submitted to one lighting treatment (T1) and control (darkness). For that, unbagged and bagged fruit of four organically grown nectarine cultivars ('Fantasia', 'Venus', 'Nectatinto' and 'Albared') were harvested at commercial maturity. After removing the bags and fruit homogenization, fruit quality and ethylene measurements were performed on the harvest day (**Figure 1**). Fruit was first incubated at high humidity conditions for 24h and then moved to postharvest storage (darkness and T1). Natural disease symptoms were recorded during 7 or 9 days (depending on the cultivar) to determine the fruit decay for each bagging condition, lighting treatment, and cultivar (**Figure 2**). After 7 or 9 days, fruit quality parameters (weight, cheek diameter, DA-meter, flesh firmness, soluble solids content, and titratable acidity) were also assessed.

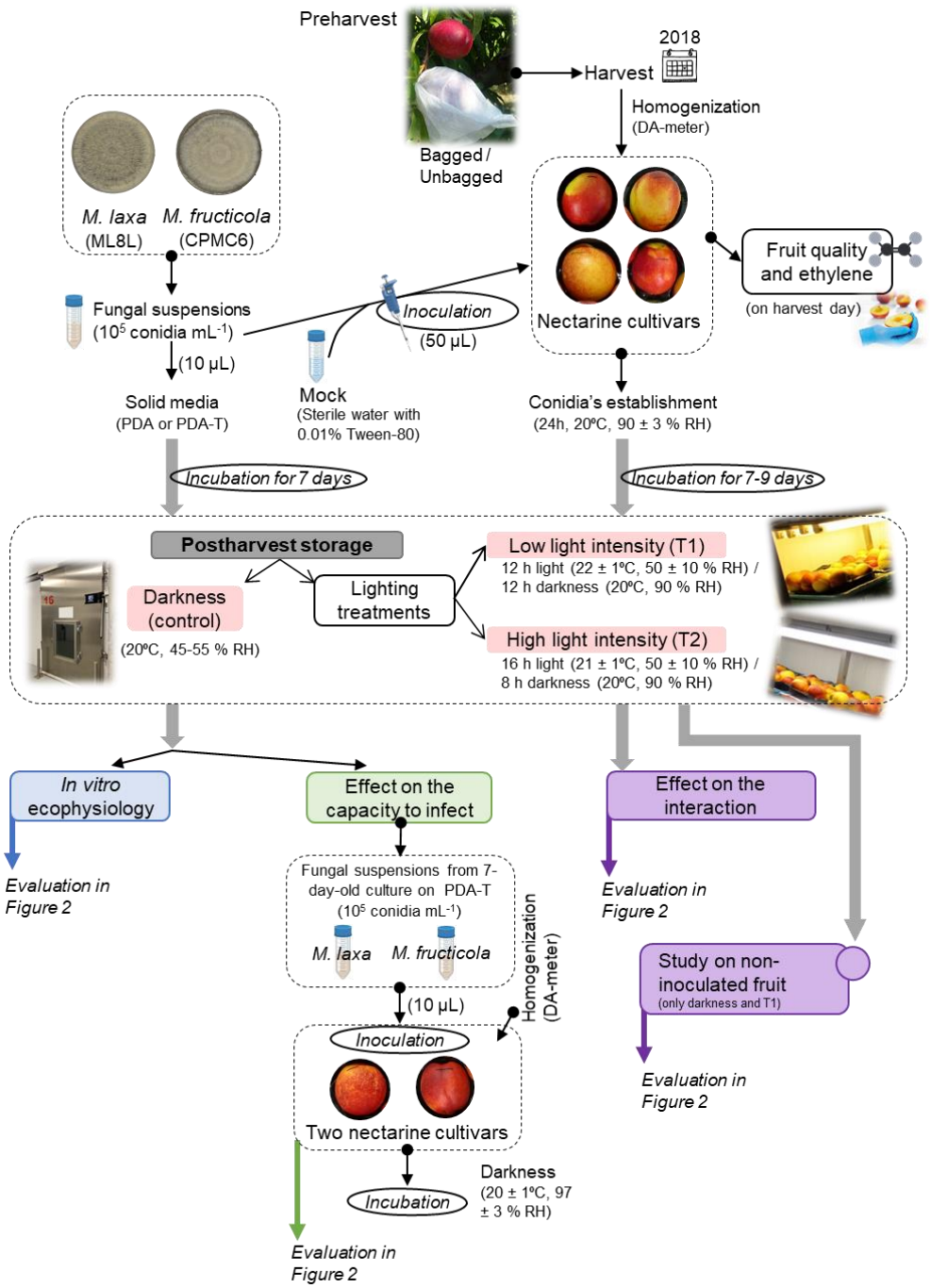

Figure 1. Effect of lighting treatments on *Monilinia* spp. in *in vitro* conditions and on *Monilinia* spp. development in four nectarine cultivars: Experimental design.

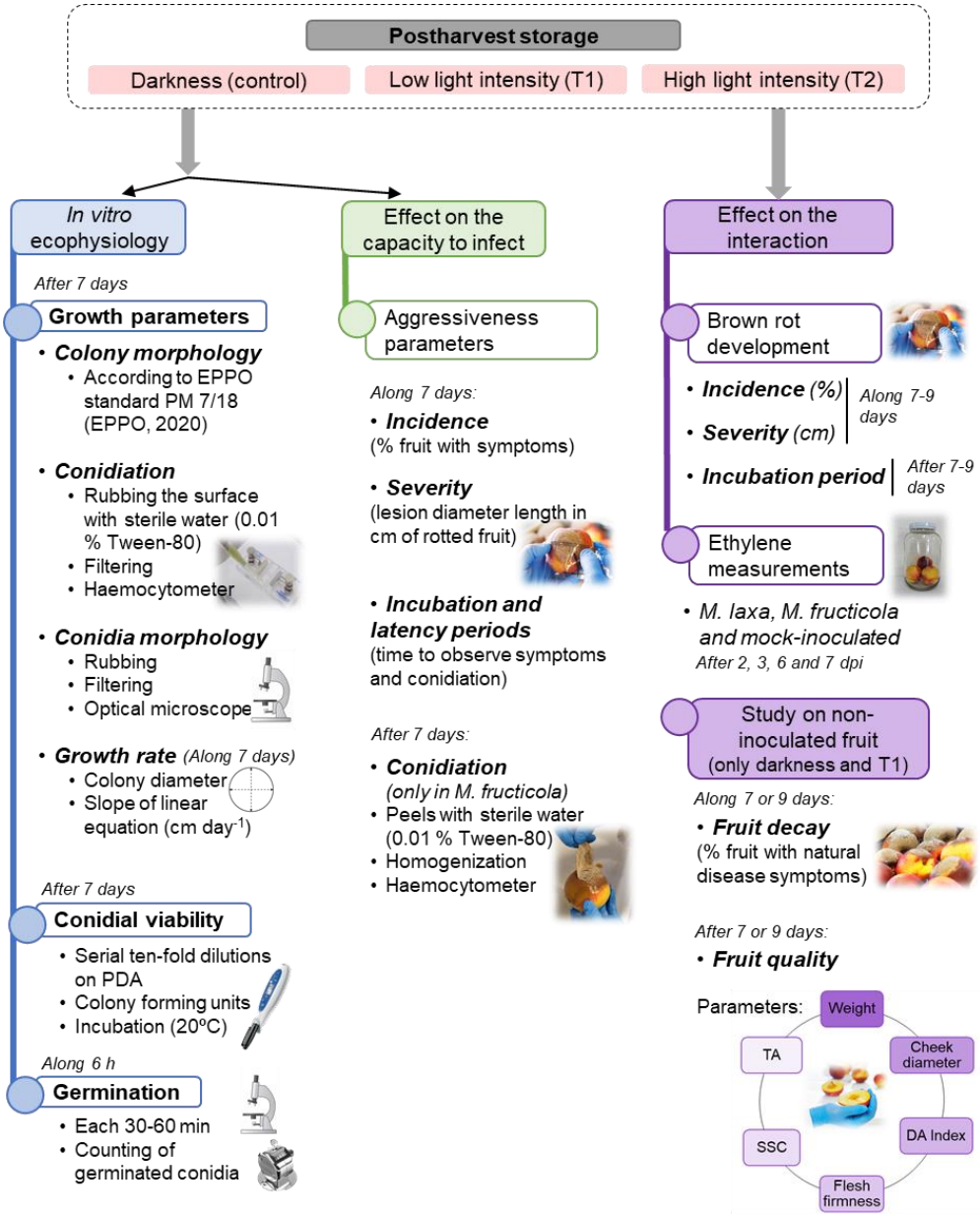

Figure 2. Effect of lighting treatments on *Monilinia* spp. in *in vitro* conditions and on *Monilinia* spp. development in four nectarine cultivars: Evaluations.

Chapter 3

Depicting the battle between nectarine and *Monilinia laxa*: the fruit developmental stage dictates the effectiveness of the host defenses and the pathogen's infection strategies

To elucidate host defense responses and pathogen virulence mechanisms, a dual RNA-Sequencing analysis was performed in two developmental stages of organic nectarines of the 'Venus' cultivar. For that, the fruit was harvested at two different fruit developmental stages, "mature" and "immature" (1 month before "mature") in 2018 (**Figure 3**). Fruit was homogenized using a portable DA-meter, and fruit quality was performed. For each developmental stage, the fruit was inoculated with 6 drops of 30 µL of fungal suspensions of *M. laxa* (10^6 conidia mL^{-1}). The same inoculation methodology using sterile water with 0.01% Tween-80 was conducted for mock treatment. Fruit was incubated under darkness and high humidity for 3 days. Along this period, one set of fruit was used to monitor the ethylene production on both *M. laxa*- and mock-inoculated fruit at 4 time points along the infection period. The rest of the fruit was used for the sampling for the molecular studies. For that, sampling of inoculated sites was conducted at 6, 14, 24, 48, and 72 h and flash-frozen in liquid nitrogen. RNA extraction was conducted to obtain fruit and fungal RNA to perform RNA-Sequencing (**Figure 4**). Libraries of cDNA were performed at UC Davis (California), following the RNA-Sequencing in an external service. Data processing, differential expression analysis, and enrichment analysis were conducted in collaboration with Dr. Barbara Blanco-Ulate (UC Davis, California). Fungal biomass on both inoculated and mock fruit was also assessed, and RNA-Sequencing validation was performed through gene expression analysis (RT-qPCR).

Chapter 4

Transcriptional profiling of the terpenoid biosynthesis pathway reveals putative roles of linalool and farnesal in nectarine resistance against brown rot

The previous RNA-sequencing analysis revealed several potential plant metabolisms involved in fruit defense. Among them, terpenoid metabolism resulted in being relevant for resistance and/or susceptibility of nectarines to *M. laxa.* An in-deep analysis of terpenoid metabolism was conducted in 'Venus' and 'Albared' cultivars. The same plant material and harvest design previously described for 'Venus' nectarines (chapter 3) were used for 'Albared'. First, a disease evaluation was conducted on both cultivars at two developmental stages (**Figure 3**). For that, fruit was inoculated with 1 drop of 30 µL of *M. laxa* suspensions (10^6 conidia mL^{-1}), and the same inoculation methodology using sterile water with 0.01% Tween-80 was conducted for mock treatment. Fruit was incubated under darkness and high humidity for 3 days, and incidence and severity were examined daily. The same inoculation methodology, incubation, sampling, and RNA extraction previously described for the 'Venus' cultivar was conducted for 'Albared' nectarines. Fungal biomass was determined on both inoculated and mock fruit through gene expression analysis (RT-qPCR) (**Figure 4**). Based on RNA-Sequencing analysis, eleven nectarine genes related to terpenoid metabolism were selected for gene expression analyses (RT-qPCR) of 'Albared' samples. Primers were mainly designed *de novo.* For 'Venus' samples, the normalized read counts obtained from the differential analysis of the RNA-Sequencing (chapter 3) were used to assess the gene expression of terpenoid biosynthetic genes.

Chapter 5

Emission of volatile organic compounds during nectarine-*Monilinia laxa* interaction and its relationship with fruit susceptibility to brown rot

An analysis of the volatile organic compounds (VOCs) profile was conducted on both 'Venus' and 'Albared' cultivars. For that, the same experimental design described for 'Venus' and 'Albared' cultivars (chapters 3 and 4) was conducted in 2020 (**Figure 5**). Fruit sampling of both cultivars at two developmental stages inoculated with either *M. laxa* or mock treatment (control) was conducted at 72 days post-inoculation (dpi), time in which cultivars presented different susceptibility to *M. laxa.* Besides, an analysis of the VOCs profile of *M. laxa* during *in vitro* growth on media based on peach juice was also conducted. For that, flasks containing 30 mL of peach juice-based-medium were inoculated with conidial suspensions to a final concentration of 2 x 10^4 conidia mL^{-1} and then incubated at 20 ± 1 ºC under darkness. Sampling was conducted at 3 and 7 dpi by extracting the mycelium and rinsing it with sterile water. Mycelia were immediately flash-frozen in liquid nitrogen. Analyses were conducted on 5 or 1.5 g of frozen homogenized plant tissue or *M. laxa* mycelium, respectively. Volatile releasing and absorption were performed through headspace solid-phase microextraction (HS-SPME). A coupled gas chromatography/mass spectrometry (GC-MS) was used for the VOCs analysis, and volatiles were identified using the NIST11.L library. Among the total VOCs detected in the nectarine-*M. laxa* study, only those most relevant for the interaction were selected for further representation and data analysis. Among the total VOCs detected in the *M. laxa in vitro* culture, those shared with the previous study were selected for further representation and data analysis.

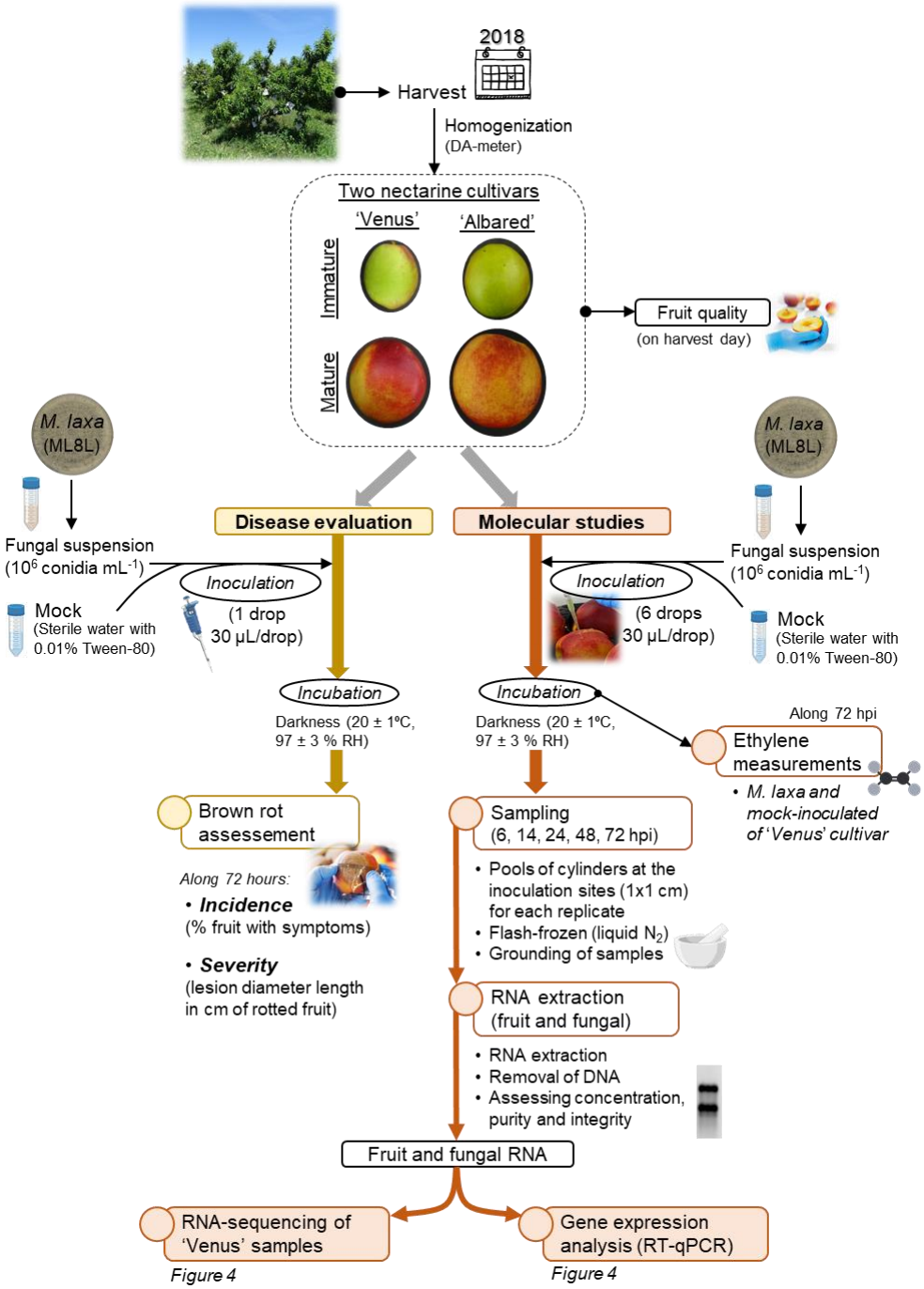

Figure 3. Host-pathogen interaction studies: Experimental design

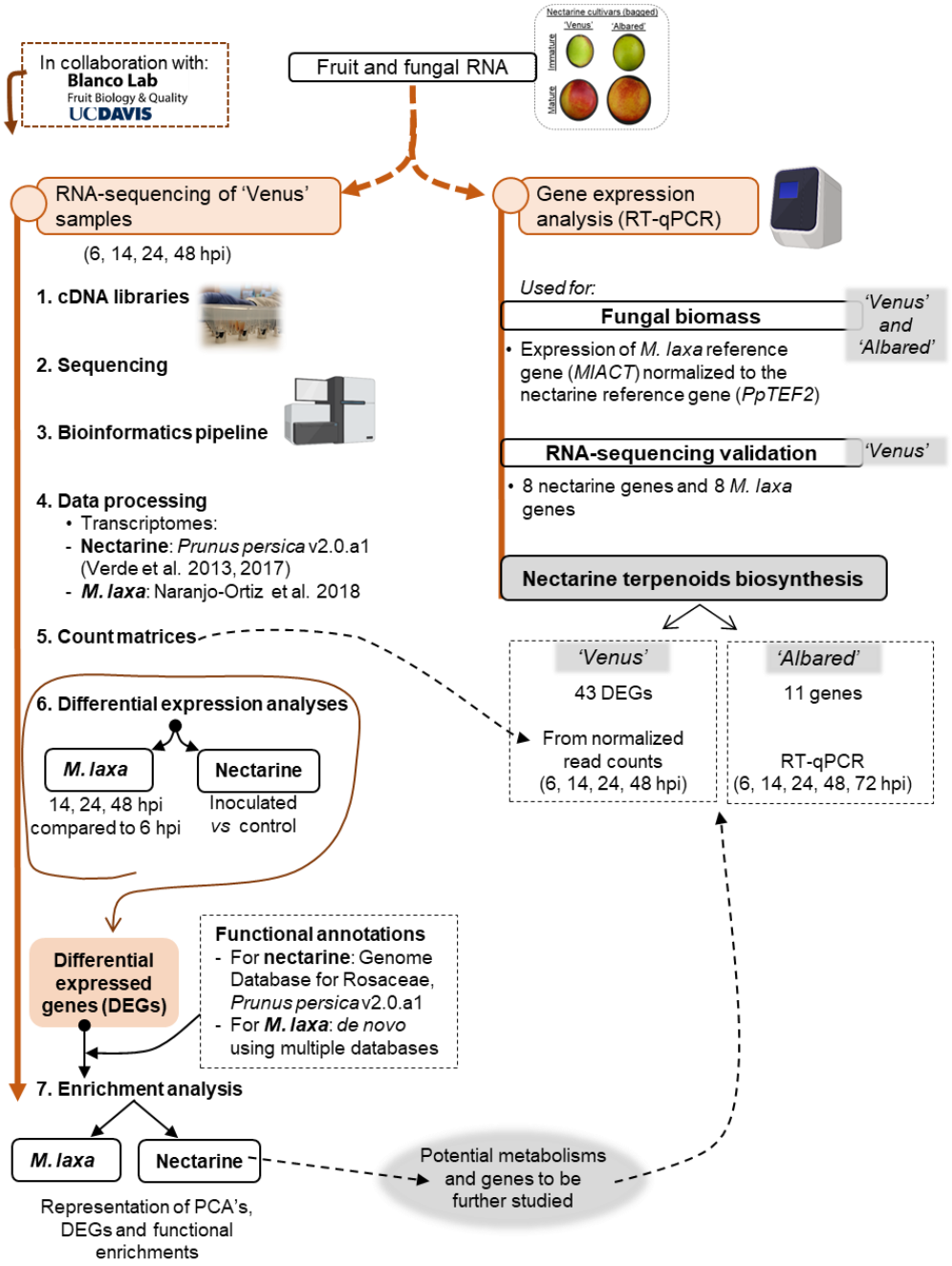

Figure 4. Host-pathogen interaction studies: Molecular analyses

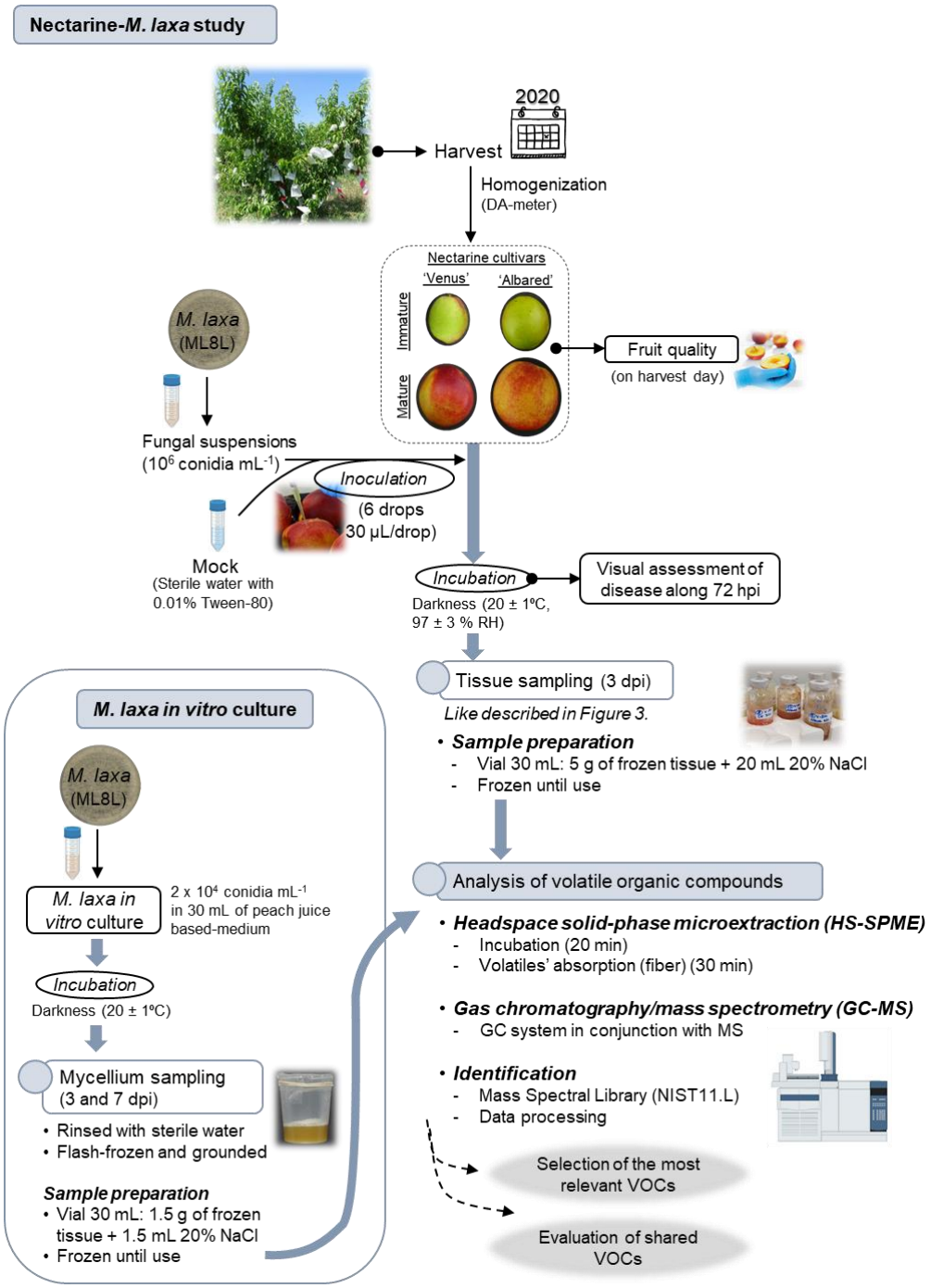

Figure 5. Host-pathogen interaction studies: Volatile organic compounds analysis

4. RESULTS

Chapter 1. Light intensity alters the behavior of *Monilinia* spp. *in vitro* and the disease development on stone fruit-pathogen interaction

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Abstract

The development of brown rot caused by the necrotrophic fungi *Monilinia* spp. in stone fruit under field and postharvest conditions depends, among others, on environmental factors. The effect of temperature and humidity are well studied but there is little information on the role of light in disease development. Herein, we studied the effect of two lighting treatments and a control condition (darkness) on: i) several growth parameters of two *Monilinia* spp. (*M. laxa* and *M. fructicola*) grown in vitro and ii) the light effect in their capacity to rot the fruit (nectarines) when exposed to the different lighting treatments. We also assessed the effect of such abiotic factors in the development of the disease on inoculated nectarines during postharvest storage. Evaluations also included testing of the effect of fruit bagging on disease development as well as on ethylene production. Under in vitro conditions, lighting treatments altered colony morphology and conidiation of *M. laxa* but this effect was less acute in *M. fructicola*. Such light-induced changes under in vitro development also altered the capacity of *M. laxa* and *M. fructicola* to infect nectarines, with *M. laxa* becoming less virulent. The performance of *Monilinia* spp. exposed to treatments was also determined in vivo by inoculating four bagged or unbagged nectarine cultivars, indicating an impaired disease progression. Incidence and lesion diameter of fruit exposed to the different lighting treatments during postharvest showed that the effect of the light was intrinsic to the nectarine cultivar but also *Monilinia* spp. dependent. While lighting treatments reduced *M. laxa* incidence, they enhanced *M. fructicola* development. Preharvest conditions such as fruit bagging also impaired the ethylene production of inoculated fruit, which was mainly altered by *M. laxa* and *M. fructicola*, while the bag and light effects were meaningless. Thus, we provide several indications of how lighting treatments significantly alter *Monilinia* spp. behavior both in vitro and during the interaction with stone fruit. This study highlights the importance of modulating the lighting environment as a potential strategy to minimize brown rot development on stone fruit and to extent the shelf-life period of fruit in postharvest, market and consumer's house.

Keywords: necrotroph, brown rot, nectarine, photomorphogenesis, preharvest, postharvest, bagging, ethylene

Introduction

Species of *Monilinia* are responsible of brown rot disease on stone fruit both in the field and during postharvest. In particular, *Monilinia laxa* is found worldwide (Obi et al., 2018) and is the main causal agent of brown rot in Europe (Rungjindamai et al, 2014), while *M. fructicola* is more virulent (Kreidl et al., 2015) and its presence has been increasing in Spanish orchards since 2006 (De Cal et al., 2009; Villarino et al., 2013). These pathogens are necrotrophic since they can colonize fruit tissues causing cellular death (Garcia-Benitez et al., 2016), rotting most parts of the tree, from buds to fruit (Villarino et al., 2010). Sources of inoculum can be primary (e.g., from mummified fruit (Gell et al., 2008) or secondary (e.g., from infected fruit (Villarino et al., 2012)), resulting in a polycyclic disease (reviewed in Oliveira Lino et al., 2016).

Environmental conditions are critical for brown rot development. Temperature and wetness period are the most studied factors and are demonstrated to influence penetration and spread of both *M. laxa* (Gell et al., 2008) and *M. fructicola* (Luo and Michailides, 2001). Solar radiation, wind speed and rainfall factors also play an important role in the spread of *M. laxa* and *M. fructicola* (Gell et al., 2009) but detailed information is scarce. During plant-pathogen interactions, light quantity and quality (Idnurm and Crosson, 2009) and photoperiod (Tisch and Schmoll, 2010) not only influence the behavior of the pathogen, but also the interaction with its hosts (Carvalho and Castillo, 2018).

Fungi are able to adapt their metabolic pathways when perceiving light (Tisch and Schmoll, 2010; Corrochano, 2019) through a complex of photoreceptors and so regulate their behavior and development (Bahn et al., 2007), such as the development of sexual or vegetative reproductive structures and tropism of unicellular structure (Corrochano, 2019). The light alters gene expression patterns of *Monilinia* spp. (De Miccolis Angelini et al., 2018) and, in fact, some photoreceptors and related regulatory proteins (e.g. velvet regulatory family) have recently been described in *M. laxa* (Rodríguez-Pires et al., 2021). However, how the fungus perceives and modulates light responses needs further investigation. For instance, *Botrytis cinerea*, a species of the same family of *Monilinia* spp., produces sclerotia in constant darkness and conidia under the light, which is enhanced when growing under light-dark cycles compared to constant light (Schumacher, 2017). Hence, the presence of light but also its intensity, quality and photoperiod can alter fungal development both under *in vitro* conditions and on fruit. Thus said, little is known regarding how light can affect the infection process of phytopathogenic fungi, and only one study incubating *M. laxa*-inoculated

stone fruit under different white light conditions and photoperiods have been conducted (Rodríguez-Pires et al., 2020).

Likewise, light regulates plant growth and development (Folta and Carvalho, 2015), including responses to biotic stresses (Roeber et al., 2020). Perception of light can control the establishment of the systemic acquired resistance, which would lead to an enhancement of disease resistance in several plant-pathogen interactions (Métraux, 2002; Roberts and Paul, 2006). When both the pathogen-host interaction and light conditions take place, plant circadian rhythm controls the pathogen host, leading to a daytime-dependent response (Griebel and Zeier, 2008). After an interaction, the host induces a hormone signaling cascade, which in turn, triggers defense mechanisms (Pandey et al., 2016). Ethylene is one of the multiple hormones which mediates the host response against necrotrophic pathogens (McDowell and Dangl, 2000), although it also modulates the response to numerous abiotic stresses (reviewed in Müller and Munné-Bosch, 2015). In fact, recent studies have demonstrated the link between the jasmonate/ethylene pathway and the photoreceptor-mediated light response, and its importance on the resistance to the pathogen *B. cinerea* (Xiang et al., 2020).

The solar radiation that fruit receives in the field varies along the year, being low at the beginning and higher at the end of the season. Nowadays, growers are implementing some alternative practices to control pests and diseases during preharvest (Usall et al., 2015) in substitution to those based on chemicals. Among them, fruit bagging (Allran, 2017) has been proved to be effective in controlling brown rot incidence in peach and plum (Keske et al., 2011, 2014). However, these alternatives, together with the use of colored shade nets (Ilić and Fallik, 2017), have also an effect on the incidence of solar radiation that fruit receives during its development, altering many fruit physicochemical properties (Sharma et al., 2014; Ilić and Fallik, 2017; Zhou et al., 2019), which ultimately could impair the fruit response to pathogens.

Therefore, the understanding of the light effect on the pathogens but also on the capacity of fruit to respond properly to infections is critical to establish an optimal practice in the field but also along the postharvest in packinghouses and through the distribution chain. Thus said, this study aimed to understand the effect of the darkness (control) and two lighting treatments on the behavior of *M. laxa* and *M. fructicola in vitro* and during the interaction with nectarine fruit. In particular, we assessed the *i*) effect of the three treatments on the ecophysiology of *Monilinia* spp. *in vitro* in two different culture media; *ii*) effect of the three treatments in the capacity of the two species after being exposed to the lighting treatments to infect fruit; *iii*) effect of fruit

bagging on fruit susceptibility at postharvest; *iv*) effect of the three treatments in the ethylene production and the development of the disease of the inoculated fruit exposed to different lighting treatments.

Materials and Methods

Fungal material and incubation treatments

The species of *Monilinia* used in this study were single-conidia strains of *M. laxa* (ML8L) and *M. fructicola* (CPMC6), deposited in the Spanish Culture Type Collection (CECT 21100 and CECT 21105, respectively). Fungal cultures and conidial suspensions were maintained and prepared as described by Baró-Montel et al. (2019c). Fungal suspensions were prepared at 10^5 conidia mL^{-1} and used to inoculate plates or fruit depending on the experiment.

Both *in vitro* and *in vivo* experiments were conducted in a growth chamber with the following incubation and lighting treatments: 1) "Control", at 20 ºC, 45-55 % RH and complete darkness; 2) "Treatment 1", consisting of 4 fluorescents of low light intensity and incandescent white TL-D 36 W/827 (Ta = 2700 K, 3350 lm, 350 - 740 nm, 630 nm max) (Philips), and photoperiod of 12 h light (22 ± 1 ºC, 50 ± 10 % RH) / 12 h dark (20 ºC, 90 % RH); 3) "Treatment 2", consisting of 4 fluorescents of high light intensity and cool white TL-D 58W/840 (Ta = 4000 K, 5000 lm, 300 - 740 nm, 550 nm max) (Philips), and photoperiod of 16 h light (21 ± 1 ºC, 50 ± 10 % RH) / 8 h dark (20 ºC, 90 % RH).

In vitro ecophysiology

To evaluate the light effect on the two strains of *Monilinia* spp., Potato Dextrose Agar (PDA; Biokar Diagnostics, 39 g L^{-1}) and/or PDA plates supplemented with 25 % tomato pulp (PDA-T) were inoculated with one drop of 10 µL of the conidial suspension (10^5 conidia mL^{-1}) of each species on the center of Petri dishes. Plates were incubated under the three incubations treatments mentioned above. During and after 7 days under each treatment, ecophysiological parameters for both species were evaluated: growth parameters (including colony morphology, conidiation, conidia morphology and growth rate), conidial viability and germination. All experiments consisted of three replicates per treatment, culture media and *Monilinia* spp. and each experiment was repeated twice.

Growth parameters

Four growth parameters were investigated for each *Monilinia* spp. grown in PDA and PDA-T media. The colony growth rate, the total conidiation, a visual inspection of colony features according to EPPO standard PM 7/18 (3) (Bulletin OEPP/EPPO, 2020) and the conidia morphology of cultures were assessed. The colony growth rate (cm day^{-1}) was determined as the slope of the lineal equation obtained from the individual measurements of the mean of the colony diameter in two perpendicular directions by plotting growth diameter (cm) *vs* time (days). Conidiation was calculated by rubbing the conidia from the surface of the PDA-T plates with a known volume of sterile water containing 0.01 % Tween-80 (*w/v*), filtering through two layers of sterile cheesecloth and then titrating the conidia using a haemocytometer. The concentration of conidiation (conidia mL^{-1}) was calculated and expressed as total conidiation in relation to control. Comparison of conidia morphology from plates subjected to different treatments was assessed by rubbing the PDA and PDA-T plates with sterile water containing 0.01 % Tween-80 (*w/v*) and filtering through two layers of sterile cheesecloth. Images at 40x magnification were taken in an optical microscope (Leica DM5000B, Leica Microsystems CMS GmbH, Germany). The images were acquired using a Leica color digital camera (Leica DFC 420).

Conidial viability

To test the conidial viability (i.e., the ability of conidia to form new colonies) after exposing the *Monilinia* species grown in PDA-T media for 7 days under the different light regimes, colony-forming units (CFUs) were measured by performing serial ten-fold dilutions on PDA medium. Plates were incubated for 3 to 4 days at 20 °C under darkness.

Germination of conidia

Percentage of germinated conidia (%) was studied under optical microscopy, as described by Casals et al. (2010) with some modifications. Droplets (10 µL) of the conidial suspension (10^5 conidia mL^{-1}) were placed around PDA plates, and immediately incubated under each treatment. Samplings were carried out each 30 min or 1 h until 6 h. To stop germination at each incubation time, 1 mL of 25 % ammonia was applied onto a filter paper placed on the cover of the Petri dish. Conidia were considered germinated when cell wall deformation forming a germ tube was observed.

Light effect on the ability of *Monilinia* spp. to infect fruit

To evaluate whether the capacity of *Monilinia* spp. to infect fruit was altered by treatments, an inoculation of nectarines with the two species previously exposed to the three treatments was conducted. Experiments were performed with two organic cultivars of nectarines (*P. persica* var. *nucipersica* (Borkh.) Schneider). 'Fantasia' and 'Venus' cultivars were obtained from an orchard located in Alfarràs and Ivars de Noguera (Lleida, Catalonia, Spain), respectively. Fruit for analysis was further homogenized by using a portable DA-Meter (TR-Turoni, Forli, Italy), based on the single index of absorbance difference.

Fruit inoculations

Cultures of *Monilinia* spp. exposed to each treatment were used to artificially inoculate nectarines. One drop (10 µL) of conidial suspensions (10^5 conidia mL^{-1}) of *M. laxa* or *M. fructicola* was placed on PDA-T plates and cultures were maintained under each afore-mentioned treatment (section "Fungal material and incubation treatments") for 7 days. Conidial suspensions of both species were prepared as described above (section "Fungal material and incubation treatments"). Non-wounded fruit was inoculated with one drop (10 µL) of conidial suspension (10^5 conidia mL^{-1}). A total of 20 fruits per cultivar, species, and treatment were used. Fruit were stored in a growth chamber, inside plastic boxes with wet filter paper (distilled water), under darkness and controlled incubation conditions (20 ± 1 °C, 97 ± 3 % RH).

Aggressiveness parameters

Disease symptoms were examined to calculate incidence (percentage of fruit with brown rot symptoms) and severity (lesion diameter length in cm of rotted fruit) along 7 days after inoculation. The incubation period (number of days to the observation of the onset of brown rot symptoms) and the latency period (number of days to the observation of conidiation) were also recorded. In fruit inoculated with *M. fructicola*, the conidiation was determined on the fruit surface after 7 days post-inoculation (dpi) for each treatment. For that, peels of the infected area of 3-4 inoculated fruits were obtained, immersed in a sterile filter bag with 40 mL sterile water containing 0.01 % Tween-80 (*w/v*) and homogenized in a Stomacher (Seward, London, UK) set at 12 strokes s^{-1} for 120 s. The filtered volume was recovered and the conidia was counted using a haemocytometer. The concentration of conidia (conidia g fresh $peel^{-1}$) was calculated as the mean of each group of 3-4 fruit.

Light effect on the *Monilinia* spp.-fruit interaction

To evaluate the light effect on the interaction of *Monilinia* spp. with nectarine, inoculated fruit with both *M. laxa* and *M. fructicola* were incubated under each aforementioned treatment. For that, experiments were conducted with organically grown cultivars of nectarines; two early-mid ('Fantasia' and 'Venus') and two late ('Nectatinto' and 'Albared') cultivars, obtained from an orchard located in Alfarràs, Ivars de Noguera, Gimenells and Alfarràs (Lleida, Catalonia, Spain), respectively. The light effect was assessed on unbagged and bagged fruit, which was bagged with white paper bags at least one month before harvest. Bagged and unbagged fruit were harvested in the same sun-side of trees due to the influence of fruit canopy position to all fruit's characteristics (Minas et al., 2018). Bags were removed just before conducting assays. Fruit for analysis was further homogenized by using a portable DA-Meter (TR-Turoni, Forli, Italy), based on the single index of absorbance difference.

Fruit inoculations and conidia establishment

Inoculation was carried out by placing one drop (50 µL) of the conidial suspension (10^5 conidia mL^{-1}) on the colored side of non-wounded fruit. A mock inoculation (mock) was performed by inoculating sterile water containing 0.01 % Tween-80 (*w/v*). Inoculated fruit was first incubated at high humidity conditions for 24 h for the establishment of conidia on the fruit surface. For that, fruit was placed on boxes covered with a wet paper and a plastic bag, and then stored in a growth chamber, at controlled conditions (20 °C, 90 ± 3 % RH). After that, fruit were immediately placed under each lighting treatment.

Aggressiveness parameters and ethylene measurements

Fruit were daily examined to calculate brown rot incidence, severity and incubation period along 7 days, as described above (section "Aggressiveness parameters"). Experiments were conducted with 4 replicates of 5 fruits each per cultivar, bag condition, treatment and *Monilinia* spp. Ethylene production of both mock-inoculated fruit and *Monilinia* spp. inoculated fruit was determined as described by Giné-Bordonaba et al. (2017). Measurements were conducted at four time points along the infection time course until 7 dpi. At each sampling point, fruit were placed in 3.8 L sealed flasks and left to incubate for 2 h. After ethylene measurements, fruit were placed back under each lighting treatment. Experiments were conducted with four replicates of three fruits each.

Statistical analysis

Data were statistically analyzed with JMP® software version 14.2.0 (SAS Institute Inc., Cary, NC, USA). Prior to the analysis, all data were checked for the assumptions of parametric statistics and transformed when needed. Data of *in vitro* assays (growth rate, total conidiation and conidial viability), conidiation on fruit surface and severity were used as original data. Incubation and latency period (dpi) were subjected to square root transformation. Data of ethylene production (µL kg^{-1} h^{-1}) were subjected to Log transformation. All these data were subjected to analysis of variance (ANOVA). Conidia germination (%) was analyzed using the generalized linear model (GLM) based on a Poisson distribution and Log-link function. Brown rot incidence (%) was analyzed using the GLM based on a binomial distribution and logit-link function. When the analysis was statistically significant, orthogonal contrasts ($P \leq 0.05$) were performed for means separation among treatments. When comparisons were conducted between two means (bagged *vs* unbagged), Student's T-test ($P \leq 0.05$) was used. For means comparison of inoculated fruit (mock, *M. laxa* and *M. fructicola*), Tukey's HSD test ($P \leq 0.05$) was conducted.

Results

Light differentially alters the phenotype of *M. laxa* and *M. fructicola*

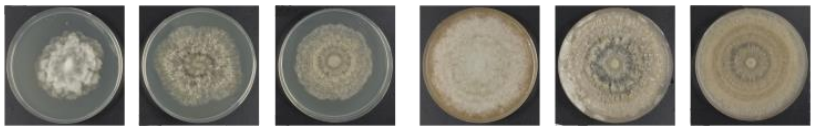
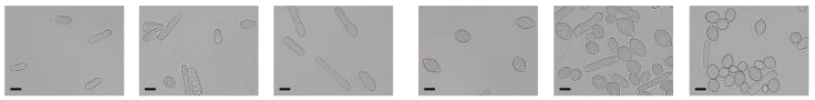
To evaluate the light effect on the *in vitro* behavior of *Monilinia* spp., we assessed several ecophysiological parameters after exposing *M. laxa* and *M. fructicola* to two lighting treatments and control condition (constant darkness) for 7 days (Figure 1). Under both treatments, colony features were very different from those grown under control condition, for each *Monilinia* spp. in both culture media (Figure 1A, B). The colonies of *M. laxa* in both culture media subjected to both lighting treatments showed more hazel colors if compared to those white and grey colors observed in the control condition. *Monilinia laxa* significantly grew faster under both lights than under control condition in both media. *Monilinia fructicola* grown on PDA-T and subjected to both lighting treatments presented lobed culture's margin, while when growing under control condition, colonies presented entire margins. Only treatment 2 was able to significantly reduce its growth rate when growing on PDA but not in PDA-T medium. Conidia morphology examination showed that, except for *M. laxa* on PDA where few conidia were detected, both treatments altered conidia shapes of both *M. laxa* and *M. fructicola* (Figure 1A, B). While conidia from control condition cultures presented the typical ovoid and limoniform morphologies, lighting treatments

induced an increase of irregular morphologies such as globose, cylindrical, or ellipsoidal (Figure 1A, B).

The visual inspection of *Monilinia* cultures demonstrated that *M. laxa* produced more conidia in PDA-T plates exposed to both treatments 1 and 2 (2.19 and 3.31-fold significantly higher, respectively) if compared to control condition (constant darkness) (Figure 1C). However, we were not able to observe *M. laxa* conidiation on the PDA medium incubated under control condition (Figure 1A). In fact, almost no conidia were visualized in microscopic inspections in PDA plates (Figure 1A) as exposed above. In contrast, conidiation of *M. fructicola* was significantly reduced in PDA-T plates exposed to both treatments 1 and 2 (0.59 and 0.71-fold, respectively) if compared to control condition (Figure 1D). Conidiation in PDA plates was like that on PDA-T plates, where both treatments 1 and 2 significantly reduced (0.43 and 0.29-fold, respectively) the number of conidia in illuminated plates compared to control condition. Regarding the conidial viability, results showed that treatment 2 significantly reduced the number of CFUs of *M. laxa*, although on treatment 1 it was slightly higher (1.12-fold) than on control condition (Figure 1C). In contrast, we did not observe any effect of lighting treatment on the conidial viability of *M. fructicola* (Figure 1D). Finally, exposition to light affected the germination's capability of neither *M. laxa* (Figure 1E) nor *M. fructicola* (Figure 1F).

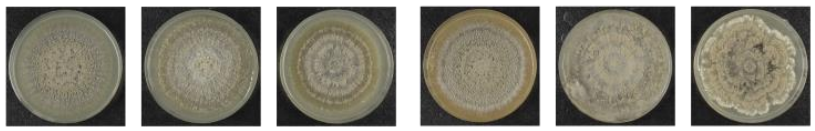
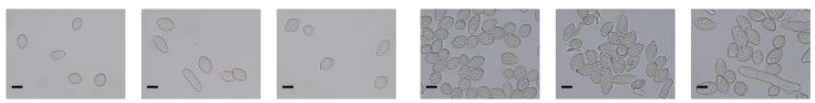
A

M. laxa

	PDA			PDA-T		
	Control	T1	T2	Control	T1	T2
						
Colony features						
Color of the culture:	Grey, white	Hazel, grey	Hazel, cream	Grey, cream	Hazel, grey	Olivaceous, hazel
Culture's margin:	Lobed rosetted	Lobed	Lobed	Lobed	Lobed	Entire
Conidiation	No	Sparse	Sparse	Sparse	Abundant	Abundant
Concentric ring of conidiation:	Yes	Yes	Yes	Yes	Yes	Yes
Formation of black stromatal arcs:	Rare	Abundant	Yes	No	Rare	Rare
Growth rate (cm day^{-1}):	0.65 ± 0.02 B	0.89 ± 0.03 A	0.80 ± 0.05 A	1.19 ± 0.04 B	1.33 ± 0.01 A	1.32 ± 0.02 A
Morphology of conidia:						

B

M. fructicola

	PDA			PDA-T		
	Control	T1	T2	Control	T1	T2
						
Colony features						
Color of the culture:	Olivaceous	Cream, hazel	Cream, olivaceous	Olivaceous	Hazel, olivaceous	Hazel, olivaceous
Culture's margin:	Entire	Entire	Entire	Entire	Lobed	Lobed
Conidiation	Abundant	Abundant	Sparse	Abundant	Abundant	Abundant
Concentric ring of conidiation:	Yes	Yes	Yes	Yes	Yes	Yes
Formation of black stromatal arcs:	Abundant	Abundant	Abundant	Rare	Abundant	Abundant
Growth rate (cm day^{-1}):	1.27 ± 0.02 A	1.26 ± 0.01 A	1.09 ± 0.02 B	1.15 ± 0.06 A	1.15 ± 0.14 A	1.14 ± 0.07 A
Morphology of conidia:						

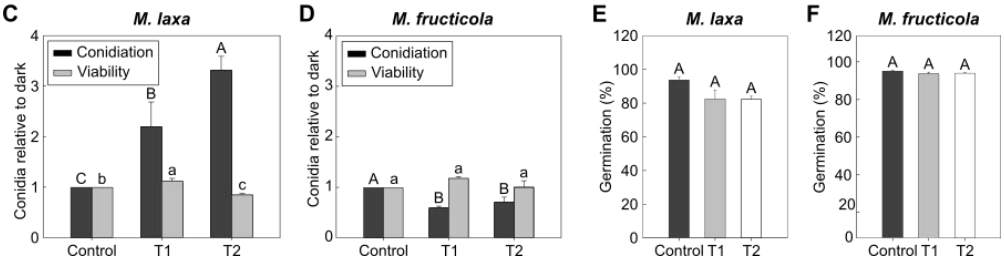


***Figure 1. In vitro ecophysiology of* Monilinia *spp. after exposure to treatments 1 and 2 and control condition** (constant darkness)**.** Images of Monilinia cultures, description of colony features, growth rate (cm day^{-1}) and microscopy images (40x) of* M. laxa ***(A)** and* M. fructicola ***(B)** grown on PDA and PDA-T and incubated under each light condition. Data for growth rate represent the mean of replicates (n = at least 4) ± standard error of the means. Different letters indicate significant differences (P ≤ 0.05) among incubation conditions according to orthogonal contrasts. Scale bar for microscopy images is indicated (10 µm). Conidiation and conidial viability of* M. laxa ***(C)** and* M. fructicola ***(D)** grown on PDA-T incubated under each light condition. Data is represented relative to the control condition (control = 1). Different uppercase and lowercase letters indicate significant differences (P ≤ 0.05) of conidiation and conidial viability, respectively, among incubation conditions according to orthogonal contrasts. Germination (%) after 6 h of* M. laxa ***(E)** and* M. fructicola ***(F)** on PDA medium. Different letters indicate significant differences (P ≤ 0.05) among incubation conditions according to orthogonal contrasts. For panels **C**, **D**, **E** and **F**, bars represent the mean of replicates (n = at least 4) and error bars represent the standard error of the means.*

Contrary to *M. fructicola, M. laxa* becomes less virulent once exposed to lighting treatment

To test how changes observed under *in vitro* ecophysiological parameters affected the capacity of both *Monilinia* spp. to infect fruit, we assessed the development of the disease on nectarines inoculated with *M. laxa* or *M. fructicola* which were previously grown under each lighting treatment. In 'Fantasia' nectarines inoculated with *M. laxa,* both treatments 1 and 2 significantly reduced incidence (55 and 61 %, respectively) and severity (2.4 and 2.0 cm, respectively) since the first time point compared to control condition (constant darkness) (90 % of incidence and 3.9 cm of severity) (Figure 2A). No differences in the incubation period were observed among treatments (Figure 2B). Only 10 % of fruit inoculated with *M. laxa* which was grown under treatment 1 and control condition showed conidiation on the fruit surface. In line with these results, fruit inoculated with *M. laxa* grown under treatment 1 revealed a higher latency period (1.17-fold) than those inoculated with the pathogen held under control condition (Figure 2B). Besides, under the treatment 2, the fungal development did not even show any conidiation (Figure 2B). Thus, although both treatments improved the behavior of *M. laxa in vitro,* they made the pathogen impair and delay its capacity to infect and in consequence, made it less virulent.

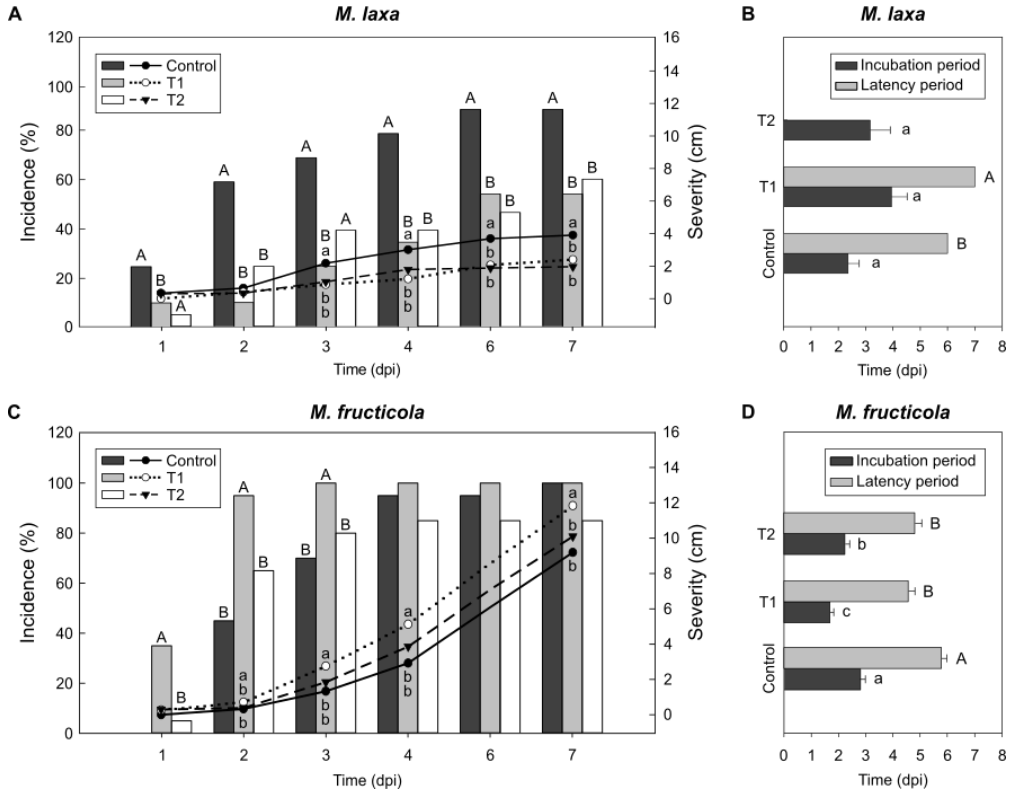


Figure 2. Light effect on the capacity of* Monilinia *spp. to infect fruit in 'Fantasia' cultivar. *Incidence (% of brown rot, bars) and severity (lesion diameter length in cm of rotted fruit, lines) of* M. laxa ***(A)*** *and* M. fructicola ***(C)*** *in 'Fantasia' nectarines along the infection time course (dpi, days post-inoculation) after growing the fungi for 7 days under treatments 1 and 2 and control condition (constant darkness). Bars represent the mean of incidence on fruit (n = 20). Lines represent the mean of diameter length of rotted fruit. Different uppercase and lowercase letters indicate significant differences (P ≤ 0.05) of incidence and severity, respectively, among incubation conditions according to orthogonal contrasts at each time point. No letters indicate no significant differences. Incubation and latency periods (dpi) of* M. laxa ***(B)*** *and* M. fructicola ***(D)*** *in 'Fantasia' nectarines after growing the fungi for 7 days under treatments 1 and 2 and control condition. Bars represent the mean of fruits with symptoms (n = 2 to 20) and error bars represent the standard error of the means. Different lowercase and uppercase letters indicate significant differences (P ≤ 0.05) of incubation and latency periods, respectively, among incubation conditions according to orthogonal contrasts.*

Regarding *M. fructicola*, the incidence of fruit inoculated with *M. fructicola* grown under treatment 1 significantly peaked at early time points (up to 100 %), although such differences completely subsided through time (Figure 2C). Interestingly, only *M. fructicola* subjected to that treatment 1 was able to cause significantly higher lesion diameter on fruit (up to 11.8 cm) than that in the two other conditions (9.2 cm under

control condition and 10.1 cm under treatment 2) (Figure 2C). In addition, both treatments accelerated the onset of disease symptoms. The incubation periods of fruit inoculated with *M. fructicola* exposed to treatment 2 and treatment 1 were significantly lower (1.25 and 1.65-fold, respectively) than when the pathogen was grown under control condition (constant darkness) Figure 2D). Between 94-100 % of inoculated fruit, irrespective of treatment in which the fungus was grown, presented conidiation on the fruit surface. However, the latency of *M. fructicola* under either lighting treatment significantly accelerated the onset of conidiation symptoms (between 4.6 and 4.8 days of average) compared to control condition (an average of 5.8 days) (Figure 2D). Finally, regarding the concentration of conidia in the fruit surface, treatment 1 induced *M. fructicola* to produce significantly more conidia on fruit (1.92-fold) compared to control condition, whereas treatment 2 was like control condition (Supplementary Figure S1). Hence, while light seemed to make *M. laxa* lose virulence, it accelerated the onset of disease symptoms and conidiation of *M. fructicola*. All these experiments were also conducted in another nectarine cultivar ('Venus') and results showed similar tendencies of fruit susceptibility to brown rot (Suppl. Figure S2).

Fruit bagging can alter its susceptibility to *Monilinia* spp. in a cultivar-dependent manner

To test the effect of fruit bagging on fruit susceptibility to brown rot, we conducted a disease evaluation of four different nectarine cultivars inoculated with *M. laxa* and *M. fructicola* and incubated under control condition (constant darkness). In inoculated fruit with either *M. laxa* or *M. fructicola*, results showed two tendencies of fruit susceptibility (Suppl. Table S1). Unbagged 'Fantasia' nectarines were more susceptible to both *Monilinia* spp. than fruit that was bagged during preharvest ("bagged fruit"). However, the other cultivars ('Venus', 'Nectatinto' and 'Albared') showed that unbagged fruit was slightly more resistant to both *Monilinia* spp. than bagged fruit. Hence, results pointed out that the effect of fruit bagging in fruit susceptibility to brown rot could be cultivar-dependent.

Light reduces *M. laxa* disease in nectarines but enhance *M. fructicola* development

To further investigate the light effect in brown rot progress at postharvest, we assessed some aggressiveness features after incubating the inoculated fruit under the lighting treatments. Results demonstrated that the effect of light on the host-pathogen interaction was cultivar-dependent. While we observed significant differences in incidence and severity of early-mid cultivars such as 'Fantasia' (Figure

3A, C; 4A, C), we detected almost no differences in late cultivars such as 'Nectatinto' and 'Albared' (data not shown). The disease behavior on the later cultivars was similar among all lighting treatments. In addition, the incubation period was slightly higher in the early cultivars than in the late ones (data not shown).

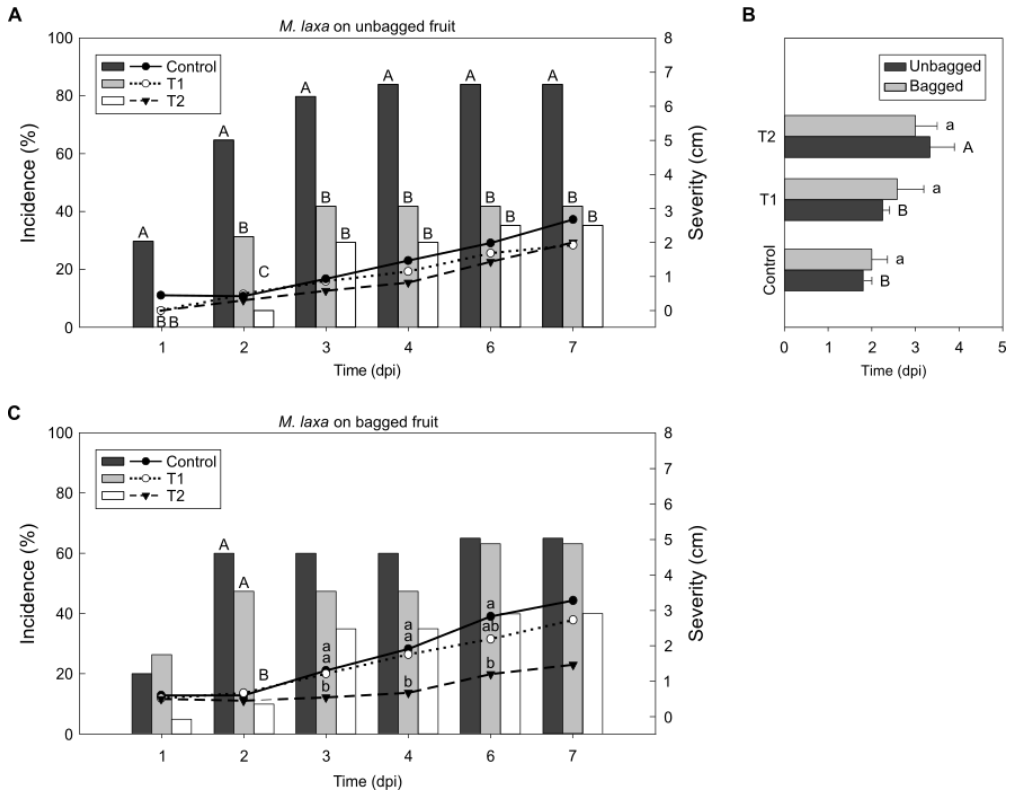


Figure 3. Light effect on the* M. laxa*-nectarine interaction. *Incidence (% of brown rot, bars) and severity (lesion diameter length in cm of rotted fruit, lines) of* M. laxa *in unbagged* ***(A)*** *and bagged* ***(C)*** *'Fantasia' nectarines along the infection time course (dpi, days post-inoculation) incubated for 7 days under treatments 1 and 2 and control condition (constant darkness). Bars represent the mean of incidence on fruits (n = 20). Lines represent the mean of diameter length of rotted fruit. Different uppercase and lowercase letters indicate significant differences (P ≤ 0.05) of incidence and severity, respectively, among incubation conditions according to orthogonal contrasts at each time point. No letters indicate no significant differences. The incubation period (dpi) of* M. laxa *in bagged and unbagged 'Fantasia' nectarines* ***(B)*** *after 7 days of incubation under treatments 1 and 2 and control condition. Bars represent the mean of fruits with symptoms (n = 2 to 20) and error bars represent the standard error of the means. Different uppercase and lowercase letters indicate significant differences (P ≤ 0.05) among incubation conditions in unbagged and bagged fruit, respectively, according to orthogonal contrasts.*

Afterwards, we selected the 'Fantasia' cultivar for further analysis. *Monilinia laxa* incidence on unbagged nectarines maintained under control condition (constant darkness) was significantly higher (84 %) than that under treatment 1 and 2 (42 % and 35 %, respectively) (Figure 3A). The lesion diameter revealed the same tendency as incidence, although with no significant differences along time (Figure 3A). In the same line, the incubation period was significantly higher in inoculated unbagged fruit exposed to the treatment 2 than treatment 1 and control condition (1.5- and 1.8-fold, respectively) (Figure 3B). Regarding bagged nectarines, there was no difference in neither incidence (ranging from 40 to 65 %) (Figure 3C) nor incubation period (between 2 and 3 days) among treatments (Figure 3B). However, the severity of *M. laxa*-inoculated fruit subjected to both control condition and treatment 1 was significantly higher than that under treatment 2 at 3 and 4 dpi (Figure 3C), although such differences subsided along the infection time course.

In 'Fantasia' unbagged nectarines inoculated with *M. fructicola*, the incidence at 2 dpi under treatment 2 (85 %) was significantly higher than those incidences under control condition and treatment 1 (65 % and 35 %, respectively). Treatment 2 also significantly increased severity (up to 12.6 cm) in unbagged fruit along time compared to that under control condition and treatment 1 (9.5 and 10.2 cm, respectively) (Figure 4A). However, the incubation period was similar among all treatments (Figure 4B). In bagged fruit, treatment 1 rose disease incidence (95 %) and was significant from 4 dpi onwards, compared to the other treatments tested (65 % both). Contrary, treatment 2 significantly increased severity (12.7 cm) compared to that under control condition and treatment 1 (10.4 and 10.5 cm, respectively) (Figure 4C). No differences were observed among treatments when analyzing the incubation period of bagged or unbagged fruit (Figure 4B). Interestingly, the *M. fructicola*-incubation period of bagged fruit incubated under control condition was significantly lower (1.4- fold) than unbagged fruit at the same condition (Figure 4B). Overall, light seemed to negatively affect the disease incidence and severity of *M. laxa* whereas it caused the opposite effect for *M. fructicola*.

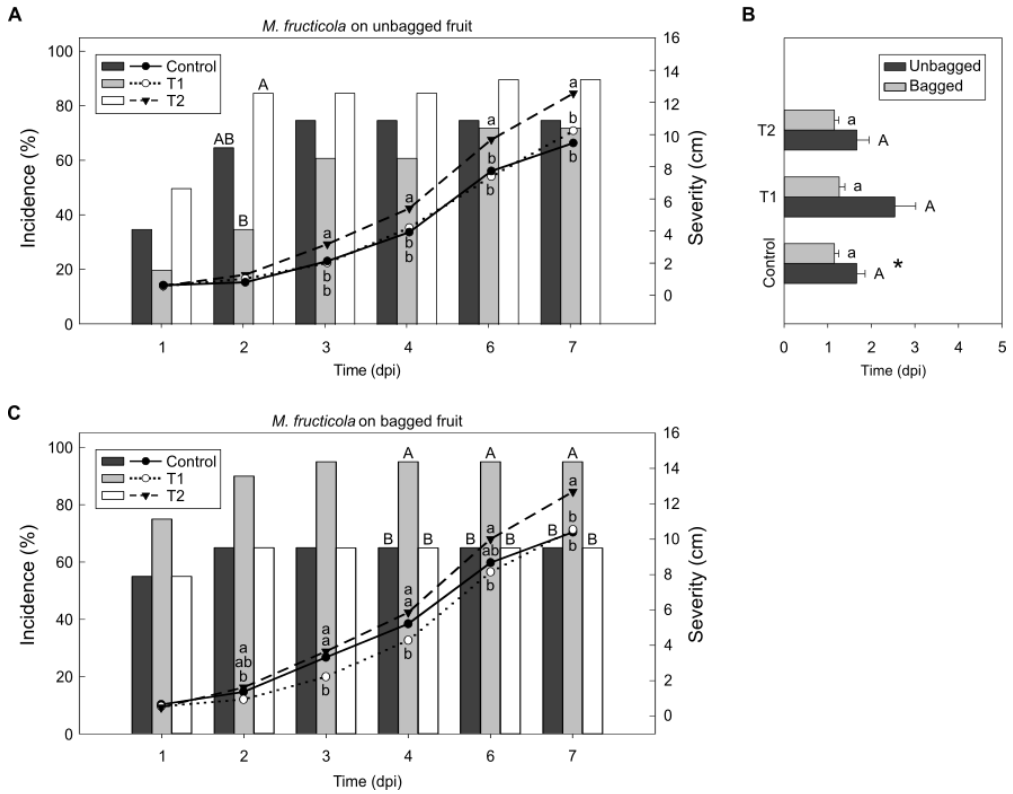


Figure 4. Light effect on the* M. fructicola*-nectarine interaction. *Incidence (% of brown rot, bars) and severity (lesion diameter length in cm of rotted fruit, lines) of* M. fructicola *in unbagged* **(A)** *and bagged* (**C**) *'Fantasia' nectarines along the infection time course (dpi, days post-inoculation) incubated for 7 days under treatments 1 and 2 and control condition (constant darkness). Bars represent the mean of incidence on fruits (n = 20). Lines represent the mean of diameter length of rotted fruit. Different uppercase and lowercase letters indicate significant differences (P ≤ 0.05) of incidence and severity, respectively, among incubation conditions according to orthogonal contrasts at each time point. No letters indicate no significant differences. The incubation period (dpi) of* M. fructicola *in bagged and unbagged 'Fantasia' nectarines* ***(B)*** *after 7 days of incubation under treatments 1 and 2 and control condition. Bars represent the mean of fruits with symptoms (n = 2 to 20) and error bars represent the standard error of the means. Different uppercase and lowercase letters indicate significant differences (P ≤ 0.05) among incubation conditions in unbagged and bagged fruit, respectively, according to orthogonal contrasts. Symbol (*****) indicates significant differences between bag conditions under control condition according to Student's T-test (P ≤ 0.05).*

Ethylene production in *M. laxa*-fruit interaction is bag and lighting treatment-dependent

In addition, to assess the development of the disease in inoculated nectarines, we also evaluated the ethylene production of the pathosystem under the different experimental treatments (Figure 5). Firstly, we determined the bagging effect on ethylene production in *Monilinia*-inoculated fruit incubated under control condition (constant darkness) (Figure 5A, B, C). Results denoted that while unbagged and bagged mock-inoculated fruit produced similar ethylene levels (Figure 5A), on *M. laxa* inoculated nectarines, the levels of ethylene produced by the unbagged fruit were significantly higher than those in the bagged fruit (Figure 5B). Contrary to *M. laxa, M. fructicola* induced a peak of ethylene at 6 dpi in both unbagged and bagged fruit and results only showed significant differences between bagging conditions at 2 dpi (Figure 5C).

We further evaluated the light effect on both unbagged and bagged fruit inoculated with each species. Results demonstrated no significant differences in the ethylene production of mock-inoculated fruit among treatments regardless of the bagging condition in which come from (Figure 5D, G). Ethylene levels of unbagged fruit inoculated with *M. laxa* were significantly higher at 7 dpi when incubated under control condition (constant darkness) than when exposed to treatments 1 and 2 (4.5 and 2.7-fold, respectively) (Figure 5E). In contrast, bagged fruit inoculated with *M. laxa* and incubated under control condition displayed an opposite ethylene pattern (Figure 5H). Under lighting treatments, fruit inoculated with *M. laxa* slowly increased ethylene production of the pathosystem along time and was significantly higher than under control condition, resulting in a 10.9- and 4.9-fold increase under treatment 1 and 2, respectively. Regarding unbagged fruit inoculated with *M. fructicola*, all incubation treatments showed similar ethylene patterns, which peaked at 6 dpi. Only at 3 dpi, fruit incubated under treatment 1 significantly produced lower ethylene levels than the other treatments (Figure 5F). Bagged fruit inoculated with *M. fructicola* revealed a similar pattern to unbagged fruit. In that case, only ethylene levels of the *Monilinia*-fruit interaction exposed to treatment 2 significantly peaked at 3 dpi (Figure 5I).

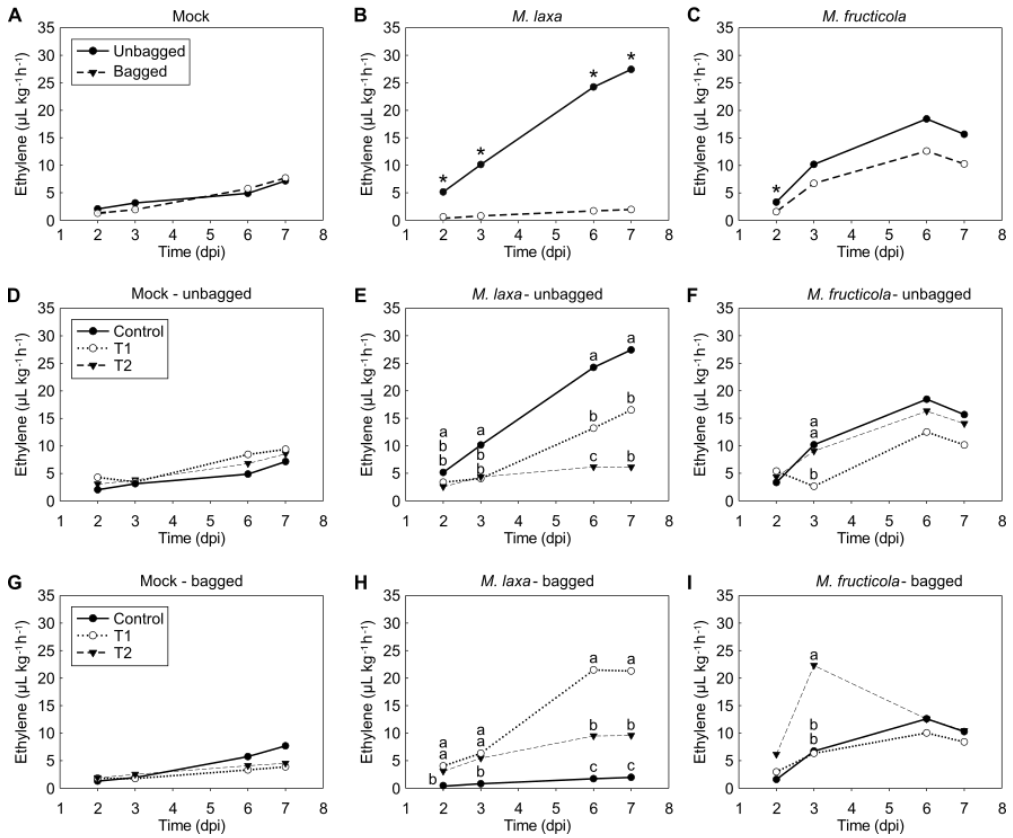


Figure 5. Ethylene production of mock fruit, M. laxa- and M. fructicola-fruit interaction among bag and lighting treatments through time in 'Fantasia' nectarines. *Ethylene measurements of mock (A), M. laxa-fruit (B) and M. fructicola-fruit (C) along the infection time course (dpi, days post-inoculation) under control condition (constant darkness). Symbols (*) indicate significant differences between bag conditions at each time point according to Student's T-test (P ≤ 0.05). Ethylene measurements in mock fruit (D, G), M. laxa-fruit (E, H) and M. fructicola-fruit (F, I) in unbagged (D, E, F) and bagged conditions (G, H, I) along the infection time course (dpi) under each lighting treatment. Different letters indicate significant differences (P ≤ 0.05) among light conditions according to orthogonal contrasts at each time point. No letters indicate no significant differences. In all graphics, values represent the mean of ethylene measurements of each replicate (n = 4).*

When comparing the ethylene emission pattern among mock-inoculated fruit and *Monilinia* spp. inoculated fruit on unbagged nectarines (Suppl. Figure S3), results clearly demonstrated that the ethylene pattern emitted by both *Monilinia* spp.-inoculated fruit incubated under control treatment was significantly higher than the one produced by mock fruit. The ethylene production of *M. laxa*-fruit interaction increased progressively along time, producing a similar pattern to mock fruit, although to a different extent, depending on the incubation treatment. In fact, *M. laxa*-

inoculated fruit maintained under control treatment produced significantly higher levels (3.8-fold) than those of the mock fruit (Suppl. Figure S3A), while a slight difference of 1.8-fold between mock and *M. laxa*-inoculated fruit was observed under the treatment 1 at 7 dpi (not statistically different) (Suppl. Figure S3B). Conversely, the presence of *M. fructicola* stimulated an ethylene peak at 6 dpi that was 3.8, 1.5 and 1.6-fold higher under control treatment, treatment 1 and 2, respectively, if compared to mock-inoculated fruit (Suppl. Figure S3). Overall, both *Monilinia* spp. induced the ethylene levels of the pathosystem but in a lighting treatment-dependent manner.

Discussion

Light is essential in both the preharvest period (i.e., solar radiation) and postharvest chain (i.e., artificial lighting) of fruit. The combination of light quality, intensity and photoperiod constitute a source of information for the fruit but also to pathogens, and in turn, can influence the onset of symptoms of the development of the disease on the fruit surface. Scarce information regarding *in vitro* development of *Monilinia* spp. or brown rot infection on stone fruit under the effect of light is available. Some studies have been conducted with discrete sections of the spectrum such as long-wave UV (De Cal and Melgarejo, 1999), in other *Monilinia* spp. such as *M. fructigena* (Marquenie et al., 2003) and the effect of visible white light in *M. laxa* isolates (Rodríguez-Pires et al., 2020; Rodríguez-Pires et al., 2021). However, a study aiming to decipher the effect of lighting treatments on the two main *Monilinia* spp. of stone fruit has never been conducted. Accordingly, we characterized for the first time, the effect of different lighting treatments on both the *in vitro* fungal development of *M. fructicola* and *M. laxa*, and during the interaction of *Monilinia* spp. – nectarine fruit, using similar artificial lighting treatment previously applied to *M. laxa*-stone fruit studies (Rodríguez-Pires et al., 2020).

Altered conidia morphology impairs the conidial viability in a *Monilinia* spp.-dependent manner

Monilinia laxa demonstrated a broader photomorphogenesis response to light than *M. fructicola* under *in vitro* conditions. In this study, cultures grown on either PDA or PDA-T media and incubated under control condition (constant darkness) were similar to other *M. laxa* or *M. fructicola* isolates grown on similar conditions (Tran et al., 2020; Rodríguez-Pires et al., 2021). After exposure to both lighting treatments, but especially under treatment 2, *M. laxa* mycelia turned mainly hazel whereas the colony color of *M. fructicola* was not altered at any condition (Figure 1A, B). To regulate fungal

biology, fungi sense light through photoreceptors and use it as an input of information (Tisch and Schmoll, 2010). One of the most common and long-term effects of light responses is the induction of pigment expression, such as carotenoid biosynthesis in many microorganisms (Fuller et al., 2015; Corrochano, 2019), and, in fact, the carotenoid production in the closely related organism *B. cinerea* has been suggested (Schumacher et al., 2014). In turn, carotenoids are highly implicated in protecting cells from reactive oxygen species (ROS) (Avalos and Limón, 2015). Light also induces the biosynthesis of other pigments such as melanin and mycosporines in several fungi (Fuller et al., 2015). The role of melanin in *M. fructicola* has been described on not only the protection against environmental stresses such as desiccation, UV irradiation, and temperature (Rehnstrom and Free, 1996), but also on the conidia turgor adjustment and full virulence to infect stone fruit (Yu et al., 2020). Visible light can cause oxidative stress in *B. cinerea* cells, which could be, in part, due to an alteration in the homeostasis of cellular ROS levels (Canessa et al., 2013). In fact, our results revealed how both treatments altered the morphology of conidia after 7 days of incubation under each light condition (Figure 1A, B) if compared to typically limoniform (or also cylindrical in the case of *M. laxa*) conidia shapes (Yin et al., 2015) observed under control condition. Therefore, these findings suggest that under these light conditions, conidia were submitted to stress that ultimately affected cell turgor. However, the impaired morphology could also rely on the result of the phototropism generated in response to light, which has been described in conidia, apothecia and conidial germ tubes of *B. cinerea* (Jarvis, 1972). Regarding conidial viability, studies on how light alters the ability to form new colonies of *Monilinia* spp. are nonexistence. We demonstrated that *M. laxa*, but not *M. fructicola,* increased its conidial viability under treatment 1 but reduced it under treatment 2 in relation to control condition (Figure 1C, D). In fact, Lafuente et al., (2018) already demonstrated that continuous blue light and complete darkness increased *Penicillium digitatum* cell viability *in vitro* compared to non-continuous light. These results are in line with what we observed for *M. laxa*, since the spectrum of lights used in this study do emit small wavelengths around blue. Alternatively, the altered conidia morphology could explain the reduction of *M. laxa* cell viability under treatment 2. Thus, the relation between turgor and the ability to form new colonies is a point of interest. Although some studies point out the role of light in controlling the conidial germination (Corrochano, 2019; Yu and Fischer, 2019), herein we did not observe an effect either on *M. laxa* or *M. fructicola* (Figure 1E, F).

Monilinia laxa coped with light stress and its *in vitro* development was favored

Light altered the *in vitro* fungal expansion, especially in *M. laxa*. Under standard conditions (growing on PDA medium at 22-25 ºC and darkness), *M. fructicola* grows faster and produces more conidia than *M. laxa* (Villarino et al., 2016; Tran et al., 2020), like observed in the present study (Figure 1). However, the light made *M. laxa* to grow and produce more conidia (compared to control condition) than *M. fructicola* on PDA-T medium. Another reported light effect, widely described in *B. cinerea* (Schumacher, 2017), is that light can regulate biological responses, such as vegetative mycelial growth and the transition from sexual to asexual reproduction (conidiation) (Corrochano, 2019). In fact, the endogenous circadian clock also controls conidiation (Hevia et al., 2015). In this line, Canessa et al., (2013) reported that a photoperiod of cool white light and control condition reduced the growth rate and increased conidiation of a strain of *B. cinerea*. Our results suggest that *M. laxa* and *M. fructicola* behaved similarly to *B. cinerea* in terms of conidiation and growth rate, respectively. *Botrytis cinerea* perceives and reacts to the entire visible spectrum and beyond, and several fungal biological responses have been described for each monochromatic section of the spectrum (Schumacher, 2017; Veloso and van Kan, 2018). Green light (around 540 nm) represses mycelial growth (Zhu et al., 2013), whereas blue (around 450 nm) and red (around 650 nm) light restrain conidiation (Tan, 1975). Both treatment 1 and 2 tested herein emit three wavelength peaks around 440, 550 and 630 nm. Remarkably, the orange/red wavelength of treatment 1 is higher than the treatment 2 one. Hence, although *M. laxa* is able to sense and express green light photoreceptors (Rodríguez-Pires et al., 2021), its growth was increased rather than repressed. Zhu et al. (2013) found that under green light, *B. cinerea* cells showed deformed mitochondria and enlarged central vacuoles, probably as a result of the vacuoles' action to eliminate cell structures damaged due to the light stress (Shoji et al., 2010), and in consequence, the growth rates of *B. cinerea* were retarded. However, under such light stress, *M. laxa* could be coping with it through autophagy of damaged organelles structures to support mycelial growth, as has been demonstrated when nutrient availability is limited (Shoji et al., 2010). A contrary effect was observed for *M. fructicola* which suggests the different ability of both species to sense and respond to light. The mechanisms underlying such differences are encouraged. An example pathway of interest related to light is the light-responsive transcription factor (LTF1), which controls development but also is required for maintenance of the redox homeostasis in mitochondria and full virulence in *B. cinerea* (Schumacher et al., 2014). Overall results showed that growth rate was in line with conidial viability and the reviewed

results evidence the different ability of both *Monilinia* spp. to cope with lighting treatments.

Blue and red light have been described to repress conidiation in *B. cinerea* (Tan, 1975). Thus, although *M. laxa* is able to sense and express blue and red-light photoreceptors (Rodríguez-Pires et al., 2021), it increased its conidiation, whereas the conidia production of *M. fructicola* seemed to be affected by these sections of the spectrum (Figure 1C, D). Recent studies have shown that red light drastically increases conidiation of *M. laxa* compared to control condition (constant darkness) while does not affect or alter *M. fructicola* conidiation when compared to control condition (Verde-Yáñez et al., unpublished). Conidiation is regulated by light-responsive transcription factors, such as FL (*fluffy*) for undifferentiated mycelia, and it is induced by blue light through the blue-light photoreceptor *WHITE COLLAR COMPLEX* in the fungal model *Neurospora crassa* (Olmedo et al., 2010). However, our results revealed a fluffy phenotype of *M. laxa* when growing on PDA-T medium and incubated under control condition. Hence, other transcription factors should be responsible for the increased conidiation in *M. laxa* and in-depth studies should be conducted. Blue light has also been shown to act as an antimicrobial agent (Kahramanoğlu et al., 2020), which could, in part, explain the reduced conidiation observed in *M. fructicola*, highlighting again the different ability of both species to respond to light.

The light-induced impaired fungal development ultimately alters their capacity to infect fruit

Light also affects the ability of pathogens to infect and rot fruit, such as described in several pathosystems (Islam et al., 1998; Lafuente et al., 2018). Among the aspects of fungal behavior and development that light can govern, light can regulate secondary metabolism, also related to the balance between sexual development towards conidia (Tisch and Schmoll, 2010; Schumacher, 2017). Our results demonstrated that after incubating *M. laxa* and *M. fructicola* under each lighting treatment for 7 days prior to fruit inoculation, both treatments reduced the ability of *M. laxa* to infect fruit, whereas only treatment 1 seemed to increase the virulence of *M. fructicola* (Figure 2). The colored phenotype and/or the altered conidia morphology observed in *M. laxa* grown on PDA-T medium maintained under lighting treatments could in part, explain its reduced capacity to infect. Similar results and hypotheses have been described for the *P. digitatum*-orange pathosystem. In line with spore viability, continuous blue light (450 nm) and complete darkness exposition of *P. digitatum* cultures lead to increased capability to infect oranges if compared to cultures submitted to non-continuous light

(Lafuente et al., 2018). The authors suggested that the anomalous morphology of spores was more responsible for the lower capacity to infect fruit rather than the other parameters evaluated (metabolic activity and ethylene production). Alternatively, mutants of *B. cinerea* producing conidia in either light or darkness are associated with reduced virulence in primary leaves of French bean (Schumacher et al., 2012). However, how these altered features ultimately impair viability and capacity to infect fruit needs further investigation. Interestingly, fruit inoculated with *M. laxa*, previously incubated under treatment 1, showed conidia on fruit surface only after 7 dpi, slightly later than under control conditions (constant darkness) (6 dpi) and no conidiation was observed under treatment 2 (Figure 2B). In the line with what observed in *P. digitatum,* opposite incubation conditions (continuous light *vs* complete darkness) can induce similar fungal phenotypes and responses, such as those observed herein regarding *M. laxa*. Contrary to *M. laxa,* in *M. fructicola,* the effect of light was mainly observed at the beginning of the infection course, showing the highest diameter length, accelerating the appearance of the onset of brown rot symptoms, and inducing more conidia on the fruit surface (Figure 2C, D; Suppl. Figure S1). Overall, results suggest that altered conidia morphology and reduced *in vitro* conidiation could positively impair its virulence on the fruit surface. Studies regarding the effect of light in photoreceptors related to conidiation (blue and red) and their signaling cascade would be interesting to be evaluated prior to and after fruit infection.

<u>The development of the disease relays on the pathogen's light effect rather than on the fruit itself</u>

Plants are continuously exposed to a variety of abiotic stresses, which could drive to a modulation of the plant phenotype. Light is one of the major and influential inputs for their physiology and is perceived through plant photoreceptors (Folta and Carvalho, 2015). For that reason, in response to light, the mechanisms to face biotic stresses can also be altered. When *Monilinia* spp.-inoculated unbagged fruit were incubated under each treatment (Figure 3A, B; 4A, B), results revealed a comparable fungal development than the one observed when the pathogens were previously incubated under each lighting treatment prior to fruit inoculation. Both lighting treatments reduced *M. laxa* incidence, whereas control condition (constant darkness) reduced *M. fructicola* in unbagged fruit, elucidating that the fruit responses were *Monilinia* spp. dependent rather than dependent on light conditions. In *Arabidopsis thaliana* plants inoculated with *B. cinerea*, constant light and a photoperiod of light/dark considerably reduced the lesion areas compared to constant darkness (Canessa et al., 2013), in

concordance with what we observed in *M. laxa*. Similar to that described in fungi, plant photoreceptors also perceive narrow-bandwidth wavelengths, which in turn activate specific internal responses (Folta and Carvalho, 2015). For instance, Zhu et al. (2013) demonstrated that white and green light decreased lesion diameter in *B. cinerea*-inoculated grapes and only green light reduced diameter in *B. cinerea*-inoculated tomatoes. Herein, we demonstrated that light had a major effect on *Monilinia* spp. rather than on fruit integrity, suggesting that pathogens are differentially modulating fruit responses.

Preharvest fruit conditions influence the disease plant response

Preharvest conditions are also crucial for fruit integrity and in turn, in its capacity to face any stress. Fruit bagging is an emerging agricultural practice mainly down to reduce the amount of fungicide on fruit surface. Bagging the fruit alters the solar radiation that irradiates fruit, and hence, influencing internal quality parameters (Sharma et al., 2014) skin color (Zhou et al., 2019) and marketable yield at harvest (Allran, 2017). Therefore, fruit bagging may result in changed defense response against pathogens. Herein, while bagged 'Fantasia' cultivar was less susceptible to brown rot, bagged fruit of the other cultivars were more susceptible to both *Monilinia* spp. under control condition (Suppl. Table S1). Hence, findings point out that different solar radiation received by the unbagged and bagged fruit can differentially affect the fruit defense mechanisms in front of brown rot in a cultivar-dependent manner. Several studies conducted to test the bagging effect have also shown contradictory results when comparing cultivars, and fruit- and cultivar-specific responses have been suggested as one of the main causes (Sharma et al., 2014). In fact, fruit have different intrinsic characteristics depending on the stone fruit cultivar that leads to a different brown rot susceptibility (Baró-Montel et al., 2019a; Obi et al., 2019). Out of the responses of the host to counteract the pathogen's intrusion, fruit activates stress responses through activating the antioxidant metabolism such as glutathione and redox-related amino acids (Balsells-Llauradó et al., 2020). Hence, analyzing intrinsic properties differing among cultivars such as quality parameters and fruit antioxidant metabolism, could ultimately be correlated with brown rot development, and thus, could shed light on the incidence differences among cultivars. In addition to that, fruit bagging can also affect to microclimate around the fruit, increasing temperature and humidity and in turn, affecting to transpiration, respiration and cuticle in peel cells (Ali et al., 2021).

The development of *Monilinia* spp. in bagged fruit, incubated under each incubation treatment (Figure 3C, 4C), was slightly different from the one observed in unbagged fruit. Hence, preventing the fruit from solar radiation may have caused not only an impact on the fruit's intrinsic characteristics but also on the response to face the pathogens. Therefore, results highlight not only the importance of the light effect in preharvest (solar radiation), but also its effect in postharvest (artificial lighting). Solar light comprises a broad range of electromagnetic waves. The red light fraction of the spectra is of interest since not only was suggested to alter the behavior of *Monilinia* spp. (Section "Altered conidia morphology impairs the conidial viability in a *Monilinia* spp.-dependent manner" and "*Monilinia laxa* coped with light stress and its *in vitro* development was favored"), but it can also have a positive effect on fruits in front of *M. laxa*, but not in front of *M. fructicola*. For instance, the previous incubation of strawberry leaves under red light significantly increased its resistance to *B. cinerea* (Meng et al., 2019). Further from the visible light, UV-C irradiation can induce resistance in several fruit and vegetables (reviewed in Romanazzi et al., 2016). Light quality can strongly modulate phenolic compounds, flavonoids, carotenoids and anthocyanins (reviewed in Ilić and Fallik, 2017), being chlorophyll and carotenoids directly activated by photons. In particular, the activation of phenylpropanoids biosynthesis is enhanced by light in *Xanthomonas oryza*-treated rice leaves (Guo et al., 1993) and by the combination of red and blue light in lettuce (Heo et al., 2012). In addition, the expression of the zeaxanthin epoxidase, a flavoprotein from the carotenoid biosynthesis, that is active under light (Latowski et al., 2000), is upregulated in inoculated-fruit with *M. laxa* compared to healthy fruit along time (Balsells-Llauradó et al., 2020). Accordingly, future studies aiming to unravel the different fruit properties such as secondary metabolites in response to light would contribute to a better understanding of the fruit's capability to face the pathogens.

Ethylene production in the host-pathogen interaction is mainly influenced by *Monilinia* spp. rather than the bag and light effect

Ethylene has been implicated in modulating the plant response not only to abiotic stresses but also to necrotrophic pathogens (McDowell and Dangl, 2000; Müller and Munné-Bosch, 2015). Hence, ethylene modulations induced by fruit bagging, lighting treatments and *Monilinia* spp. were assessed on the nectarine-*Monilinia* spp. interaction. Several studies have described that light affects ethylene levels and other hormones (e.g., cytokinins) and suggest a crosstalk among light and both hormones (reviewed in Zdarska et al., 2015), influencing plant development. However, our results

showed that the ethylene produced by mock-inoculated fruit was affected by neither the bag nor the light conditions analyzed (Figure 5A, D, G). Specifically, ethylene emission increased along time, following the production pattern of a climacteric fruit until ripening (Oetiker and Yang, 1995). In other crops, such as grapes, lighting treatment does neither induce ethylene compared to dark (Zhu et al., 2012). Only Gong et al. (2015) found that blue light can induce changes in ethylene production to accelerate postharvest ripening in peaches, although the lighting treatments tested herein only emit a short intensity of blue light wavelength. Hence, 'Fantasia' cultivar was not affected by these abiotic conditions in terms of ethylene production.

Some fungi can also produce ethylene although its function in fungal development or as a virulence factor is inconclusive (Chague, 2010). Recently, white and blue lights have been shown to significantly increase the ethylene production rate of several fungi (such as *B. cinerea*) under *in vitro* conditions compared to dark, and that even *B.* cinerea could be the ethylene producer in an interaction with *A. thaliana* seedlings (Guo et al., 2020). However, the ethylene production by *Monilinia* spp. has not been deciphered to date. Herein, overall changes on the ethylene pattern of the pathogen-fruit pathosystem were due to the interaction with the pathogen and to bag and lighting treatments. Among all the host responses that plant ethylene mediates (McDowell and Dangl, 2000), this hormone is also implicated in ripening and senescence processes, which can be conducive to disease susceptibility (Liu et al., 2015; Pandey et al., 2016). In fact, a different ethylene pattern was observed for both pathogens in interaction with fruit (Figure 5; Suppl. Figure S3), pointing out to either a different response of the host to cope with the two *Monilinia* spp. or a different *Monilinia* species-dependent modulation to avoid the ethylene-mediated defense response. Other studies also reported a different modulation of ethylene production by both *Monilinia* spp.-fruit interaction in artificially inoculated peaches (Baró-Montel et al., 2019b) but also, in peach petals (Vall-llaura et al., 2020). Although several hypotheses have been suggested, its role in promoting defense or susceptibility is still controversial (van Loon et al., 2006). In addition, results highlighted that the incubation under lighting treatments and the presence of the bag did alter the ethylene production, especially in *M. laxa*-inoculated fruit. These results could in turn explain the altered fruit's capability to respond to these species (Figure 5B, E, H; Suppl. Figure S3A, B). Accordingly, unbagged fruit incubated under control condition (constant darkness) demonstrated an increased *M. laxa* incidence and a lower incubation period (Figure 3), revealing that this species took advantage of the increased ethylene production. However, in *M. laxa*-inoculated bagged fruit both lighting treatments

significantly induced ethylene production, such as the ethylene-induced in the *B. cinerea*-grapes pathosystem (Zhu et al., 2012). In addition to the plant ethylene role in biotic interactions, Xiang et al. (2020) suggested that the main downstream regulators of phytochromes (the phytochrome-interacting factors, PIFs) acted upstream of the ethylene response factor 1 (ERF1) to negatively regulate the resistance to *B. cinerea* in *A. thaliana*. With that, those authors suggested that the PIF-mediated defense against the pathogen is closely related to the jasmonate/ethylene signaling pathway. Thus, molecular studies related to the signaling downstream phytochromes need further investigation to understand the dual ethylene responses occurring during the nectarine-*Monilinia* spp. interaction under light conditions.

Concluding remarks

To avoid or delay the appearance of brown rot symptoms and conidiation on the fruit surface and hence, reduce economic losses driven from contamination through conidia spreading along the postharvest chain, environmental light conditions should be considered. Our study highlights the different behavior of *M. laxa* and *M. fructicola* in both *in vitro* and *in vivo* development and further studies aiming to investigate the differences that underlie the impaired photomorphogenesis due to lighting treatments, such genes related to conidiation, of both species is encouraged. White light has not only impaired the fungal development but also the host response to the pathogen attack. Light received for the fruit during preharvest modifies its intrinsic properties that ultimately would influence its capability to prevent or overcome the infection caused by *Monilinia* spp. During postharvest, light incidence also affected the nectarine-*Monilinia* spp. interaction since fungal development was altered in a species-dependent manner. Thus, deciphering the light-dependent modulation of the fruit properties that will give rise to improved defense response, but also the light-effect that triggers fungal development, will allow contributing to the development of new strategies to control brown rot at both preharvest and postharvest.

Author Contributions

JU, RT and NV conceived and designed the experiments. MBL, NT and CC carried out fruit inoculations, *in vitro* studies, pathological studies, and ethylene measurements. MBL, NV and RT wrote the article and all remaining authors contributed to improving the final version of the manuscript.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Ali, M. M., Anwar, R., Yousef, A. F., Li, B., Luvisi, A., De Bellis, L., et al. (2021). Influence of bagging on the development and quality of fruits. *Plants* 10, 1–17. doi:10.3390/plants10020358.

Allran, J. (2017). Investigation of peach fruit bagging to produce high quality fruit and to manage pests and diseases. Available at: https://tigerprints.clemson.edu/all_theses/2695%0AThis.

Avalos, J., and Limón, M. C. (2015). Biological roles of fungal carotenoids. *Curr. Genet.* 61, 309–324. doi:10.1007/s00294-014-0454-x.

Bahn, Y. S., Xue, C., Idnurm, A., Rutherford, J. C., Heitman, J., and Cardenas, M. E. (2007). Sensing the environment: Lessons from fungi. *Nat. Rev. Microbiol.* 5, 57–69. doi:10.1038/nrmicro1578.

Balsells-Llauradó, M., Silva, C. J., Usall, J., Vall-llaura, N., Serrano-Prieto, S., Teixidó, N., et al. (2020). Depicting the battle between nectarine and *Monilinia laxa*: the fruit developmental stage dictates the effectiveness of the host defenses and the pathogen's infection strategies. in *Horticulture Research* (Springer US). doi:10.1038/s41438-020-00387-w.

Baró-Montel, N., Torres, R., Casals, C., Teixidó, N., Segarra, J., and Usall, J. (2019a). Developing a methodology for identifying brown rot resistance in stone fruit. *Eur. J. Plant Pathol.* 154, 287–303. doi:10.1007/s10658-018-01655-1.

Baró-Montel, N., Vall-llaura, N., Giné-Bordonaba, J., Usall, J., Serrano-Prieto, S., Teixidó, N., et al. (2019b). Double-sided battle: The role of ethylene during *Monilinia* spp. infection in peach at different phenological stages. *Plant Physiol. Biochem.* 144, 324–333. doi:10.1016/j.plaphy.2019.09.048.

Baró-Montel, N., Vall-llaura, N., Usall, J., Teixidó, N., Naranjo-Ortíz, M. A., Gabaldón, T., et al. (2019c). Pectin methyl esterases and rhamnogalacturonan hydrolases: weapons for successful *Monilinia laxa* infection in stone fruit? *Plant Pathol.* 68, 1381–93. doi:10.1111/ppa.13039.

Bulletin OEPP/EPPO (2020). PM 7/18 (3) *Monilinia fructicola*. *EPPO Bull.* 50, 5–18. doi:10.1111/epp.12609.

Canessa, P., Schumacher, J., Hevia, M. A., Tudzynski, P., and Larrondo, L. F. (2013). Assessing the effects of light on differentiation and virulence of the plant pathogen *Botrytis cinerea*: Characterization of the white collar complex. *PLoS One* 8. doi:10.1371/journal.pone.0084223.

Carvalho, S. D., and Castillo, J. A. (2018). Influence of light on plant–phyllosphere interaction. *Front. Plant Sci.* 9, 1–16. doi:10.3389/fpls.2018.01482.

Casals, C., Vinas, I., Torres, R., Griera, C., and Usall, J. (2010). Effect of temperature and water activity on *in vitro* germination of *Monilinia* spp. *J. Appl. Microbiol.* 108, 47–54.

Chague, V. (2010). "Ethylene production by fungi: biological questions and future developments towards a sustainable polymers industry," in *Handbook of Hydrocarbon and Lipid Microbiology*, ed. K. N. Timmis (Springer-Verlag Berlin Heidelberg,), 3011–3020. doi:10.1007/978-3-540-77587-4_224.

Corrochano, L. M. (2019). Light in the Fungal World: From Photoreception to Gene Transcription and beyond. *Annu. Rev. Genet.* 53, 149–170. doi:10.1146/annurev-genet-120417-031415.

De Cal, A., Gell, I., Usall, J., Viñas, I., and Melgarejo, P. (2009). First report of brown rot caused by *Monilinia fructicola* in peach orchards in Ebro Valley, Spain. *Plant Dis.* 93, 763. doi:10.1094/PDIS-93-7-0763A.

De Cal, A., and Melgarejo, P. (1999). Effects of long-wave UV light on *Monilinia* growth and identification of species. *Plant Dis.* 83, 62–65. doi:10.1094/pdis.1999.83.1.62.

De Miccolis Angelini, R. M., Abate, D., Rotolo, C., Gerin, D., Pollastro, S., and Faretra, F. (2018). De novo assembly and comparative transcriptome analysis of *Monilinia fructicola*, *Monilinia laxa* and *Monilinia fructigena*, the causal agents of brown rot on stone fruits. *BMC Genomics* 19, 1–21. doi:10.1186/s12864-018-4817-4.

Folta, K. M., and Carvalho, S. D. (2015). Photoreceptors and control of horticultural plant traits. *HortScience* 50, 1274–1280. doi:10.21273/hortsci.50.9.1274.

Fuller, K. K., Loros, J. J., and Dunlap, J. C. (2015). Fungal photobiology: visible light as a signal for stress, space and time. *Curr. Genet.* 61, 275–288. doi:10.1007/s00294-014-0451-0.

Garcia-Benitez, C., Melgarejo, P., De Cal, A., and Fontaniella, B. (2016). Microscopic analyses of latent and visible *Monilinia fructicola* infections in nectarines. *PLoS One* 11, e0160675. doi:10.1371/journal.pone.0160675.

Gell, I., De Cal, A., Torres, R., Usall, J., and Melgarejo, P. (2008). Relationship between the incidence of latent infections caused by Monilinia spp. and the incidence of brown rot of peach fruit: Factors affecting latent infection. *Eur. J. Plant Pathol.* 121, 487–498. doi:10.1007/s10658-008-9268-3.

Gell, I., De Cal, A., Torres, R., Usall, J., and Melgarejo, P. (2009). Conidial density of *Monilinia* spp. on peach fruit surfaces in relation to the incidences of latent infections and brown rot. *Eur. J. Plant Pathol.* 2009, 415–424.

Giné-Bordonaba, J., Echeverria, G., Ubach, D., Aguiló-Aguayo, I., López, M. L., and Larrigaudière, C. (2017). Biochemical and physiological changes during fruit development and ripening of two sweet cherry varieties with different levels of cracking tolerance. *Plant Physiol. Biochem.* 111, 216–225. doi:10.1016/j.plaphy.2016.12.002.

Gong, D., Cao, S., Sheng, T., Shao, J., Song, C., Wo, F., et al. (2015). Effect of blue light on ethylene biosynthesis, signalling and fruit ripening in postharvest peaches. *Sci. Hortic. (Amsterdam).* 197, 657–664. doi:10.1016/j.scienta.2015.10.034.

Griebel, T., and Zeier, J. (2008). Light regulation and daytime dependency of inducible plant defenses in Arabidopsis: Phytochrome signaling controls systemic acquired resistance rather than local defense. *Plant Physiol.* 147, 790–801. doi:10.1104/pp.108.119503.

Guo, A., Reimers, J., and Leach, J. E. (1993). Effect of light on incompatible interactions between Xanthomonas oryzae pv oryzae and rice. *Physiol. Mol. Plant Pathol.* 42, 413–425.

Guo, H., Liu, A., Wang, Y., Wang, T., Zhang, W., Zhu, P., et al. (2020). Measuring light-induced fungal ethylene production enables non-destructive diagnosis of disease occurrence in harvested fruits. *Food Chem.* 310, 125827. doi:10.1016/j.foodchem.2019.125827.

Heo, J.-W., Kang, D.-H., Bang, H.-S., Hong, S.-G., Chun, C.-H., and Kang, K.-K. (2012). Early Growth, Pigmentation, Protein Content, and Phenylalanine Ammonia-lyase Activity of Red Curled Lettuces Grown under Different Lighting Conditions. *Korean J. Hortic. Sci. Technol.* 30, 6–12. doi:10.7235/hort.2012.11118.

Hevia, M. A., Canessa, P., Müller-Esparza, H., and Larrondo, L. F. (2015). A circadian oscillator in the fungus *Botrytis cinerea* regulates virulence when infecting *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. U. S. A.* 112, 8744–8749. doi:10.1073/pnas.1508432112.

Idnurm, A., and Crosson, S. (2009). The photobiology of microbial pathogenesis. *PLoS Pathog.* 5, 11–13. doi:10.1371/journal.ppat.1000470.

Ilić, Z. S., and Fallik, E. (2017). Light quality manipulation improves vegetable quality at harvest and postharvest: A review. *Environ. Exp. Bot.* 139, 79–90. doi:10.1016/j.envexpbot.2017.04.006.

Islam, S. Z., Honda, Y., and Sonhaji, M. (1998). Phototropism of conidial germ tubes of *Botrytis cinerea* and its implication in plant infection processes. *Plant Dis.* 82, 850–856. doi:10.1094/PDIS.1998.82.8.850.

Jarvis, W. R. (1972). Phototropism in *Botrytis cinerea*. *Trans. Br. Mycol. Soc.* 58, 526-IN16. doi:10.1016/s0007-1536(72)80108-6.

Kahramanoğlu, I., Nisar, M. F., Chen, C., Usanmaz, S., Chen, J., and Wan, C. (2020). Light: An alternative method for physical control of postharvest rotting caused by fungi of citrus fruit. *J. Food Qual.* 2020. doi:10.1155/2020/8821346.

Keske, C., Amorim, L., and Mio, L. (2011). Peach brown rot incidence related to pathogen infection at different stages of fruit development in an organic peach production system. *Crop Prot.* 30, 802–806. doi:10.1016/j.cropro.2011.03.005.

Keske, C., Treutter, D., and Neumüller, M. (2014). Effect of bagging on brown rot incidence in European Plum. *Ecofruit. 16th Int. Conf. Org. Grow. Proceedings, 17-19 Febr. 2014, Hohenheim, Ger. 2014 pp.228-231 ref.7*, 228–231. doi:9783000450716.

Kreidl, S., Edwards, J., and Villalta, O. N. (2015). Assessment of pathogenicity and infection requirements of *Monilinia* species causing brown rot of stone fruit in Australian orchards. *Australas. Plant Pathol.* 44, 419–430. doi:10.1007/s13313-015-0362-7.

Lafuente, M. T., Alférez, F., and González-Candelas, L. (2018). Light-emitting diode blue light alters the ability of *Penicillium digitatum* to infect citrus fruits. *Photochem. Photobiol.* 94, 1003–1009. doi:10.1111/php.12929.

Latowski, D., Burda, K., and Strzałka, K. (2000). A mathematical model describing kinetics of conversion of violaxanthin to zeaxanthin via intermediate antheraxanthin by the xanthophyll cycle enzyme violaxanthin de-epoxidase. *J. Theor. Biol.* 206, 507–514. doi:10.1006/jtbi.2000.2141.

Liu, M., Pirrello, J., Chervin, C., Roustan, J. P., and Bouzayen, M. (2015). Ethylene control of fruit ripening: Revisiting the complex network of transcriptional regulation. *Plant Physiol.* 169, 2380–2390. doi:10.1104/pp.15.01361.

Luo, Y., and Michailides, T. J. (2001). Factors affecting latent infection of prune fruit by *Monilinia fructicola*. *Phytopathology* 91, 864–872.

Marquenie, D., Geeraerd, A. H., Lammertyn, J., Soontjens, C., Van Impe, J. F., Michiels, C. W., et al. (2003). Combinations of pulsed white light and UV-C or mild heat treatment to inactivate conidia of *Botrytis cinerea* and *Monilia fructigena*. *Int. J. Food Microbiol.* 85, 185–196. Available at: http://www.elsevier.com/locate/ijfoodmicro;

McDowell, J. M., and Dangl, J. L. (2000). Signal transduction in the plant immune response. *Trends Biochem. Sci.* 25, 79–82. doi:10.1016/S0968-0004(99)01532-7.

Meng, L., Höfte, M., and Van Labeke, M. C. (2019). Leaf age and light quality influence the basal resistance against *Botrytis cinerea* in strawberry leaves. *Environ. Exp. Bot.* 157, 35–45. doi:10.1016/j.envexpbot.2018.09.025.

Métraux, J. P. (2002). Systemic Acquired Resistance. *Euphytica* 124, 237–243. doi:10.1016/B978-0-12-374984-0.01509-6.

Minas, I. S., Tanou, G., and Molassiotis, A. (2018). Environmental and orchard bases of peach fruit quality. *Sci. Hortic. (Amsterdam).* 235, 307–322. doi:10.1016/j.scienta.2018.01.028.

Müller, M., and Munné-Bosch, S. (2015). Ethylene response factors: A key regulatory hub in hormone and stress signaling. *Plant Physiol.* 169, 32–41. doi:10.1104/pp.15.00677.

Obi, V. I., Barriuso, J. J., and Gogorcena, Y. (2018). Peach brown rot: Still in search of an ideal management option. *Agriculture* 8, 1–34. doi:10.3390/agriculture8080125.

Obi, V. I., Barriuso, J. J., Usall, J., and Gogorcena, Y. (2019). Breeding strategies for identifying superior peach genotypes resistant to brown rot. *Sci. Hortic. (Amsterdam).* 246, 1028–1036. doi:10.1016/j.scienta.2018.10.027.

Oetiker, J. H., and Yang, S. F. (1995). The role of ethylene in fruit ripening. *Acta Hortic.* 398, 167–178. doi:10.17660/actahortic.1995.398.17.

Oliveira Lino, L., Pacheco, I., Mercier, V., Faoro, F., Bassi, D., Bornard, I., et al. (2016). Brown rot strikes *Prunus* fruit: An ancient fight almost always lost. *J. Agric. Food Chem.* 64, 4029–4047. doi:10.1021/acs.jafc.6b00104.

Olmedo, M., Ruger-Herreros, C., and Corrochano, L. M. (2010). Regulation by blue light of the *fluffy* gene encoding a major regulator of conidiation in *Neurospora crassa*. *Genetics* 184, 651–658. doi:10.1534/genetics.109.109975.

Pandey, D., Rajendran, S. R. C. K., Gaur, M., Sajeesh, P. K., and Kumar, A. (2016). Plant defense signaling and responses against necrotrophic fungal pathogens. *J. Plant Growth Regul.* 35, 1159–1174. doi:10.1007/s00344-016-9600-7.

Rehnstrom, A. L., and Free, S. J. (1996). The isolation and characterization of melanin-deficient mutants of *Monilinia fructicola*. *Physiol. Mol. Plant Pathol.* 49, 321–330. doi:10.1006/pmpp.1996.0057.

Roberts, M. R., and Paul, N. D. (2006). Seduced by the dark side: Integrating molecular and ecological perspectives onfence against pests and pathogens. *New Phytol.* 170, 677–699. doi:10.1111/j.1469-8137.2006.01707.x.

Rodríguez-Pires, S., De Cal, A., Espeso, E. A., Rasiukeviciut, N., and Melgarejo, P. (2021). Light-photoreceptors and proteins related to *Monilinia laxa*. *J. Fungi* 7. doi:10.3390/jof7010032.

Rodríguez-Pires, S., Garcia-Companys, M., Espeso, E. A., Melgarejo, P., and De Cal, A. (2020). Influence of light on the *Monilinia laxa* stone fruit interaction. *Plant Pathol.*, 1–10. doi:10.1111/ppa.13294.

Roeber, V. M., Bajaj, I., Rohde, M., Schmülling, T., and Cortleven, A. (2020). Light acts as a stressor and influences abiotic and biotic stress responses in plants. *Plant Cell Environ.*, 1–20. doi:10.1111/pce.13948.

Romanazzi, G., Sanzani, S. M., Bi, Y., Tian, S., Gutiérrez Martínez, P., and Alkan, N. (2016). Induced resistance to control postharvest decay of fruit and vegetables. *Postharvest Biol. Technol.* 122, 82–94. doi:10.1016/j.postharvbio.2016.08.003.

Rungjindamai, N., Jeffries, P., and Xu, X.-M. (2014). Epidemiology and management of brown rot on stone fruit caused by *Monilinia laxa*. *Eur. J. Plant Pathol.* 140, 1–17. doi:10.1007/s10658-014-0452-3.

Schumacher, J. (2017). How light affects the life of *Botrytis*. *Fungal Genet. Biol.* 106, 26–41. doi:10.1016/j.fgb.2017.06.002.

Schumacher, J., Pradier, J. M., Simon, A., Traeger, S., Moraga, J., Collado, I. G., et al. (2012). Natural variation in the *VELVET* gene *bcvel1* affects virulence and light-dependent differentiation in *Botrytis cinerea*. *PLoS One* 7. doi:10.1371/journal.pone.0047840.

Schumacher, J., Simon, A., Cohrs, K. C., Viaud, M., and Tudzynski, P. (2014). The transcription factor BcLTF1 regulates virulence and light responses in the necrotrophic plant pathogen *Botrytis cinerea*. *PLoS Genet.* 10. doi:10.1371/journal.pgen.1004040.

Sharma, R. R., Reddy, S. V. R., and Jhalegar, M. J. (2014). Pre-harvest fruit bagging: A useful approach for plant protection and improved post-harvest fruit quality - A review. *J. Hortic. Sci. Biotechnol.* 89, 101–113. doi:10.1080/14620316.2014.11513055.

Shoji, J. Y., Kikuma, T., Arioka, M., and Kitamoto, K. (2010). Macroautophagy-mediated degradation of whole nuclei in the filamentous fungus Aspergillus oryzae. *PLoS One* 5. doi:10.1371/journal.pone.0015650.

Tan, K. K. (1975). Interaction of near-ultraviolet, blue, red, and far-red light in sporulation of *Botrytis cinerea*. *Trans. Br. Mycol. Soc.* 64, 215–222. doi:10.1016/s0007-1536(75)80105-7.

Tisch, D., and Schmoll, M. (2010). Light regulation of metabolic pathways in fungi. *Appl. Microbiol. Biotechnol.* 85, 1259–1277. doi:10.1007/s00253-009-2320-1.

Tran, T. T., Li, H., Nguyen, D. Q., Sivasithamparam, K., Jones, M. G. K., and Wylie, S. J. (2020). Comparisons between genetic diversity, virulence and colony morphology of *Monilinia fructicola* and *Monilinia laxa* isolates. *J. Plant Pathol.* 102, 743–751. doi:10.1007/s42161-020-00498-2.

Usall, J., Casals, C., Sisquella, M., Palou, L., and De Cal, A. (2015). Alternative technologies to control postharvest diseases of stone fruits. *Stewart Postharvest Rev.* 11, 1–6. doi:10.2212/spr.2015.4.2.

Vall-llaura, N., Giné-Bordonaba, J., Usall, J., Larrigaudière, C., Teixidó, N., and Torres, R. (2020). Ethylene biosynthesis and response factors are differentially modulated during the interaction of peach petals with *Monilinia laxa* or *Monilinia fructicola*. *Plant Sci.* 299, 110599. doi:10.1016/j.plantsci.2020.110599.

van Loon, L. C., Rep, M., and Pieterse, C. M. J. (2006). Significance of inducible defense-related proteins in infected plants. *Annu. Rev. Phytopathol.* 44, 135–162. doi:10.1146/annurev.phyto.44.070505.143425.

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

Villarino, M., Eguen, B., Lamarca, N., Segarra, J., Usall, J., Melgarejo, P., et al. (2013). Occurrence of *Monilinia laxa* and *M. fructigena* after introduction of *M. fructicola* in peach orchards in Spain. *Eur. J. Plant Pathol.* 137, 835–845. doi:10.1007/s10658-013-0292-6.

Villarino, M., Melgarejo, P., and De Cal, A. (2016). Growth and aggressiveness factors affecting *Monilinia* spp. survival peaches. *Int. J. Food Microbiol.* 227, 6–12. doi:10.1016/j.ijfoodmicro.2016.01.023.

Villarino, M., Melgarejo, P., Usall, J., Segarra, J., and De Cal, A. (2010). Primary inoculum sources of *Monilinia* spp. in Spanish peach orchards and their relative importance in brown rot. *Plant Dis.* 94, 1048–1054. doi:10.1094/pdis-94-8-1048.

Villarino, M., Melgarejo, P., Usall, J., Segarra, J., Lamarca, N., and De Cal, A. (2012). Secondary inoculum dynamics of *Monilinia* spp. and relationship to the incidence of postharvest brown rot in peaches and the weather conditions during the growing season. *Eur. J. Plant Pathol.* 133, 585–598. doi:10.1007/s10658-011-9931-y.

Xiang, S., Wu, S., Zhang, H., Mou, M., Chen, Y., Li, D., et al. (2020). The PIFs redundantly control plant defense response against *Botrytis cinerea* in *Arabidopsis*. *Plants* 9, 1–13. doi:10.3390/plants9091246.

Yin, L. F., Chen, S. N., Chen, G. K., Schnabel, G., Du, S. F., Chen, C., et al. (2015). Identification and characterization of three *Monilinia* species from plum in China. *Plant Dis.* 99, 1775–1783. doi:10.1094/PDIS-12-14-1308-RE.

Yu, F.-Y., Chiu, C.-M., Lee, Y.-Z., Lee, S.-J., Chou, C.-M., You, B.-J., et al. (2020). Polyketide synthase gene expression in relation to chloromonilicin and melanin production in *Monilinia fructicola*. *Phytopatholog*, 1–64. doi:10.1094/phyto-02-20-0059-r.

Yu, Z., and Fischer, R. (2019). Light sensing and responses in fungi. *Nat. Rev. Microbiol.* 17, 25–36. doi:10.1038/s41579-018-0109-x.

Zdarska, M., Dobisová, T., Gelová, Z., Pernisová, M., Dabravolski, S., and Hejátko, J. (2015). Illuminating light, cytokinin, and ethylene signalling crosstalk in plant development. *J. Exp. Bot.* 66, 4913–4931. doi:10.1093/jxb/erv261.

Zhou, H., Yu, Z., and Ye, Z. (2019). Effect of bagging duration on peach fruit peel color and key protein changes based on iTRAQ quantitation. *Sci. Hortic. (Amsterdam).* 246, 217–226. doi:10.1016/j.scienta.2018.10.072.

Zhu, P., Xu, L., Zhang, C., Toyoda, H., and Gan, S. S. (2012). Ethylene produced by *Botrytis cinerea* can affect early fungal development and can be used as a marker for infection during storage of grapes. *Postharvest Biol. Technol.* 66, 23–29. doi:10.1016/j.postharvbio.2011.11.007.

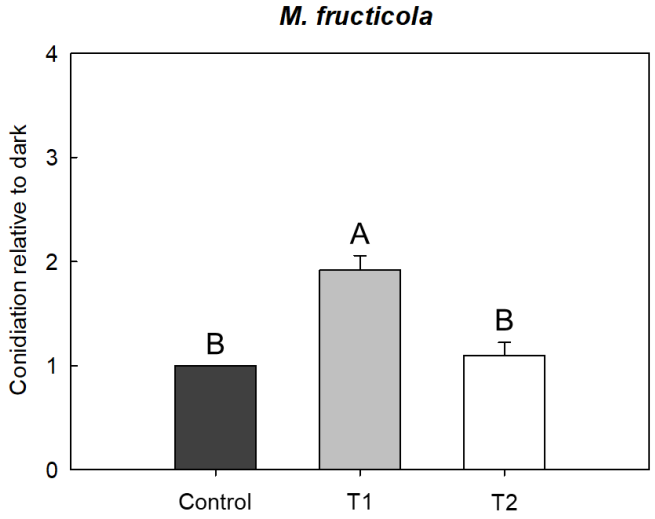
Zhu, P., Zhang, C., Xiao, H., Wang, Y., Toyoda, H., and Xu, L. (2013). Exploitable regulatory effects of light on growth and development of *Botrytis cinerea*. *J. Plant Pathol.* 95, 509–517. doi:10.4454/JPP.V95I3.038.

Supplementary Material

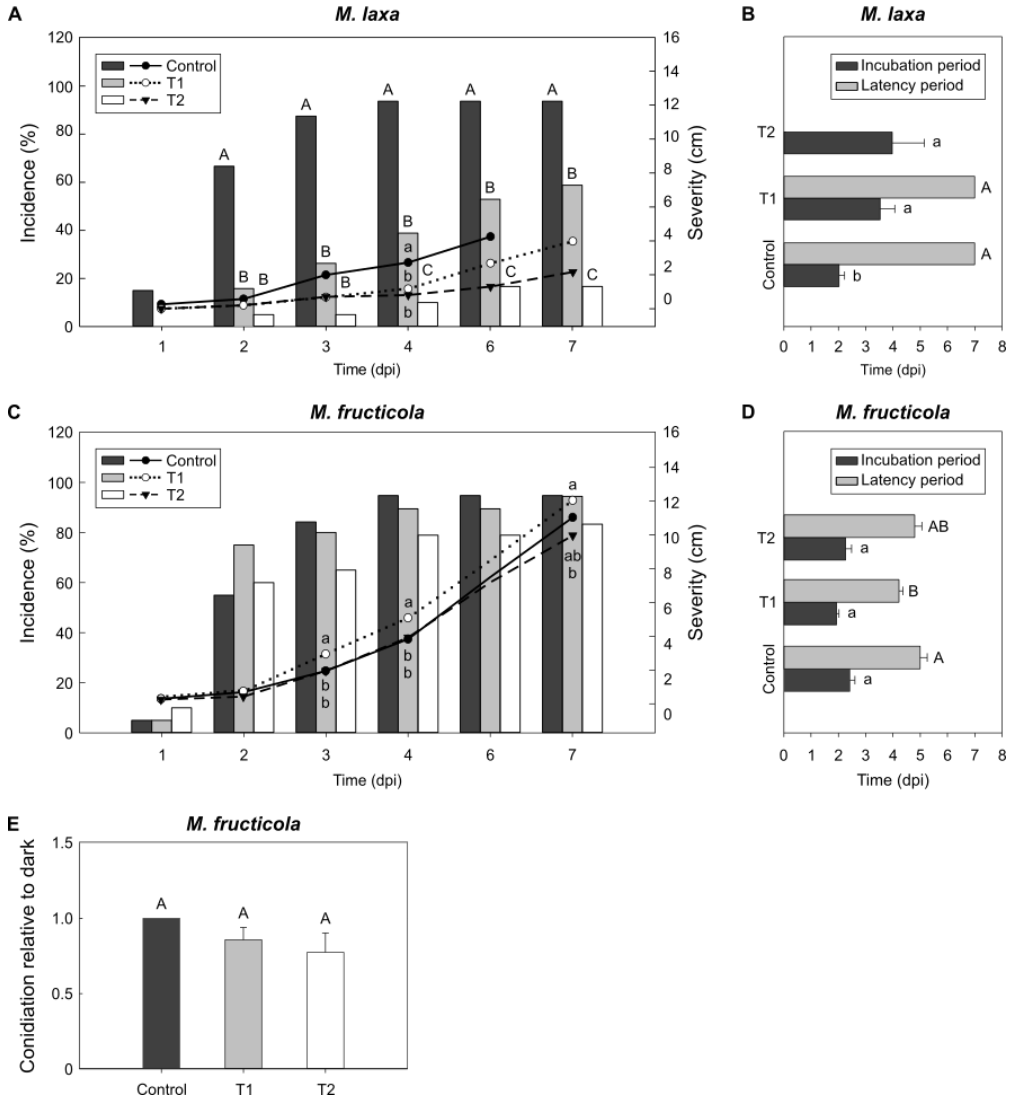
Supplementary Table S1. *Monilinia* spp. incidence (%) on unbagged and bagged fruit from different cultivars after 7 days of incubation under control condition (dark). No significant differences between bagging conditions were found according to generalized linear model (GLM).

Cultivar	*Monilinia* spp.	Fruit condition	Incidence
'Fantasia'	*M. laxa*	Unbagged	84.2 %
'Fantasia'	*M. laxa*	Bagged	65.0 %
'Fantasia'	*M. fructicola*	Unbagged	75.0 %
'Fantasia'	*M. fructicola*	Bagged	65.0 %
'Venus'	*M. laxa*	Unbagged	15.0 %
'Venus'	*M. laxa*	Bagged	26.3 %
'Venus'	*M. fructicola*	Unbagged	82.4 %
'Venus'	*M. fructicola*	Bagged	89.5 %
'Nectatinto'	*M. laxa*	Unbagged	70.0 %
'Nectatinto'	*M. laxa*	Bagged	93.75 %
'Nectatinto'	*M. fructicola*	Unbagged	89.5 %
'Nectatinto'	*M. fructicola*	Bagged	94.4 %
'Albared'	*M. laxa*	Unbagged	75.0 %
'Albared'	*M. laxa*	Bagged	100.0 %
'Albared'	*M. fructicola*	Unbagged	100.0 %
'Albared'	*M. fructicola*	Bagged	100.0 %

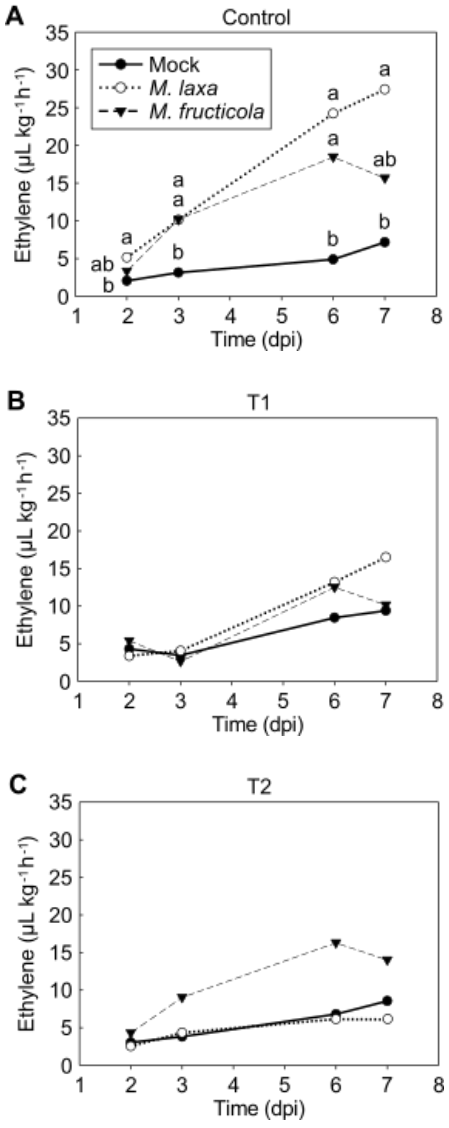
Supplementary Figure S1. Conidiation of *M. fructicola* on 'Fantasia' cultivar surface. The concentration of conidia is represented relative to control condition (dark). Different letters indicate statistically differences among treatments according to orthogonal contrasts ($P < 0.05$).



Supplementary Figure S2. **Light effect on the capacity of *Monilinia* spp. to infect fruit in 'Venus' cultivar**. Incidence (% of brown rot, bars) and severity (lesion diameter length in cm of rotted fruit, lines) of *M. laxa* **(A)** and *M. fructicola* **(C)** in 'Venus' nectarines along the infection time course (dpi, days post inoculation) after growing the fungi during 7 days under control and treatments 1 and 2. Bars represent the mean of incidence on fruit (n = 20). Lines represent the mean of diameter length of rotted fruit. Different uppercase and lowercase letters indicate significant differences ($P \leq 0.05$) of incidence and severity, respectively, among treatments according to orthogonal contrasts at each time point. No letters indicate no significant differences. Incubation and latency periods (days) of *M. laxa* **(B)** and *M. fructicola* **(D)** in 'Venus' nectarines after growing the fungi during 7 days under control and treatments 1 and 2. Bars represent the mean of fruits with symptoms (n = 1 to 20) and error bars represent the standard error of the means. Different lowercase and uppercase letters indicate significant differences ($P \leq 0.05$) of incubation and latency periods, respectively, among treatments according to orthogonal contrasts. Conidiation of *M. fructicola* on fruit surface (**E**). The concentration of conidia is represented relative to control (dark). Different letters indicate statistically differences among treatments according to orthogonal contrasts ($P < 0.05$).


A
M. laxa
Control
T1
T2
Incidence (%)
Severity (cm)
Time (dpi)
B
M. laxa
Incubation period
Latency period
T2
T1
Control
Time (dpi)
C
M. fructicola
Control
T1
T2
Incidence (%)
Severity (cm)
Time (dpi)
D
M. fructicola
Incubation period
Latency period
T2
T1
Control
Time (dpi)
E
M. fructicola
Conidiation relative to dark
Control
T1
T2

Supplementary Figure S3. **Ethylene production of mock-inoculated fruit, *M. laxa* and *M. fructicola*-inoculated fruit on unbagged nectarines.** Ethylene measurements of mock, *M. laxa*, *M. fructicola*-inoculated fruit incubated during 7 days under control condition (**A**) and treatments 1 (**B**) and 2 (**C**). Different letters indicate significant differences among inoculums at each time point according to Tukey's HSD test ($P \leq 0.05$). No letters indicate no significant differences. In all graphics, values represent the mean of ethylene measurements of each replicate (n = 4).



Chapter 2. Impact of fruit bagging and postharvest storage conditions on quality and decay of organic nectarines

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Abstract

Abiotic factors such as light influence the physicochemical properties of fruit and may alter their responses to the environment. This study aimed to reveal for the first time the effect of two postharvest storage conditions on the overall quality and natural fungal disease incidence (fruit decay) of organic nectarines. Experiments were conducted with four organic nectarine cultivars (two early-mid season and two late-season) that were unbagged or bagged during preharvest, and after harvest, were stored for 7-9 days in the control treatment or under a lighting treatment (T1). Quality parameters (weight, diameter, firmness, soluble solids content, titratable acidity, and single index of absorbance difference), ethylene production, and fruit decay (as a percentage of rot incidence) were evaluated. Preharvest bagging reduced fruit decay in the late-season cultivars, in which storage in control treatment reduced fungal decay (up to 100%) more than storage under T1 treatment (47.1% of reduction). Bagging altered initial fruit quality, but values were within official recommendations. Storage conditions reduced differences attributed to bagging, especially under T1 storage. This work highlighted the importance of modulating the light, both in the field by fruit bagging and during postharvest, to reduce fruit decay and improve fruit quality. This may serve as a tool for both farmers and postharvest chain managers.

Keywords: stone fruit; paper bags; postharvest chain; darkness; light; photoperiod; fungal diseases; *Rhizopus*; *Monilinia*

Introduction

Peach, nectarine, plum, cherry, and apricot (*Prunus* genus) are the most economically important species of stone fruit (Mari et al., 2019). The worldwide production of peach and nectarine was 25.7 MT in 2019, and China, Spain, Italy, and Greece were the main producers (FAO, 2021). Stone fruit can suffer pathological diseases and physiological disorders which lead to fruit losses (Mari et al., 2019; Manganaris and Crisosto, 2020). Fruit decay can occur both preharvest and during the postharvest chain (Eckert and Ratnayake, 1983), although postharvest losses tend to be greater than orchard losses (Porat et al., 2018). The most destructive and economically important fungal disease is brown rot, caused by *Monilinia* spp. (Mari et al., 2019; Mustafa et al., 2021), producing up to 7% and over 60% of incidence at harvest and after postharvest, respectively, in the lower Ebro Valley of Spain (Villarino et al., 2012). Other relevant diseases are caused by pathogens such as *Rhizopus* spp., *Mucor* spp., and *Geotrichum candidum* (Mari et al., 2019).

Currently, diseases are mainly controlled with a combination of cultural practices (e.g., tree management and removing natural inoculum sources) (Villarino et al., 2012; Bussi et al., 2015; Casals et al., 2015), biological control, and chemical fungicide programs applied in the orchard (De Oliveira Lino et al., 2016; Mari et al., 2019). Nevertheless, health concerns related to the environmental footprint and toxicological risks have led to a demand for chemical-free fresh fruit (Usall et al., 2015), encouraging more sustainable systems and organic agriculture.

Fruit bagging is an environmentally friendly strategy for plant protection in organic production that is extensively used in several fruit crops (e.g., apple, pears, mango) (Sharma et al., 2014). This mechanical technique consists of introducing the fruit into a bag during the stone hardening phase until harvest, when it is removed. Bags can be made of many materials (e.g., paraffin, plastic, paper) and can be of different colors (e.g., white, yellow, brown) (Ali et al., 2021). Bagging reduces physical injuries, fruit decay (e.g., brown rot), and cracking and russeting incidence in peaches (Keske et al., 2014; Sharma et al., 2014; Campbell et al., 2021), as well as improving visual quality (e.g., color development) and altering fruit quality (Zhou et al., 2019; Ali et al., 2021) by affecting the solar radiation that fruit receives on the tree. However, the results of this strategy are contradictory among investigations, probably due to external factors (i.e., type of bag and storage conditions) or the fruit's intrinsic properties (Sharma et al., 2014).

After harvest, the conditions in which stone fruit is stored are crucial to avoiding disease and physiological disorders (Manganaris and Crisosto, 2020). Temperature and relative humidity have been extensively studied. Still, the effect of white artificial lighting along the postharvest chain (i.e., packinghouses, markets, and consumers' homes) on fruit quality and disease incidence (fruit decay) has not been studied. Artificial lighting can alter many physicochemical fruit properties and improve fruit quality in peaches. For example, blue light increases total sugar content in peaches (Gong et al., 2015), and UV-B radiation reduces firmness, but it does not affect the soluble solids content and titratable acidity (Santin et al., 2019). UV-B radiation also affects plant defense signaling (Ballaré, 2014) and the peach phenolic response to *Monilinia fructicola* (Santin et al., 2018). Recently, Balsells-Llauradó et al. (Balsells-Llauradó et al., 2021) studied the effect of postharvest storage under a photoperiod of unbagged and bagged fruit in response to artificial inoculations of *Monilinia* spp., but the effect of photoperiod and fruit bagging on fruit quality after postharvest storage remains unknown.

Fruit quality includes all aspects related to physical, mechanical, sensory, nutritive, and appearance properties, and properties related to food safety (Crisosto and Costa, 2008). The purposes of this study were *i*) to evaluate the effect of bagging on fruit quality and ethylene production of four nectarine cultivars at harvest, *ii*) to assess the effect of fruit bagging on natural fungal disease incidence (fruit decay) under two postharvest storage treatments (control and T1), *iii*) to decipher the effect of these postharvest storage treatments on fruit quality.

Materials and Methods

Plant material and fruit bagging

Four yellow-fleshed cultivars of nectarines (*P. persica* var. *nucipersica* (Borkh.) Schneider) were used for the studies. Two early-mid season ('Fantasia' and 'Venus') and two late season ('Albared' and 'Nectatinto') cultivars were obtained from organic orchards located in Lleida (Catalonia, Spain). The incidence of fruit decay and the effect of postharvest storage conditions (control and lighting treatment) were assessed on unbagged fruit and fruit that was bagged in the orchard ("bagged fruit"). Commercial single layer white paper bags (16.5 x 21.5 cm, 32 g m^{-2}) (Gràfiques Salaet, Gandesa, Spain), impregnated with paraffin wax, were used to bag fruit before harvest (185, 172,

185 and 197 Julian days[1] for 'Fantasia', 'Venus', 'Albared' and 'Nectatinto', respectively) using a staple to fasten the bag to the branch. Harvest date was at commercial fruit maturity based on the grower's recommendations. Fruit was harvested at 218, 221, 250 and 260 Julian days for 'Fantasia', 'Venus', 'Albared' and 'Nectatinto', respectively. Bagged and unbagged fruit from the same sun-side of trees to avoid fruit position effects (Minas et al., 2018) were randomly harvested. Fruit was homogenized based on the single index of absorbance difference (DA index) using a portable DA-Meter (TR-Turoni, Forli, Italy). A lux meter was used to assess the incident solar radiation inside the bags. Bags were removed upon arrival at the laboratory, before conducting the assays and postharvest storage.

Storage conditions and evaluation of decay losses in postharvest

Fruit was stored as described by Balsells-Llauradó et al. (Balsells-Llauradó et al., 2021). Briefly, fruit was stored at high humidity for 24 h and then placed in a postharvest chamber under two storage conditions. The control treatment was at complete darkness, 20 °C and 50 ± 5% RH; the lighting treatment T1 consisted of a photoperiod of 12 h light (4 incandescent white TL-D 36 W/827 fluorescent lights; Ta = 2700 K, 3350 lm, 350 - 740 nm, 630 nm max; Philips, Madrid, Spain) at 22 ± 1 °C, 50 ± 10% RH and 12 h darkness at 20 °C, 90% RH. Experiments were conducted with 4 replicates of 5 fruits each in each bagging condition × postharvest storage × cultivar. Fruit was examined daily to detect rot tissue. The evaluation was recorded for 9 days in early-mid season cultivars. Due to the early and high perishability in late-season cultivars, evaluations were conducted for up to 7 days. The incidence of fruit decay was calculated as the percentage of fruit with natural disease symptoms. Identification of fungal agents was carried out following the EPPO standard PM 7/18 (3) (Bulletin OEPP/EPPO, 2020) and Mari et al. (Mari et al., 2019).

An economic evaluation between bagged and unbagged fruit was conducted considering the production of an organic orchard of one hectare, prices (bags, fruit, workers in Ebro Valley area), and the postharvest losses due to fruit decay.

Quality characteristics and ethylene measurements

Quality characteristics were measured according to Baró-Montel et al. (Baró-Montel et al., 2019), i.e., weight, cheek diameter (CD), flesh firmness (FF), soluble solids content (SSC), titratable acidity (TA) and the DA Index. These measurements were performed

[1] Julian days (e.g., January 1st is considered as day 1)

on the harvest day (initial fruit quality) and at the end of the postharvest storage period. Changes in quality were calculated after postharvest storage as percentages in relation to initial fruit quality for each cultivar, bagging condition and postharvest storage. Ethylene measurements of fruit at harvest were determined as described by Giné-Bordonaba et al. (Giné-Bordonaba et al., 2017). Fruit was placed in 3.8 L sealed flasks for 2 h. After ethylene measurements, the fruit was returned to its respective postharvest storage condition. Ethylene was measured using four replicates of 3 fruit each, from each cultivar and bagging condition.

Statistical analysis

JMP® software version 14.2.0 (SAS Institute Inc., Cary, NC, USA) was used to statistically analyze the data. All data were checked for the assumptions of parametric statistics and were transformed when needed. Ethylene production data (nL kg^{-1} h^{-1}) were subjected to Log transformation. Analysis of variance (ANOVA) was applied to the data, and when the analysis was statistically significant, Tukey's HSD test ($p \leq 0.05$) was used to compare the incidence of fruit decay at each time point for each cultivar. To compare the two different means of bagging conditions or the two postharvest storage conditions, Student's T-test ($p \leq 0.05$) was used.

Results

Preharvest fruit bagging slightly impaired fruit quality and ethylene at harvest

To assess the effect of fruit bagging on quality, and the decay of nectarines after postharvest storage, we first assessed the quality at harvest (initial quality). A first general overview revealed slight differences among the cultivars. For instance, the late cultivars were slightly larger than the early-mid season ones (Table 1). The unbagged 'Fantasia' nectarines had the highest ethylene production, whereas both bagged and unbagged 'Venus' fruit had the lowest. 'Albared' nectarines had the lowest FF (59.6 – 58.9 N) and the highest SSC (14.8 – 13.7 °Brix) (Table 1). 'Nectatinto' cultivar had the lowest TA (2.9 – 3.0 g L^{-1}) and the highest SSC/TA ratio values (4.1 - 4.2).

Table 1. Fruit quality on harvest day of four nectarine cultivars. *Quality parameters are listed with their measurement unit in brackets. Weight, cheek diameter (CD), single index of absorbance difference (DA Index), flesh firmness (FF), soluble solids content (SSC), titratable acidity (TA), SSC/TA ratio, and ethylene levels of preharvest bagged fruit (B) and unbagged fruit (UB). Values represent the mean (4 replicates, 5 fruits each) ± the standard error of the mean. Lower case letters indicate significant differences ($p \leq 0.05$) between bagging conditions, within each cultivar. No letter indicates no significant differences.*

Cultivar	Pre-harvest	Weight (g)	CD (mm)	DA Index	FF (N)	SSC (°Brix)	TA (g L^{-1})	SSC / TA ratio
'Fantasia'	B	144.5 ± 7.1	63.4 ± 1.1	0.9 ± 0.0	75.7 ± 1.2 a	12.2 ± 0.1 a	8.1 ± 0.6	1.6 ± 0.1
	UB	160.1 ± 7.2	65.4 ± 1.0	1.0 ± 0.0	70.2 ± 1.8 b	11.9 ± 0.1 b	9.7 ± 0.4	1.3 ± 0.1
'Venus'	B	178.9 ± 9.8 b	67.3 ± 1.3 b	0.7 ± 0.0 a	68.8 ± 1.3 b	10.6 ± 0.2 b	6.6 ± 0.6 b	1.5 ± 0.1
	UB	211.7 ± 10.2 a	72.0 ± 1.2 a	0.6 ± 0.0 b	74.1 ± 2.1 a	11.5 ± 0.2 a	8.6 ± 0.2 a	1.3 ± 0.0
'Nectatinto'	B	249.0 ± 11.1	77.8 ± 1.2	1.1 ± 0.0 b	73.7 ± 3.7	11.7 ± 0.3	2.9 ± 0.1	4.2 ± 0.2
	UB	221.2 ± 10.7	75.4 ± 1.0	1.2 ± 0.0 a	68.9 ± 3.8	12.3 ± 0.3	3.0 ± 0.1	4.1 ± 0.1
'Albared'	B	251.5 ± 7.1 a	77.1 ± 0.7 a	0.7 ± 0.0 b	59.6 ± 2.0	14.8 ± 0.3	7.9 ± 0.5	2.0 ± 0.1
	UB	200.0 ± 8.3 b	71.1 ± 1.0 b	0.8 ± 0.0 a	58.9 ± 3.1	13.7 ± 0.6	7.3 ± 0.7	1.9 ± 0.1

Paper bags allowed up to 76% of the light intensity to pass on the south-south-east side of the trees. This led to significant differences in all quality parameters based on different bagging conditions of cultivars (Table 1). In cv 'Venus', bagged fruit had significantly smaller weight and CD than unbagged fruit (15.5 and 6.5% lower, respectively), whereas only 'Albared' bagged fruit was significantly larger (20 and 7.8% higher weight and CD, respectively) than unbagged fruit. Bagging the fruit also significantly ($p \leq 0.05$) impaired the DA Index (e.g., reduced maturity) in the early-mid season cv 'Venus' (Table 1). In contrast, in the late season cultivars the DA index was significantly smaller (more mature fruit) in the bagged fruit than in the unbagged fruit. Fruit bagging also altered FF in both early-mid season cultivars, although not in the same direction (Table 1). Ethylene levels differed significantly in 'Fantasia' only, i.e., unbagged fruit produced 6.9-fold higher ethylene levels than bagged fruit.

Fruit bagging reduced fruit decay during postharvest and was cost-effective

In unbagged fruit, the incidence of disease was higher in the late cultivars (up to 75 - 85%) than in the early-mid season ones (up to 30 - 35%) (Figure 1). The onset of disease was observed earlier (one day after storage) in the late cultivars than in the early-mid ones (4 – 6 days after storage). The fungal pathogens detected were mainly *Monilinia* spp. (especially *M. fructicola*), and *Rhizopus* spp.

Overall, fruit bagging reduced and even prevented the appearance of decay during postharvest, in some cases to 0 (Fig. 1). In unbagged cv 'Fantasia', fruit decay was found to be 5% under control treatment and 10% under T1 storage, which was slightly higher than bagged fruit (0 and 5%, respectively) at the end of storage, although not statistically significant (Fig. 1A). Unbagged nectarines of cv 'Venus' had more disease under both control and T1 (35 and 20%, respectively) than bagged fruit (0% in both postharvest conditions) after 9 days of storage (Fig. 1B). This represents a 100% reduction in both postharvest conditions.

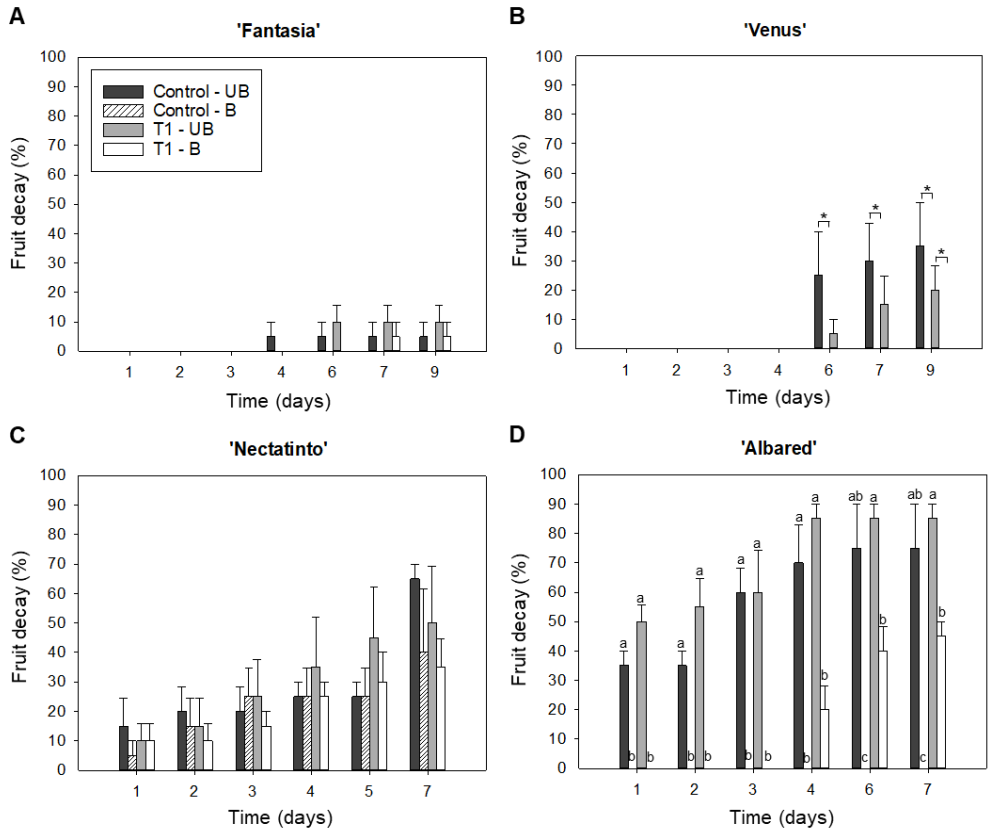

Figure 1. Fruit decay of nectarines after control and T1 postharvest conditions. *Incidence of fruit decay (%) during storage in unbagged (UB) and preharvest bagged (B) fruit stored under control or T1, of 'Fantasia' (A), 'Venus' (B), 'Nectatinto' (C) and 'Albared' (D) cultivars. Bars represent the mean of fruits with disease symptoms (n = 4 replicates, 5 fruits per replicate) and error bars represent the standard error of the means. Different lower-case letters indicate significant differences (p ≤ 0.05) of fruit decay incidence among postharvest storage × bagging conditions at each time point. No letters indicate no significant differences. Asterisks indicate significant differences (p ≤ 0.05) at each time point between bagged and unbagged fruit for each postharvest condition.*

In both late season cultivars, fruit decay was observed from the first day of storage, and gradually increased along storage time (Fig. 1C, D). Fruit decay was observed in cv 'Nectatinto' in all conditions on all days of storage. In unbagged fruit of 'Nectatinto', decay was already 15 and 10% under control and T1 after 1 day of storage, respectively, and increased steadily thereafter. At day 7, decay of fruit ranged from 35 to 65%, although the differences among bagging and storage conditions were not significant on any individual day. In cv 'Albared', the disease incidence in unbagged fruit was prominent at day one of storage in both treatments (35 and 50%,

respectively; Fig. 1D). Decay increased with increasing storage time and reached 75 and 85%, respectively, at 7 days. Interestingly, no decay was observed in the bagged fruit stored in control treatment. Contrarily, the bagged fruit stored in the T1 treatment developed disease symptoms on day 4 of storage. Decay in unbagged fruit was significantly higher ($p \leq 0.05$) than bagged fruit under both postharvest treatments (100 and 47.1% of reduction, respectively).

The economic evaluation indicated that fruit bagging is cost-effective for the four nectarine cultivars tested herein. For example, one hectare of organic nectarine orchard produces approximately 22.5 T of fruit (36 kg tree^{-1}). At a market price of 1.20 € Kg^{1} (e.g., in Spain) the sale price would be 27 K€. In our studies, unbagged early-mid cultivars showing 25% of postharvest losses (after 6 days of storage under control treatment at 20 °C) would represent a loss of 6,750 €. Similarly, late cultivars displaying postharvest losses up to 60% (after 3 days of storage under control treatment at 20 °C) would represent a loss up to 16,200 €. Considering the cost of paper bags, workers, and bag removal, fruit bagging is still worthwhile compared to production without bags, being much more cost-effective when considering late cultivars (+ 10,575 €) than early-mid cultivars (+ 1,125 €).

Postharvest storage minimized fruit quality differences between bagging conditions

The effect of bagging was also evaluated in terms of fruit quality changes suffered after postharvest storage. Under control treatment, most of the quality parameters were similar in bagged and unbagged fruit, although there were small but significant differences in two cultivars (Table 2). Unbagged cv 'Fantasia' fruit was significantly larger (i.e., weight and CD) and had lower DA Index and FF compared to bagged fruit. However, in cv 'Albared', unbagged fruit had significantly higher DA Index, FF, and SSC/TA ratio but lower SSC and TA than bagged fruit.

Under T1 storage, the quality of bagged and unbagged fruit was more uniform in all cultivars (Table 3). Bagged fruit of cv 'Fantasia' had significantly higher firmness, SSC and TA values than unbagged fruit (9 vs 6.6 N, 12.7 vs 11.3 °Brix and 7.5 vs 5.8 g L^{-1}, respectively). However, no effect attributable to bagging was observed in cv 'Venus' under T1, and only a significant difference in weight or CD was observed in the late cultivars (Table 3).

Table 2. Fruit quality of four nectarine cultivars after postharvest storage under control treatment. *The storage period was 9 days for 'Fantasia' and 'Venus' cultivars, and 7 days for 'Nectatinto' and 'Albared' cultivars. Weight, cheek diameter (CD), single index of absorbance difference (DA Index), flesh firmness (FF), soluble solids content (SSC), titratable acidity (TA) and SSC/ TA ratio. Values represent the mean (4 replicates, 5 fruits each) ± the standard error of the mean. Lower case letters indicate significant differences ($p \leq 0.05$) between bagging conditions, within each cultivar. No letter indicates no significant differences.*

Cultivar	Pre-harvest	Weight (g)	CD (mm)	DA Index	FF (N)	SSC (°Brix)	TA (g L^{-1})	SSC / TA ratio
'Fantasia'	B	116.9 ± 6.4 b	57.3 ± 1.0 b	0.3 ± 0.0 a	13.9 ± 1.7 a	12.7 ± 0.2	7.1 ± 0.6	1.8 ± 0.2
	UB	134.7 ± 5.5 a	60.6 ± 0.9 a	0.2 ± 0.0 b	7.4 ± 0.4 b	12.6 ± 0.2	8.1 ± 0.3	1.6 ± 0.1
'Venus'	B	160.1 ± 7.9	62.3 ± 1.2	0.2 ± 0.0	7.7 ± 0.6 a	12.8 ± 0.4	7.3 ± 0.2	1.8 ± 0.1
	UB	177.6 ± 5.7	66.7 ± 0.9	0.2 ± 0.0	6.1 ± 0.3 b	12.5 ± 0.5	7.8 ± 0.5	1.6 ± 0.1
'Nectatinto'	B	168.0 ± 8.0	66.7 ± 1.2	0.7 ± 0.1	15.5 ± 1.1	14.2 ± 0.5	3.3 ± 0.1	4.5 ± 0.3
	UB	161.2 ± 8.6	66.7 ± 1.2	0.5 ± 0.1	17.2 ± 1.4	13.0 ± 0.5	3.1 ± 0.2	4.2 ± 0.2
'Albared'	B	175.0 ± 6.2	65.3 ± 1.0	0.1 ± 0.0 b	6.0 ± 0.4 b	15.7 ± 0.3 a	7.0 ± 0.4 a	2.2 ± 0.1 b
	UB	174.0 ± 5.9	66.2 ± 0.8	0.1 ± 0.0 a	8.4 ± 0.8 a	14.7 ± 0.4 b	4.5 ± 0.3 b	3.4 ± 0.4 a

Table 3. Fruit quality of four nectarine cultivars after postharvest storage under T1 treatment. *The storage period was 9 days for 'Fantasia' and 'Venus' cultivars, and 7 days for 'Nectatinto' and 'Albared' cultivars. Weight, cheek diameter (CD), single index of absorbance difference (DA Index), flesh firmness (FF), soluble solids content (SSC), titratable acidity (TA), and SSC/ TA ratio. Values represent the mean (4 replicates, 5 fruits each) ± the standard error of the mean. Lower case letters indicate significant differences ($p \leq 0.05$) between bagging conditions, within each cultivar. No letter indicates no significant differences.*

Cultivar	Pre-harvest	Weight (g)	CD (mm)	DA Index	FF (N)	SSC (°Brix)	TA (g L^{-1})	SSC / TA ratio
'Fantasia'	B	137.4 ± 7.9	62.4 ± 0.9	0.3 ± 0.1	9.0 ± 0.7 a	12.7 ± 0.2 a	7.5 ± 0.2 a	1.70± 0.0
	UB	152.9 ± 4.8	63.8 ± 0.9	0.2 ± 0.0	6.6 ± 0.4 b	11.9 ± 0.2 b	5.8 ± 0.2 b	2.0 ± 0.1
'Venus'	B	168.4 ± 6.1	64.8 ± 1.0	0.2 ± 0.0	7.6 ± 0.6	11.8 ± 0.2	8.2 ± 0.3	1.5 ± 0.1
	UB	181.8 ± 7.3	67.1 ± 0.9	0.2 ± 0.0	7.1 ± 0.5	12.8 ± 0.2	7.5 ± 0.3	1.7 ± 0.1
'Nectatinto'	B	166.6 ± 10.6	66.8 ± 1.4 b	0.5 ± 0.1	15.9 ± 1.5	12.5 ± 0.5	3.2 ± 0.3	4.2 ± 0.6
	UB	185.5 ± 6.0	70.1 ± 0.9 a	0.6 ± 0.1	15.0 ± 1.1	13.5 ± 0.4	3.0 ± 0.2	4.8 ± 0.3
'Albared'	B	227.1 ± 12.3 a	72.3 ± 1.3	0.1 ± 0.0	7.8 ± 0.7	15.6 ± 0.5	4.9 ± 0.3	3.2 ± 0.2
	UB	183.1 ± 7.2 b	69.0 ± 1.0	0.1 ± 0.1	7.1 ± 1.1	14.7 ± 0.7	4.1 ± 0.2	3.3 ± 0.0

Changes in fruit quality under control vs T1 in relation to harvest day

To evaluate which postharvest storage condition triggered a greater change in fruit quality, the percentage of reduction or increase was calculated relative to the initial quality for each postharvest condition, bagging condition, and quality parameter. There were differences between storage conditions within each bagging condition in some cultivars (Fig. 2). Size parameters (weight and CD) were least altered in comparison to initial quality; reductions were below 19% for all cultivars except 'Nectatinto' (16 – 33%) and 'Albared' bagged and stored in control treatment (30%). In contrast, reductions in FF and DA Index were the highest (40 to 92% reduction).

In bagged fruit, the reductions of weight and CD under control treatment were significantly greater than under T1, in both 'Fantasia' and 'Albared' nectarine cultivars (Fig. 2A). The reduction in DA Index under control treatment was significantly lower than under T1 in cv 'Nectatinto', and the reduction in FF under control was significantly lower than under T1 in cv 'Fantasia' (Fig. 2B). In addition, the SSC/TA ratio increased in 'Fantasia' and 'Nectatinto' cultivars under both storage conditions. However, the changes in SSC/TA ratio in control vs T1 were significant in 'Venus' and 'Albared', but in opposite directions (Fig. 2C).

Changes observed in unbagged fruit were like those observed for bagged fruits. The reduction of weight and CD under control treatment was significantly higher than under T1 in both 'Fantasia' and 'Nectatinto' nectarines (Fig. 2D). Although there were no differences in DA Index, the reduction of FF was significantly smaller in cv 'Fantasia' under control than T1 (Fig. 2E). The increase of SSC/TA ratio under T1 was higher than under control in all unbagged cultivars, although the difference was significant only in cv 'Fantasia' (Fig. 2F).

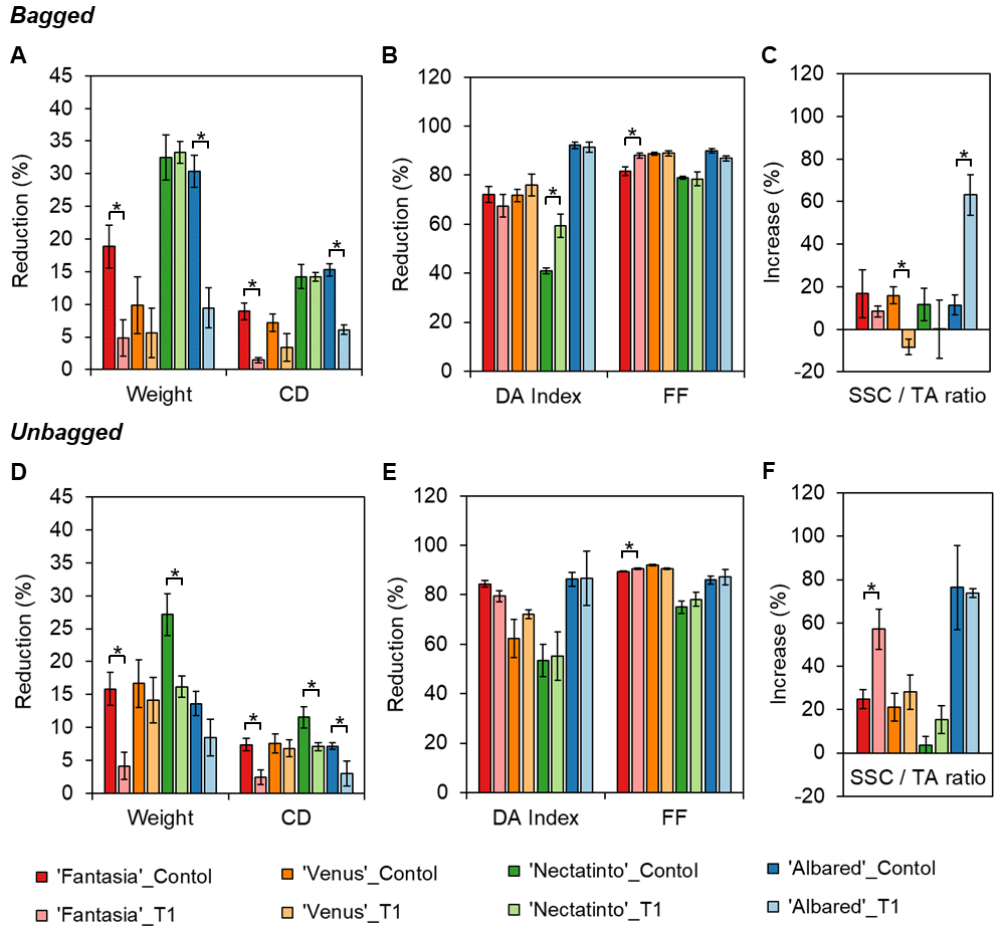

Figure 2. Changes in quality characteristics relative to initial fruit quality of nectarine cultivars after postharvest storage. *Percentage change calculated for bagged (A-C) and unbagged (D-F) fruit, stored in control treatment or T1. Weight and cheek diameter (CD) (A, D); DA Index and flesh firmness (FF) (B, E); SSC/ TA ratio (C, F). Bars represent the mean (n = 4 replicates, 5 fruits each) and error bars represent the standard error of the means. Asterisks indicate significant difference (p ≤ 0.05) between postharvest storage conditions, within each cultivar and bagging condition.*

Discussion

Effects of preharvest fruit conditions on fruit quality at harvest

Fruit undergoes physiological changes throughout its development, and external factors are crucial in determining fruit quality. Bagging fruit during its development influences the quantity (intensity) of solar irradiation that it receives in the field but also the light quality (wavelength of electromagnetic spectrum, i.e., color) that

irradiates the fruit. In the work reported herein, the reduction of light intensity was around 24%, suggesting that bagging could have impaired some fruit quality parameters. This could explain the differences between bagged and unbagged fruit in FF and SSC in 'Fantasia' and 'Venus' cvs, and the relatively small effect in 'Nectatinto' and 'Albared'. In a study conducted with UV-B radiation, it was shown that UV-B radiation reduces the activity of cell wall-modifying enzymes (e.g., pectin methylesterase and polygalacturonase), leading to loss of firmness, but without affecting the SSC and the titratable acidity (Santin et al., 2019). This suggests that a better understanding of the mechanisms underlying the effects of white light quality on FF and sugar content could help to ensure desired quality.

In organic peaches, fruit bagging also alters DA Index (Campbell et al., 2021), and DA Index values are correlated with chlorophyll content (Spadoni et al., 2016). In the presented work, DA Index in 3 out of 4 bagged cultivars was strongly differentiated on the day of harvesting. It could also be explained by the cultivar-dependent effect which influence the fruit quality (e.g., FF, SSC and TA) (Iglesias and Echeverría, 2009). Bagging process as well as its shortened duration can delay chlorophyll degradation and improve the anthocyanin content in peach peel, respectively (Zhou et al., 2019). Overall, changes related to FF, chemical content, and pigmentation were related to the presence or absence of bags in the field. This suggests that the producer should consider the type of bag and the specific cultivar response before bagging the fruit.

Altered fruit quality and fruit decay incidence

Host susceptibility to pathogens can depend on the ongoing physicochemical and physiological changes during fruit development and ripening, as well as the fruit characteristics intrinsic to the cultivar (Baró-Montel et al., 2020). In the work reported here, for example, in cv 'Albared', TA was negatively correlated with fruit decay (R^2 = - 0.97, p = 0.026) whereas the correlation between SSC/TA ratio and decay was positive (R^2 = 0.93, p = 0.067; Fig. 1, Table 2,3). Sugars are the major soluble solids in fruit juice and have been implicated in biotic (Kou et al., 2018) and abiotic stress responses (Wang et al., 2013). The development of brown rot, the main disease of stone fruit, has been positively associated with sucrose (Baró-Montel et al., 2020) and SSC (Gradziel, 1994), as nutrients for fungal growth. Hence, fruit quality of different cultivars stored under different conditions, either favored or restricted the onset of fruit decay.

Ethylene is also involved in responses to abiotic (Müller and Munné-Bosch, 2015) and biotic stresses, either acting against necrotrophic pathogens (Glazebrook, 2005) or being conductive to disease susceptibility (Van Der Ent and Pieterse, 2012). Here, ethylene production was reduced by fruit bagging in cv 'Fantasia' (by 6.9-fold in unbagged compared to bagged fruit) but slightly increased in the other cultivars tested (Table 1). Ethylene is required for fruit softening (Hayama et al., 2006) and, as expected, the highest ethylene production was accompanied by a reduced FF. The high ethylene production in cv 'Fantasia' unbagged fruit may have increased susceptibility to fruit decay, as well as the high ethylene production in late-season cultivars, which presented an increased fruit decay incidence. In nectarines artificially inoculated with *Monilinia* spp., fruit bagging altered ethylene production during postharvest, but all fruit was susceptible to *Monilinia* spp. under both treatments (Balsells-Llauradó et al., 2021). In the study reported here, ethylene production in late cultivars may have favored ripening-associated events, such as loss of FF, which made the fruit more susceptible to decay.

The reduction of fruit decay by fruit bagging is cultivar- and postharvest storage-dependent, but is cost-effective

Infections occurring along the postharvest chain can remain quiescent or cause latent infections until favorable factors trigger disease development (Luo et al., 2005; Garcia-Benitez et al., 2020). Incubation in humidity with photoperiod lighting favors naturally occurring diseases in peaches (Villarino et al., 2012). Herein, the incubation period of the observed decay suggests that early-mid season cultivars probably had relatively more quiescent conidia that developed later in time. In contrast, the late cultivars probably had relatively more field-occurring infections that remained briefly latent and were visible early in storage (Fig. 1A, B). For peaches, bagging is common in late cultivars which are exposed to more favorable climatic conditions for pests and diseases than early cultivars, to protect the fruit against insects such as the Mediterranean fly (Faci et al., 2014) and other fungal diseases such as brown rot (Mari et al., 2019). However, in orchards with high brown rot disease pressure, neither biological nor chemical treatment is completely effective (Casals et al., 2021). Thus, the low efficacy of bagging could be attributed to a high inoculum pressure in the field, especially in the cv 'Nectatinto'.

Herein, exposure to control treatment also reduced fruit decay, in a cultivar- and bagging-dependent manner (Fig. 1B, D). Roeber et al. (Roeber et al., 2021) found that impaired solar radiation affects both abiotic and biotic stress-triggered responses. UV-B radiation can also regulate plant metabolism such as gene expression of terpene synthases and the content of terpenoids and phytoalexins in peaches (Liu et al., 2017; Santin et al., 2021). In particular, the expression of terpenoids and phenylpropanoids has been implicated in both susceptibility and resistance of nectarines to brown rot (Balsells-Llauradó et al., 2020). Hence, in our study, the distinct level of solar radiation caused by fruit bagging may have induced changes in fruit that differentially affected their ability to face pathogens under different postharvest storage conditions. Deciphering the role of secondary metabolites (e.g., phenolics, terpenoids and phenylpropanoids) in the response to both pathogens and lighting conditions could improve our understanding of the disease development.

Scarce information exists related to the economic viability of fruit bagging (Blasi et al., 2017), which does not specify whether or not the losses occurring during the postharvest chain are considered. The economical evaluation conducted herein to test the differences between an orchard with or without bagged fruit, suggested that if fruit bagging is applied in similar orchards to the ones reported in this study (i.e., Ebro Valley area), bagging would be cost-effective especially in late-season cultivars.

Fruit quality parameters are within official and recommended ranges

All cultivars were harvested at commercial maturity date according to grower's recommendation, and all quality characteristics on harvest day and after either storage condition were within international recommendations (OECD, 2010; European Commission, 2019). Fruit size on harvest day and after the different storage conditions (Tables 1, 2, 3) was within specifications and accepted tolerances (European Commission, 2019). There are no official recommendations for DA Index, but all cultivars were within the limits for nectarines at harvest date (0.3- 1.5), as described by Reig et al. (Reig et al., 2012). Values of DA Index after postharvest storage were also within commercial maturity limits (0 – 1.5) described by Spadoni et al. (Spadoni et al., 2016). Published studies report that FF values should range from 40 to 62 N after harvest, depending on the intended use, and decrease during postharvest storage (Iglesias and Echeverría, 2009; Reig et al., 2012; Manganaris et al., 2017). These are slightly below our results except for cv 'Albared' (Table 1), but we also found that FF decreased during storage. The recommended FF at consumption ranges from 3 to 13

N (Crisosto, 2002; Bonany et al., 2014), in line with our results (except for cv 'Nectatinto'), after both storage conditions.

SSC should be ≥ 8 °Brix (OECD, 2010; European Commission, 2019), although some studies suggest at least 10 °Brix for consumer acceptance (e.g., Crisosto and Crisosto, 2005). Initial TA values were high, ranging from 3.3 to 10 (which includes the range of sweet and nonsweet nectarine cultivars; (Reig et al., 2012)), except for 'Nectatinto', which had TA < 3.3, placing this cultivar in the sub-acid category (Reig et al., 2012). However, eating quality is better described by the sugar-to-acid ratio (SSC:TA) rather than TA or SSC alone (Crisosto et al., 2006; Iglesias and Echeverría, 2009; Bonany et al., 2014). After storage, 'Nectatinto' remained in the sub-acid cultivar under both postharvest storage conditions due to its high SSC/TA ratio (> 2). After storage at both postharvest conditions, also 'Albared' nectarines became sub-acid, especially after T1 storage for both bagged and unbagged fruit.

T1 storage keeps the fruit quality better

After postharvest storage under control treatment, bagging condition has more pronounced effects on the quality of fruits stored under control than those stored under T1 in comparison to the initial quality (Tables 2, 3). Bag effect was conspicuous mainly in 'Fantasia' and 'Albared' cvs under control and T1. In addition to changes attributable to cultivar (Iglesias and Echeverría, 2009), a recurrent photoperiod can reduce the response to subsequent stresses (Roeber et al., 2021). This suggests that the effect of sunlight on fruit may have subsided after storage under control or T1 in 'Fantasia' and 'Albared' cvs.

The percentage change relative to initial quality suggested which postharvest storage condition had a greater effect on fruit quality for each bagging condition. A moderate weight loss was observed after both treatment storages, with some exceptions (Fig. 2). Loss of 5 to 8% of the fruit's water content may cause visual shriveling in peaches and nectarines, although the degree of shriveling is cultivar-dependent (Crisosto et al., 2020). In cv 'Nectatinto', which presented the highest weight loss in almost all conditions, shriveling was barely appreciable (data not shown). Interestingly, T1 storage induced a lower weight reduction than control in half of the cultivars, suggesting that T1 may have maintained fruit integrity. However, further research integrating all factors that affect water loss in fruit is needed (Lufu et al., 2020).

The DA index and FF values differed little between storage conditions within each bagging condition. The DA Index values decreased greatly in all cultivars (53 and 92%), probably because of the ongoing ripening during shelf life (Manganaris et al., 2017), causing a decrease in the chlorophyll content and an increase in other pigments, such anthocyanins (Bassi and Monet, 2008; Ramina et al., 2008). No studies report the effect of white lighting on these quality parameters, but a combination of white, blue, and green light irradiation increases the anthocyanin content and phenylalanine ammonia lyase activity in sweet cherries (Kokalj et al., 2019). In the work reported here, FF was also reduced sharply (75 to 92%) in all cultivars. Flesh firmness is regulated by a variety of cell wall modifications, including depolymerization and modifications of polymers (Brummell et al., 2004). Beyond the white light spectrum, blue light treatment reduces firmness in peaches during storage (Gong et al., 2015). Hence, investigation of the effects of white light on factors related to ripening (e.g., DA Index and FF) is needed.

Depending on the bagging condition and cultivar, T1 storage increased the SSC/TA ratio in some cases (Fig. 2), suggesting that light irradiation can favor the conversion of starch to sugars, and hence, decrease the acidity. Although there are no previous reports of the effect of white artificial lighting on fruit quality, a treatment with artificial blue light enhances total sugar content in peaches during storage (Gong et al., 2015). Hence, the results reported here suggest that lighting during the postharvest chain influences the fruit quality, although dependent on the preharvest conditions (bagging or not).

Conclusions

Results demonstrated that fruit bagging reduces the incidence of fruit decay during postharvest storage, especially in fruits from orchards with high inoculum pressure (e.g., late-season cultivars). Fruit bagging was cost-effective for both late and early-mid cultivars. Postharvest storage under T1 increased fruit losses, and hence, storage under control treatment is preferable. Fruit quality on harvest day and after storage were within international recommendations, irrespective of bagging conditions. Therefore, both preharvest and postharvest management (bagged fruit and postharvest storage like the described control condition) should be considered by growers and distributors for sustainable fruit production and to ensure desirable fruit quality for the marketplace.

Author Contributions

Marta Balsells-Llauradó: Conceptualization, Investigation, Formal Analysis, Writing – original draft preparation, Writing- Reviewing and Editing. Núria Vall-llaura: Conceptualization, Methodology, Data Curation, Writing – review & editing. Josep Usall: Supervision, Funding acquisition, Writing – review & editing. Carla Casals: Investigation, Writing – review & editing. Neus Teixidó: Investigation, Resources, Writing – review & editing. Rosario Torres: Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration.

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Conflicts of Interest

The authors declare no conflict of interest.

References

Ali, M. M., Anwar, R., Yousef, A. F., Li, B., Luvisi, A., De Bellis, L., et al. (2021). Influence of bagging on the development and quality of fruits. *Plants* 10, 1–17. doi:10.3390/plants10020358.

Ballaré, C. L. (2014). Light regulation of plant defense. *Annu. Rev. Plant Biol.* 65, 335–363. doi:10.1146/annurev-arplant-050213-040145.

Balsells-Llauradó, M., Silva, C. J., Usall, J., Vall-llaura, N., Serrano-Prieto, S., Teixidó, N., et al. (2020). Depicting the battle between nectarine and *Monilinia laxa*: the fruit developmental stage dictates the effectiveness of the host defenses and the pathogen's infection strategies. *Hortic. Res.* 7, 1–15. doi:10.1038/s41438-020-00387-w.

Balsells-Llauradó, M., Torres, R., Vall-llaura, N., Casals, C., Teixidó, N., and Usall, J. (2021). Light intensity alters the behavior of *Monilinia* spp. *in vitro* and the disease development on stone fruit-pathogen interaction. *Front. Plant Sci.* 12, 1–12. doi:10.3389/fpls.2021.666985.

Baró-Montel, N., Giné-Bordonaba, J., Torres, R., Vall-llaura, N., Teixidó, N., and Usall, J. (2020). Scrutinising the relationship between major physiological and compositional changes during 'Merrill O'Henry' peach growth with brown rot susceptibility. *Food Sci. Technol. Int.* 27, 366–379. doi:10.1177/1082013220959988.

Baró-Montel, N., Torres, R., Casals, C., Teixidó, N., Segarra, J., and Usall, J. (2019). Developing a methodology for identifying brown rot resistance in stone fruit. *Eur. J. Plant Pathol.* 154, 287–303. doi:10.1007/s10658-018-01655-1.

Bassi, D., and Monet, R. (2008). "Botany and taxonomy.," in *The peach: botany, production and uses*, eds. D. R. Layne and D. Bassi (Wallingford: CAB International), 1–36. doi:10.1079/9781845933869.0001.

Blasi, E., Pancino, B., Passeri, N., and Franco, S. (2017). Environmental and economic benefits of the preharvest fruit bagging technique: Trade-off evaluation in a Mediterranean area. *Acta Hortic.* 1160, 313–318. doi:10.17660/ActaHortic.2017.1160.45.

Bonany, J., Carbó, J., Echeverria, G., Hilaire, C., Cottet, V., Iglesias, I., et al. (2014). Eating quality and European consumer acceptance of different peach (*Prunus persica* (L.) Batsch) varieties. *J. Food, Agric. Environ.* 12, 67–72.

Brummell, D. A., Dal Cin, V., Crisosto, C. H., and Labavitch, J. M. (2004). Cell wall metabolism during maturation, ripening and senescence of peach fruit. *J. Exp. Bot.* 55, 2029–2039. doi:10.1093/jxb/erh227.

Bulletin OEPP/EPPO (2020). PM 7/18 (3) *Monilinia fructicola*. *EPPO Bull.* 50, 5–18. doi:10.1111/epp.12609.

Bussi, C., Plenet, D., Merlin, F., Guillermin, A., and Mercier, V. (2015). Limiting brown rot incidence in peach with tree training and pruning. *Fruits* 70, 303–309. doi:10.1051/fruits/2015030.

Campbell, D., Sarkhosh, A., Brecht, J. K., Gillett-Kaufman, J. L., Liburd, O., Melgar, J. C., et al. (2021). Bagging organic peaches reduces physical injuries and storage decay with minimal effects on fruit quality. *HortScience* 56, 52–58. doi:10.21273/HORTSCI15391-20.

Casals, C., Guijarro, B., De Cal, A., Torres, R., Usall, J., Perdrix, V., et al. (2021). Field validation of biocontrol strategies to control brown rot on stone fruit in several European countries. *Pest Manag. Sci.* 77, 2502–2511. doi:10.1002/ps.6281.

Casals, C., Segarra, J., De Cal, A., Lamarca, N., and Usall, J. (2015). Overwintering of *Monilinia* spp. on mummified stone fruit. *J. Phytopathol.* 163, 160–167. doi:10.1111/jph.12298.

Crisosto, C. H. (2002). How do we increase peach consumption? in *Acta Horticulturae*, 601–605. doi:10.17660/ActaHortic.2002.592.82.

Crisosto, C. H., and Costa, G. (2008). "Preharvest factors affecting peach quality," in *The Peach: Botany, Production and Uses*, eds. D. R. Layne and D. Bassi (Cambridge, MA, USA: CAB International), 536–549. doi:10.1079/9781845933869.0536.

Crisosto, C. H., and Crisosto, G. M. (2005). Relationship between ripe soluble solids concentration (RSSC) and consumer acceptance of high and low acid melting flesh peach and nectarine (*Prunus persica* (L.) Batsch) cultivars. *Postharvest Biol. Technol.* 38, 239–246. doi:10.1016/j.postharvbio.2005.07.007.

Crisosto, C. H., Crisosto, G. M., and Garner, D. (2006). Understanding tree fruit consumer acceptance. *Acta Hortic.* 682, 865–870. doi:10.17660/ActaHortic.2005.682.112.

Crisosto, C. H., Echeverría, G., and Manganaris, G. A. (2020). "Peach and Nectarine," in *Manual on postharvest handling of Mediterranean tree fruits and nuts*, eds. C. H. Crisosto and G. M. Crisosto (CAB International), 53–87.

De Oliveira Lino, L., Génard, M., Signoret, V., and Quilot-Turion, B. (2016). Physical host factors for brown rot resistance in peach fruit. *Acta Hortic.* 1137, 105–112. doi:10.17660/ActaHortic.2016.1137.15.

Eckert, J. W., and Ratnayake, M. (1983). *Post-Harvest Physiology and Crop Preservation*. , ed. M. Lieberman Boston, MA: Springer, Boston, MA doi:10.1007/978-1-4757-0094-7.

European Commission (2019). COMMISSION DELEGATED REGULATION (EU) 2019/428.

Faci, J. M., Medina, E. T., Martínez-Cob, A., and Alonso, J. M. (2014). Fruit yield and quality response of a late season peach orchard to different irrigation regimes in a semi-arid environment. *Agric. Water Manag.* 143, 102–112. doi:10.1016/j.agwat.2014.07.004.

FAO (2021). Database of Food and Agriculture Organization of the United Nations. Available at: http://www.fao.org/faostat/en/#data/QC/visualize [Accessed April 29, 2021].

Garcia-Benitez, C., Casals, C., Usall, J., Sánchez-Ramos, I., Melgarejo, P., and De Cal, A. (2020). Impact of postharvest handling on preharvest latent infections caused by *Monilinia* spp. in nectarines. *J. Fungi* 6, 1–14. doi:10.3390/jof6040266.

Giné-Bordonaba, J., Echeverria, G., Ubach, D., Aguiló-Aguayo, I., López, M. L., and Larrigaudière, C. (2017). Biochemical and physiological changes during fruit development and ripening of two sweet cherry varieties with different levels of cracking tolerance. *Plant Physiol. Biochem.* 111, 216–225. doi:10.1016/j.plaphy.2016.12.002.

Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annu. Rev. Phytopathol.* 43, 205–227. doi:10.1146/annurev.phyto.43.040204.135923.

Gong, D., Cao, S., Sheng, T., Shao, J., Song, C., Wo, F., et al. (2015). Effect of blue light on ethylene biosynthesis, signalling and fruit ripening in postharvest peaches. *Sci. Hortic. (Amsterdam).* 197, 657–664. doi:10.1016/j.scienta.2015.10.034.

Gradziel, T. M. (1994). Changes in susceptibility to brown rot with ripening in three clingstone peach genotypes. *J. Amer. Soc. Hort. Sci* 119, 101–105. doi:10.21273/JASHS.119.1.101.

Hayama, H., Shimada, T., Fujii, H., Ito, A., and Kashimura, Y. (2006). Ethylene-regulation of fruit softening and softening-related genes in peach. *J. Exp. Bot.* 57, 4071–4077. doi:10.1093/jxb/erl178.

Iglesias, I., and Echeverría, G. (2009). Differential effect of cultivar and harvest date on nectarine colour, quality and consumer acceptance. *Sci. Hortic. (Amsterdam).* 120, 41–50. doi:10.1016/j.scienta.2008.09.011.

Keske, C., Treutter, D., and Neumüller, M. (2014). Effect of bagging on brown rot incidence in European Plum. *Ecofruit. 16th Int. Conf. Org. Grow. Proceedings, 17-19 Febr. 2014, Hohenheim, Ger.*, 228–231. doi:9783000450716.

Kokalj, D., Zlatić, E., Cigić, B., and Vidrih, R. (2019). Postharvest light-emitting diode irradiation of sweet cherries (*Prunus avium* L.) promotes accumulation of anthocyanins. *Postharvest Biol. Technol.* 148, 192–199. doi:10.1016/j.postharvbio.2018.11.011.

Kou, J., Wei, Y., He, X., Xu, J., Xu, F., and Shao, X. (2018). Infection of post-harvest peaches by *Monilinia fructicola* accelerates sucrose decomposition and stimulates the Embden–Meyerhof–Parnas pathway. *Hortic. Res.* 5. doi:10.1038/s41438-018-0046-x.

Liu, H., Cao, X., Liu, X., Xin, R., Wang, J., Gao, J., et al. (2017). UV-B irradiation differentially regulates terpene synthases and terpene content of peach. *Plant Cell Environ.* 40, 2261–2275. doi:10.1111/pce.13029.

Lufu, R., Ambaw, A., and Opara, U. L. (2020). Water loss of fresh fruit: Influencing pre-harvest, harvest and postharvest factors. *Sci. Hortic. (Amsterdam).* 272, 109519. doi:10.1016/j.scienta.2020.109519.

Luo, Y., Michailides, T. J., Morgan, D. P., Krueger, W. H., and Buchner, R. P. (2005). Inoculum dynamics, fruit infection, and development of brown rot in prune orchards in California. *Phytopathology* 95, 1132–1136. doi:10.1094/PHYTO-95-1132.

Manganaris, G. A., and Crisosto, C. H. (2020). "Stone fruits: Peaches, nectarines, plums, apricots," in *Controlled and Modified Atmospheres for Fresh and Fresh-Cut Produce*, eds. M. Gil and R. Beaudry (Elsevier Inc.), 311–322. doi:10.1016/b978-0-12-804599-2.00017-x.

Manganaris, G. A., Drogoudi, P., Goulas, V., Tanou, G., Georgiadou, E. C., Pantelidis, G. E., et al. (2017). Deciphering the interplay among genotype, maturity stage and low-temperature storage on phytochemical composition and transcript levels of enzymatic antioxidants in *Prunus persica* fruit. *Plant Physiol. Biochem.* 119, 189–199. doi:10.1016/j.plaphy.2017.08.022.

Mari, M., Spadaro, D., Casals, C., Collina, M., De Cal, A., and Usall, J. (2019). "Stone Fruits," in *Postharvest Diseases of Fresh Horticultural Produce*, eds. L. Palou and J. L. Smilanick (CRC Press), 111–140.

Minas, I. S., Tanou, G., and Molassiotis, A. (2018). Environmental and orchard bases of peach fruit quality. *Sci. Hortic. (Amsterdam).* 235, 307–322. doi:10.1016/j.scienta.2018.01.028.

Müller, M., and Munné-Bosch, S. (2015). Ethylene response factors: A key regulatory hub in hormone and stress signaling. *Plant Physiol.* 169, 32–41. doi:10.1104/pp.15.00677.

Mustafa, M. H., Bassi, D., Corre, M.-N., Lino, L. O., Signoret, V., Quilot-Turion, B., et al. (2021). Phenotyping brown rot susceptibility in stone fruit: A literature review with emphasis on peach. *Horticulturae* 7, 115. doi:10.3390/horticulturae7050115.

OECD (2010). Peaches and nectarines, International Standards for Fruit and Vegetables. OECD Publishing, Paris doi:https://doi.org/10.1787/9789264084926-en-fr.

Porat, R., Lichter, A., Terry, L. A., Harker, R., and Buzby, J. (2018). Postharvest losses of fruit and vegetables during retail and in consumers' homes: Quantifications, causes, and means of prevention. *Postharvest Biol. Technol.* 139, 135–149. doi:10.1016/j.postharvbio.2017.11.019.

Ramina, A., Tonutti, P., and Mcglasson, W. (2008). "Ripening, Nutrition and Postharvest Physiology," in *The Peach: Botany, Production and Uses*, eds. D. R. Layne and D. Bassi, 550–574.

Reig, G., Alegre, S., Iglesias, I., Echeverría, G., and Gatius, F. (2012). Fruit quality, colour development and index of absorbance difference (I_{AD}) of different nectarine cultivars at different harvest dates. *Acta Hortic.* 934, 1117–1126. doi:10.17660/ActaHortic.2012.934.150.

Roeber, V. M., Bajaj, I., Rohde, M., Schmülling, T., and Cortleven, A. (2021). Light acts as a stressor and influences abiotic and biotic stress responses in plants. *Plant. Cell Environ.* 44, 645–664. doi:10.1111/pce.13948.

Santin, M., Giordani, T., Cavallini, A., Bernardi, R., Castagna, A., Hauser, M. T., et al. (2019). UV-B exposure reduces the activity of several cell wall-dismantling enzymes and affects the expression of their biosynthetic genes in peach fruit (*Prunus persica* L., cv. Fairtime, melting phenotype). *Photochem. Photobiol. Sci.* 18, 1280–1289. doi:10.1039/c8pp00505b.

Santin, M., Neugart, S., Castagna, A., Barilari, M., Sarrocco, S., Vannacci, G., et al. (2018). UV-B Pre-treatment alters phenolics response to Monilinia fructicola infection in a structure-dependent way in peach skin. *Front. Plant Sci.* 9. doi:10.3389/fpls.2018.01747.

Santin, M., Ranieri, A., Hauser, M.-T., Miras-Moreno, B., Rocchetti, G., Lucini, L., et al. (2021). The outer influences the inner: Postharvest UV-B irradiation modulates peach flesh metabolome although shielded by the skin. *Food Chem.* 338, 127782. doi:10.1016/j.foodchem.2020.127782.

Sharma, R. R., Reddy, S. V. R., and Jhalegar, M. J. (2014). Pre-harvest fruit bagging: A useful approach for plant protection and improved post-harvest fruit quality - A review. *J. Hortic. Sci. Biotechnol.* 89, 101–113. doi:10.1080/14620316.2014.11513055.

Spadoni, A., Cameldi, I., Noferini, M., Bonora, E., Costa, G., and Mari, M. (2016). An innovative use of DA-meter for peach fruit postharvest management. *Sci. Hortic. (Amsterdam).* 201, 140–144. doi:10.1016/j.scienta.2016.01.041.

Usall, J., Casals, C., Sisquella, M., Palou, L., and De Cal, A. (2015). Alternative technologies to control postharvest diseases of stone fruits. *Stewart Postharvest Rev.* 11, 1–6. doi:10.2212/spr.2015.4.2.

Van Der Ent, S., and Pieterse, C. M. J. (2012). "Ethylene: multi-tasker in plant-attacker interactions," in *Annual Plant Reviews*, ed. Michael T. McManus (Blackwell Publishing Ltd), 343–377. doi:10.1002/9781118223086.ch13.

Villarino, M., Melgarejo, P., Usall, J., Segarra, J., Lamarca, N., and De Cal, A. (2012). Secondary inoculum dynamics of *Monilinia* spp. and relationship to the incidence of postharvest brown rot in peaches and the weather conditions during the growing season. *Eur. J. Plant Pathol.* 133, 585–598. doi:10.1007/s10658-011-9931-y.

Wang, K., Shao, X., Gong, Y., Zhu, Y., Wang, H., Zhang, X., et al. (2013). The metabolism of soluble carbohydrates related to chilling injury in peach fruit exposed to cold stress. *Postharvest Biol. Technol.* 86, 53–61. doi:10.1016/j.postharvbio.2013.06.020.

Zhou, H., Yu, Z., and Ye, Z. (2019). Effect of bagging duration on peach fruit peel color and key protein changes based on iTRAQ quantitation. *Sci. Hortic. (Amsterdam).* 246, 217–226. doi:10.1016/j.scienta.2018.10.072.

Chapter 3. Depicting the battle between nectarine and *Monilinia laxa*: the fruit developmental stage dictates the effectiveness of the host defenses and the pathogen's infection strategies

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Abstract

Infections by the fungus *Monilinia laxa*, the main cause of brown rot in Europe, result in considerable losses of stone fruit. Herein, we present a comprehensive transcriptomic approach to unravel strategies deployed by nectarine fruit and *M. laxa* during their interaction. We used *M. laxa*-inoculated immature and mature fruit, which were resistant and susceptible to brown rot, respectively, to perform a dual RNA-Seq analysis. In immature fruit, host responses, pathogen biomass, and pathogen transcriptional activity peaked at 14 – 24 hours post inoculation (hpi), at which point *M. laxa* appeared to switch its transcriptional response to either quiescence or death. Mature fruit experienced an exponential increase in host and pathogen activity beginning at 6 hpi. Functional analyses in both host and pathogen highlighted differences in stage-dependent strategies. For example, in immature fruit, *M. laxa* unsuccessfully employed carbohydrate-active enzymes (CAZymes) for penetration, which the fruit was able to combat with tightly regulated hormone responses and an oxidative burst that challenged the pathogen's survival at later time points. In contrast, in mature fruit, *M. laxa* was more dependent on proteolytic effectors than CAZymes and was able to invest in filamentous growth early during the interaction. Hormone analyses of mature fruit infected with *M. laxa* indicated that, while jasmonic acid activity was likely useful for defense, high ethylene activity may have promoted susceptibility through induction of ripening processes. Lastly, we identified *M. laxa* genes that were highly induced in both quiescent and active infections and may serve as targets for control of brown rot.

Keywords: brown rot, fruit disease, postharvest pathology, plant hormones, secondary metabolism, necrotrophic fungal pathogen, quiescent infection.

Introduction

Monilinia laxa is the main causal agent of brown rot in Europe, leading to important losses of stone fruit in the field and postharvest (Rungjindamai et al., 2014). The worldwide yearly losses are estimated to be 1.7 M euros for peach and nectarine (Martini and Mari, 2014) and 170 M USD for peach, cherry, and plum production (RosBREED). The disease is controlled using several cultural practices (e.g., removing the overwintering inoculum), chemical fungicides in the orchard, treatments onto mummified fruit and postharvest storage at low temperatures (Rungjindamai et al., 2014; Usall et al., 2015). However, the gradual withdrawal of some fungicides driven by concerns about their negative impact on the environment and human health, the constant threat of the emergence of fungicide resistance, and the appearance of novel virulence alleles demonstrate the need for alternative methods for managing brown rot (Ma et al., 2005; Byrne, 2012; Usall et al., 2015). Prior to infection, *M. laxa* can remain latent or quiescent on flowers and fruit surfaces until favorable host factors (i.e., fruit developmental stage (Mari et al., 2003)), and environmental factors and other characteristics intrinsic to the stone fruit variety (Gununu et al., 2019), trigger the disease cycle (Luo et al., 2005).

During fruit infection, *M. laxa* can overcome the need of wounds to infect and penetrate the plant cell. As a necrotrophic pathogen, *M. laxa* relies on the secretion of cell wall degrading enzymes (CWDEs), such as pectin methyl esterases (De Miccolis Angelini et al., 2018), and possibly phytotoxins, although these compounds have not been fully identified yet (Garcia-Benitez et al., 2019). After penetration, *M. laxa* colonizes the epidermis of the fruit with hyphae (Lee and Bostock, 2006) causing the collapse and disruption of cells, lysogenic cavities, and total degradation of the cuticle and epidermis, similar to the lesions caused by *M. fructicola* (Garcia-Benitez et al., 2016).

Overall, fruit can be infected at any growth stage, but their susceptibility to brown rot increases with maturation, which results in a short postharvest life (Mari et al., 2019). Hence, the activation of immune responses alongside the physicochemical properties of the fruit may determine the pathogen's ability to infect and spread. Although these underlying mechanisms have not been fully elucidated, possible explanations could depend on changes in cell wall composition, volatiles, organic acids and phenolic compounds (Lee and Bostock, 2007; Villarino et al., 2011).

We hypothesize that *M. laxa* is able to adapt its infection strategies according to the nectarine developmental stage, resulting in either quiescent or disease progression, while the plant host can only establish effective defenses to restrict pathogen growth in fruit tissues that have not yet reached full maturity. Here, the fruit responses and pathogenicity mechanisms in the nectarine-*M. laxa* interaction were investigated as a function of the host developmental stage and time. Nectarine fruit were harvested at two different developmental stages (immature and mature) and inoculated with *M. laxa*. Disease development and ethylene production were assessed for 3 days. Thanks to the recent availability of the *M. laxa* 8L genome (Naranjo-Ortíz et al., 2018), a comparative transcriptomics study was conducted on the nectarine-*M. laxa* pathosystem across four time points. This approach allowed us to identify not only host defense responses that were uniquely or highly induced in immature fruit during early infections, which may partially explain why these tissues are resistant to brown rot, but also key strategies employed by the fungus to either become established in tissues or colonize them, which may be targeted to control brown rot.

Materials and methods

Plant material and fungal culture

'Venus' nectarines (*P. persica* var. *nucipersica* (Borkh.) Schneider) were obtained from an organic orchard located in Raïmat (Lleida, Spain). Fruit were bagged 6 weeks before the last harvest and then harvested at two different fruit developmental stages, "mature" (211 Julian days) and "immature" (184 Julian days), and used immediately after harvest. Injured or deformed fruit were discarded, and fruit for analysis were further homogenized by using a portable DA-Meter (TR-Turoni, Forli, Italy), based on the single index of absorbance difference (I_{AD} = 1.99 – 2.26 for immature fruit and I_{AD} = 0.25 – 1.60 for mature fruit). Other assessments of quality parameters were performed on 20 randomly selected fruit (weight, cheek diameter, flesh firmness, soluble solids content and titratable acidity), according to the method of Baró-Montel et al. (2019a)

The *M. laxa* single-spore strain 8L (ML8L, Spanish Culture Type Collection number CECT 21100) was used for all experiments. Fungal conidial suspensions were maintained and prepared as described by Baró-Montel et al. (2019c).

Fruit inoculations

Each fruit was inoculated with the application of six 30 µL drops of a conidial suspension at concentration of 10^6 conidia mL^{-1} on the fruit surface. Mock inoculated fruit were equally treated with sterile water containing 0.01% (w/v) Tween-80. Fruit were placed in closed containers with a relative humidity of 97 ± 3% at 20 ± 1°C. Four replicates consisting of five fruit per treatment were obtained at each sampling point (6, 14, 24, 48 and 72 hpi). Six cylinders of peel and pulp tissue (1 cm diameter and depth) encompassing the inoculation sites were sampled from each fruit and pooled for each replicate. Samples were immediately flash frozen in liquid nitrogen and stored at -80 ºC until extraction. For symptom analysis, inoculated fruit was imaged at the set time points. Ethylene production of both mock *and M. laxa* inoculated immature and mature fruit was determined as described by Baró-Montel et al. (2019b).

Fruit and fungal RNA extraction

Frozen samples were ground using a mortar and pestle. Total RNA was extracted following the protocol described previously (Baró-Montel et al., 2019c). Contaminant DNA was removed by treating RNA extracts with Turbo DNA-free DNase (Ambion, TX, USA). RNA concentration and purity were assessed with the Qubit® 3.0 Fluorometer (Invitrogen, USA). Gel electrophoresis on an agarose gel stained with GelRed™ Nucleic Acid Gel Stain (Biotium, Hayward, CA, USA) was used to confirm the RNA was free of DNA and not degraded.

cDNA libraries preparation and RNA sequencing

A total of 48 samples were analyzed by RNA sequencing, using 3 replicates of each treatment and stage at 4 of the sampled time points (6, 14, 24 and 48 hpi). cDNA libraries were prepared using the Illumina TruSeq RNA Sample Preparation Kit v2 (Illumina, USA). Quality control of the cDNA libraries was performed with the High Sensitivity DNA Analysis Kit in the Agilent 2100 Bioanalyzer (Agilent Technologies, USA). Paired-end libraries of 150-bp were sequenced on the Illumina HiSeq 4000 platform in IDSEQ INC (Davis, CA, USA).

RNA-Seq bioinformatics pipeline and data processing

Quality and adapter trimming on raw reads was performed with Trimmomatic v0.33 (Bolger et al., 2014) with the following parameters: maximum seed mismatches = 2, palindrome clip threshold = 30, simple clip threshold = 10, minimum leading quality = 3, minimum trailing quality = 3, window size = 4, required quality = 15, and

minimum length = 36. Basic quality measurements were assessed with FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) before and after quality trimming. Mapping of parsed reads to a combined transcriptome of nectarine and *M. laxa* was performed using Bowtie2 (Langmead and Slazberg, 2012). The nectarine transcriptome was obtained for peach (*Prunus persica* v2.0.a1) from the Genome Database for Rosaceae (Verde et al., 2013, 2017) (https://www.rosaceae.org/species/prunus_persica/genome_v2.0.a1) as no nectarine genome was available. The transcriptome of *M. laxa* was previously obtained by our group (Naranjo-Ortíz et al., 2018).

Count matrices were made from the Bowtie2 results using sam2counts.py v0.919 (https://github.com/vsbuffalo/sam2counts) and are available in Suppl. Table S6 and S7 for nectarine and *M. laxa*, respectively. Differential expression (DE) analyses were conducted with the Bioconductor package DESeq2 (Love et al., 2014) in R. Reads were first normalized for library size. Differentially expressed genes (DEGs) were considered to be those with an adjusted *p-value* less than or equal to 0.05. Two principal component analyses (PCA) were constructed with DESeq2 using the "plotPCA" function after normalized data sets were transformed with the "vst" function separately for nectarine and *M. laxa*.

Functional analysis of nectarine genes

Functional annotations for the nectarine transcriptome were downloaded and processed from the Genome Database for Rosaceae version Peach v2.0.a1 (v2.1) (Verde et al., 2013, 2017). Once differential expression analysis was combined with the functional annotations, enrichment analysis of KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways was performed using Fisher's exact test ($P \leq 0.05$).

Functional annotation and analysis of *M. laxa* genes

Transcripts were annotated with multiple databases. Gene Ontology (GO) terms were obtained via Blast2GO (https://www.blast2go.com/). Additional BLAST searches were carried out to the Transporter Classification Database (TCDB, http://www.tcdb.org/) and the Pathogen-Host Interactions database (PHI, http://www.phi-base.org/). Custom HMMER alignment results for HMM profiles from the Protein Families database (Pfam), the Carbohydrate-active enzyme annotation database (dbCAN, http://csbl.bmb.uga.edu/dbCAN/) and the fungal peroxidases database (fPox, http://peroxidase.riceblast.snu.ac.kr/) were similarly included. The presence of secretion signal peptides was evaluated for all genes in the transcriptome using

SignalP v.4.0 (Petersen et al., 2011). An e-value of 1e-3 was used as the cutoff value across all methods described. All enrichments carried out for *M. laxa* were performed as previously described for nectarine.

Gene expression analysis with RT-qPCR and primer design

To determinate the fungal biomass in all samples and to validate RNA-Seq results, gene expression analyses with RT-qPCR were carried out. First-strand cDNA was synthesized on 1 µg of RNA using the M-MLV Reverse Transcriptase (Promega, USA) in the SimpliAmp Thermal Cycler (Applied Biosystems, USA). Expression of the reference genes was quantified through Real-Time Quantitative PCR (RT-qPCR) using KAPA SYBR® Fast qPCR Master Mix (Kapa Biosystems, Inc., Wilmington, USA) in the 7500 Real Time PCR System (Applied Biosystems, USA) with 2 µL of cDNA. Relative expression levels for fungal biomass determination were calculated according to the relative gene expression of the *M. laxa* reference gene *ACT* normalized to the nectarine reference gene expression *TEF2*. Primers for genes of interest were obtained from literature or designed *de novo* and are available in Suppl. Table S8. Primer efficiency was determined by the serial dilution method, using a mix of all cDNA samples as a template.

Data availability

The raw sequencing reads, and the read mapping count matrices have been deposited in the National Center for Biotechnology Information Gene Expression Omnibus database under the accession GSE146293.

Results

Nectarine susceptibility to brown rot is developmentally controlled

We visually assessed the development of brown rot over time at two maturity stages of nectarine (Figure 1a). Quality parameters were measured and summarized in Suppl. Table S1. Overall, the disease progressed in mature tissues while only surface discoloration was observed in immature tissues. At the mature stage, tissue maceration was observed on the surface of the fruit at 14 hpi followed by the pathogen penetration of the pericarp tissues between 14 and 24 hpi, and increasing lesion spread at 48 and 72 hpi. Fungal biomass was also estimated in both inoculated and control (mock inoculated) fruit to complement the visual assessments (Fig. 1b). Although no symptoms of brown rot disease were visible on the immature fruit surface at any time point, the *M. laxa* biomass increased from 6 to 14 hpi, when the highest

quantity was detected, and then significantly decreased until 72 hpi. Although at early stages of infection (6 to 14 hpi), the fungal biomass was not significantly different between immature and mature tissues, it increased exponentially ($y = 0.2119e^{0.0596t}$, $R^2 = 0.9075$) in the mature fruit at later time points, reaching levels approximately twenty times more than the maximum observed in immature fruit. In control tissues, a negligent quantity of the fungal biomass was detected across all time points in both stages.

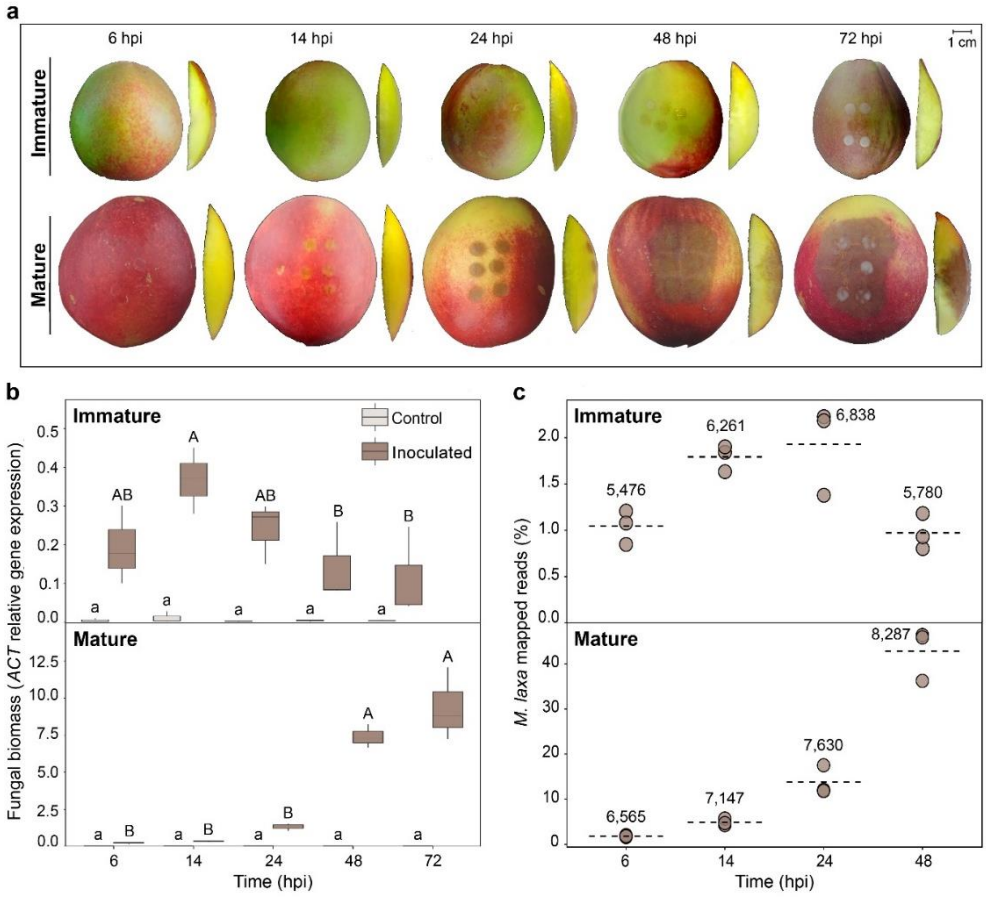

Figure 1. Fungal behavior development in 'Venus' nectarines. a) *Brown rot spread development in immature and mature tissues at different time points after inoculation (6, 14, 24, 48 and 72 hpi). Two different viewpoints are shown (left image - entire fruit showing 6 drops; right image – perpendicular section of the fruit to discern fungus penetration if observable).* ***b)*** *Determination of pathogen biomass by relative gene expression of the* M. laxa *reference gene* (ACT), *normalized to the expression of the nectarine reference gene* (TEF2) *in both stages (immature and mature) of both control (light brown) and inoculated (dark brown) tissues. The box plot represents the mean of 3 biological replicates with its*

interquartile range. Lowercase and uppercase letters indicate significance differences (P < 0.05, Student's T-test) in control and inoculated tissues, respectively. ***c)*** *Abundance (%) of* M. laxa *mapped reads in inoculated tissue out of the total amount of reads at each time point in both tissues. Each dot represents the number of mapped reads for each of the three biological replicates. The dashed line represents the average of the mapped reads in each group. Numbers represent the average of genes that were obtained at each time point in both tissues.*

A dual RNA-seq study revealed the dynamics of the fruit-pathogen interaction at early (6 hpi and 14 hpi) and late (24 hpi and 48 hpi) infection time points. The expression of 21,334 nectarine genes (79.39% of total transcriptome) and 8,364 *M. laxa* genes (87.30% of total transcriptome) was detected across all developmental stages and time points (Suppl. Table S2). The proportion of total (i.e., from both host and pathogen) mapped reads for each sample that corresponded to *M. laxa* (Fig. 1c) strongly correlated (r = 0.996) with the measurements of fungal biomass. Remarkably, more than 6,000 genes were found to be expressed in inoculated immature fruit at 14 hpi and 24 hpi, indicating that the pathogen was active in these tissues but yet it could not cause disease. More genes were detected in mature fruit, increasing across time, from 6,565 at 6 hpi up to 8,287 at 48 hpi, reflecting the progression of pathogen growth and host tissue colonization.

Nectarine and *M. laxa* synchronize their transcriptional responses during their interaction

The principal component analyses (PCA) revealed that in nectarine, PC1 and PC2 (89% cumulative variance) clearly separated the samples based on their developmental stage and infection status (Fig. 2a). Notably, at both development stages, 14 hpi was the time point when the inoculated samples appeared to experience a significant change in their expression profiles compared to the controls. These results demonstrate that early time points are critical for dictating the outcome of the interaction. For *M. laxa*, PC1 (53%) distinguished the samples based on the fruit developmental stage, while PC2 (16%) mainly divided the samples between early and late inoculation time points (Fig. 2b). In immature fruit, there was an evident switch in the pathogen's transcriptional profile after 14 hpi, coinciding with the decrease in fungal biomass, and then continued to change up to 48 hpi. In mature fruit, *M. laxa* showed a change in gene expression between 6 and 14 hpi, when disease symptoms were first noticed on the fruit surface. Then, between 14 and 24 hpi, the pathogen altered its gene expression in mature fruit once again and retained most of these changes up to 48 hpi. Remarkably, the expression patterns of *M. laxa* at late time

points of infection were highly divergent when infecting immature and mature tissues, suggesting that the pathogen utilizes different survival or infection mechanisms depending on the host developmental stage.

A differential gene expression (DE) analysis was performed to determine the responses of immature and mature fruit to *M. laxa*, and to identify specific strategies used by the pathogen at specific times of infection. Nectarine DE genes (DEGs) were identified in comparisons between inoculated and control fruit for each maturity stage and time point (Fig. 2c; Suppl. Table S3). A total of 4,005 DEGs were detected in immature fruit across all time points, and of these the majority (63.60%) were up-regulated in inoculated tissues. In immature fruit, the number of DEGs (up- and down-regulated) progressively increased over time and peaked at 24 hpi; then, the changes in gene expression appeared to reach a slightly lower plateau at 48 hpi. Mature fruit displayed a stronger transcriptional response to *M. laxa* infection since a total of 13,855 DEGs (3.5-fold that from immature fruit) were detected at early and late time points. The number of DEGs in mature fruit continuously increased from 6 hpi to 48 hpi, indicating that the host tissues were undergoing a large transcriptional reprograming as the disease progressed.

Monilinia laxa DEGs were detected by comparing the expression profiles of the fungus at each time point against 6 hpi for immature and mature fruit, respectively (Fig. 2d; Suppl. Table S4). These comparisons allowed us to depict how the pathogen modified its transcriptional response based on the initial time point of the interaction when gene expression profiles of *M. laxa* were similar between immature and mature fruit (Fig. 2b). A total of 3,160 DEGs (*P-adj* ≤ 0.05) were detected, with 895 DEGs identified in immature fruit and 2,842 in mature fruit. A closer inspection of these DEGs corroborated the divergence observed in the PCA at latter time points (Fig. 2d). For example, the largest group of *M. laxa* unique DEGs consisted of down-regulated genes in mature tissue at 48 hpi, followed by the up-regulated ones in the same conditions. The DE data was further validated by RT-qPCR using 8 nectarine (r = 0.892, P = 2.2 x 10^{-16}) and 8 *M. laxa* (r = 0.915, P = 2.2 x 10^{-16}) DEGs as shown in Suppl. Table S5.

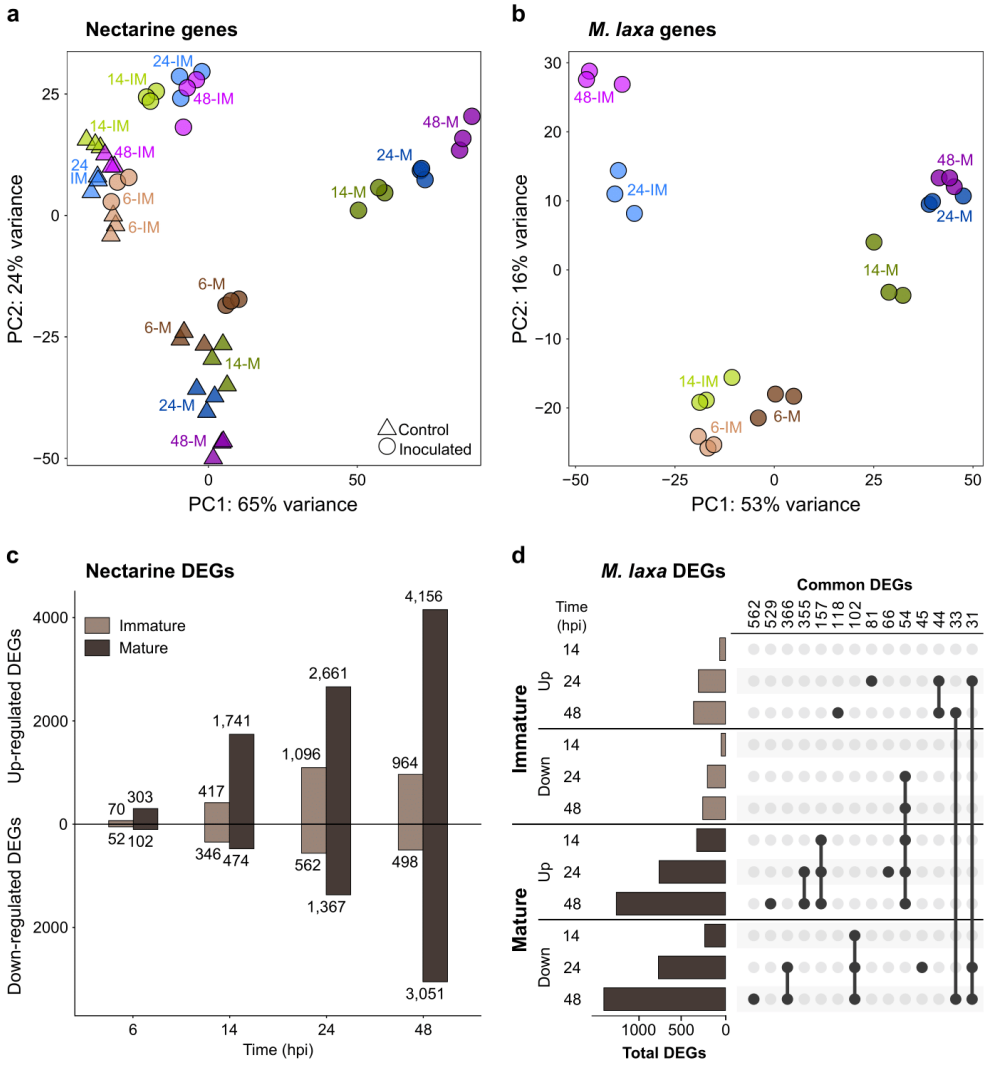

Figure 2.* M. laxa *and nectarine gene expression profiles. a, b) *Patterns of gene expression represented by Principal Component Analysis (PCA) plots of normalized count matrices for nectarine (a) and* M. laxa *(b), generated by DESeq2 through Differential Expression Analysis for both control (△) and inoculated tissue (◯). Labels indicate the time point (6, 14, 24 and 48 hpi) in both immature (IM) and mature (M) stage.* ***c)*** *Amount of nectarine differentially expressed genes (DEGs) as a result of pairwise comparison of inoculated vs control tissue obtained in DESeq2 (*P-adj *value ≤ 0.05). The upper part shows the up-regulated DEGs and the lower, the down-regulated ones, of all the 4 time points analyzed for the immature (light brown) and the mature (dark brown). The number of DEGs in each set are shown.* ***d)*** *Amount of* M. laxa *DEGs obtained through pairwise comparisons between 14, 24 and 48 hpi compared to 6 hpi in both immature (medium brown) and mature (dark brown) tissue. The highest groups of DEGs number in each set are indicated. Dots and lines represent the common DEGs that were found between time points in each stage.*

Susceptible mature fruit display a stronger transcriptional response to *M. laxa* infection than resistant immature fruit

To study host metabolic pathways altered during *M. laxa* progression, we performed a functional enrichment analysis for KEGG terms in the up-regulated nectarine DEGs at each time point for immature and mature fruit (Suppl. Table S3). Figure 3a depicts KEGG terms that were significantly enriched (*P-adj* ≤ 0.05) in at least 4 out of the 8 comparisons (i.e., between mature and immature tissues and the 4 time points). In immature fruit, enriched pathways were more evident at or after 24 hpi. In contrast, multiple pathways were enriched in mature fruit, as shown by early time points, which suggests an overall activation of stress responses associated with biotic challenge and tissue breakdown. These time-dependent responses to *M. laxa* were also evident when quantifying the number of DEGs for enriched categories related to plant defense (Fig. 3b), which confirmed that immature fruit had the highest gene expression induction at 24 hpi, and that mature fruit had a larger number of genes induced than immature fruit as early as 6 hpi. DEGs related to the plant-pathogen interaction pathway (e.g., *CERK1*, *PTI1*, *MAP2K1*, *WRKY33*) were largely absent from the immature fruit response, with the exception of 24 hpi, but were quite abundant in the mature fruit response starting at 14 hpi (Suppl. Table S3). Hormone signaling was enriched early in fruit at both developmental stages, though it appeared to become less relevant in immature fruit at 48 hpi. Cysteine and methionine metabolism and α-linolenic acid metabolism pathways, associated with ethylene (ET) biosynthesis and jasmonic acid (JA) biosynthesis, respectively, were enriched in both immature and mature fruit, though more prominently in the latter. Pathways related to the biosynthesis of terpenoids was also found to be enriched at early time points in immature (14 hpi) and mature fruit (6 hpi), but their enrichment was higher in immature than mature tissue. Other pathways that appeared to be relevant for nectarine responses against *M. laxa* included the phenylpropanoid and glutathione metabolism, which were highly induced in the mature fruit, likely utilized as antioxidants.

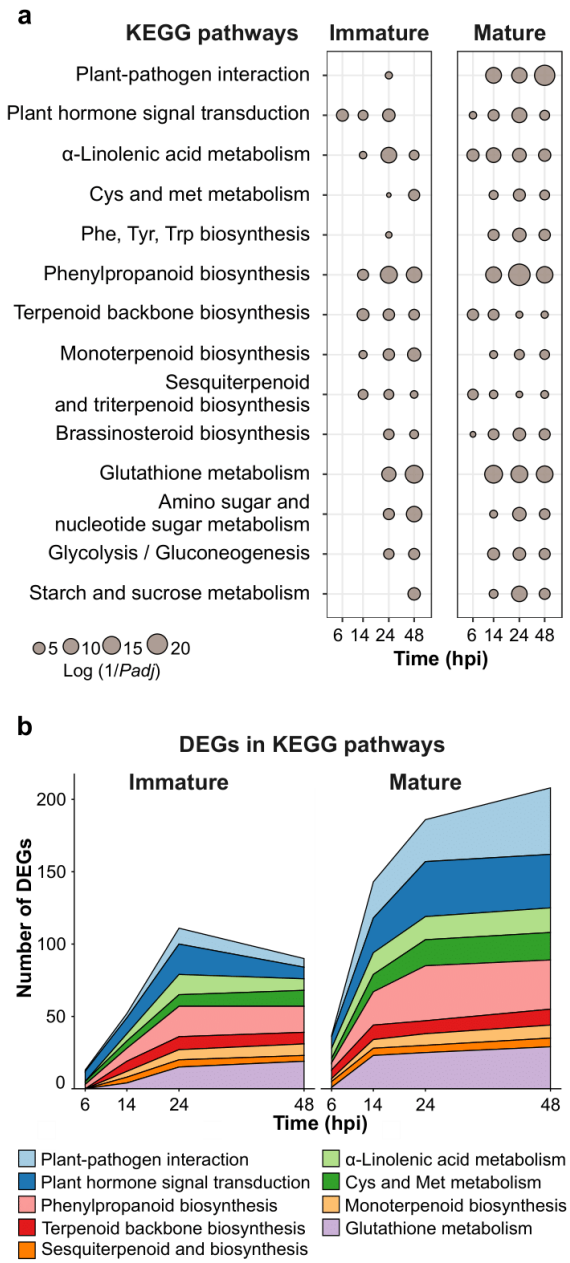

Figure 3. KEGG enrichments of up-regulated genes in nectarine. a*) Metabolic pathways from the KEGG database that were found in at least half of the 8 comparisons obtained in the differential expression analyses (inoculated vs control). The dot size represents the log of the inverted* P-adj *value obtained in the KEGG enrichment analyses along all the time points in both stages (*P-adj *value ≤ 0.05) (Suppl. Table S7).* ***b****) The magnitude of the fruit response in terms of number of DEGs that have KEGG annotations for the selected metabolic pathways in both stages through time. Each color represents one different KEGG pathway.*

Ethylene and jasmonic acid pathways are activated in response to *M. laxa* inoculations of nectarine

Given the enrichment of genes involved in plant hormone signaling transduction during early infection and the activation of methionine and α-linolenic metabolism in both fruit tissues across time, a targeted analysis of ET and JA pathways was conducted. The transcriptional activation of JA biosynthesis was evident in immature and mature fruit, with special emphasis in the induction of multiple genes encoding the initial biosynthetic steps (Fig. 4a), from lipoxygenase (*LOX*) to 12-oxophytodienoic acid reductase (*OPR3*). Later steps of the biosynthesis pathway were only moderately activated in both tissues. In mature tissues at 48 hpi, a down-regulation of the JA-amino synthetase (*JAR1*) gene was observed, involved in the production of the active form of JA, and of the homolog of the JA receptor coronatine-insensitive protein 1 (*COI1*). Two out of the five paralogs of the signaling repressor JA ZIM domain (*JAZ*) appeared to be activated in immature and mature tissues at multiple time points. The three paralogs encoding the transcriptional activator of JA responses, *MYC2*, were strongly induced in mature fruit after 14 hpi and up-regulated in immature fruit only at 14 hpi and 24 hpi. In fact, the *MYC2* gene expression level of the third paralog (*Prupe.5G130700.1*) was significantly higher in inoculated immature than mature tissue, but then, its expression was significantly higher in mature than immature tissue at both 24 and 48 hpi (Suppl. Table S3).

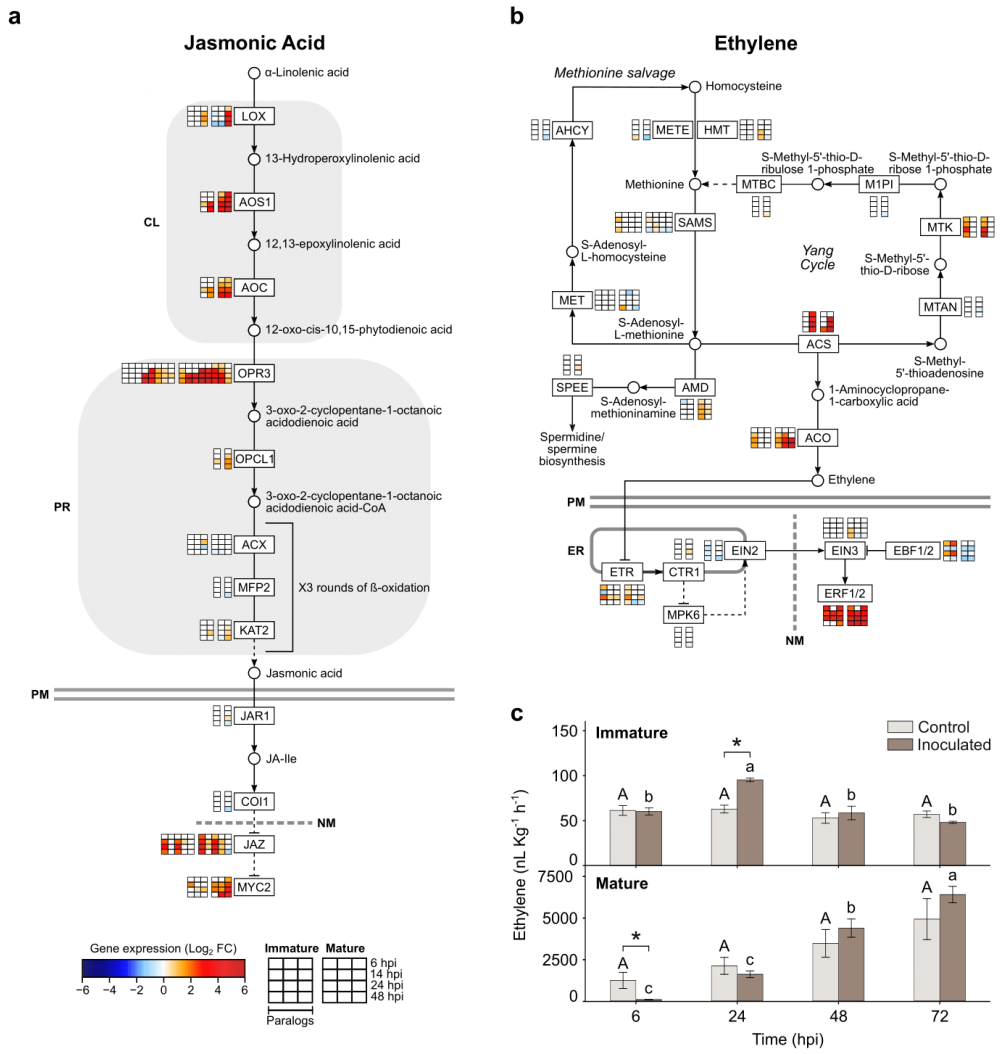

***Figure 4. Activation of jasmonic and ethylene pathways in nectarine fruit after inoculations with* M. laxa.** *a, b) Jasmonic acid and ethylene pathways are shown with substrates (◯) and enzymes (boxes) and include 32 and 41 DEGs for JA and ET, respectively. The scale color of the heat maps represents the intensity of the significant expression changes (Log2FC), which resulted from the pairwise comparison of inoculated vs control samples (*P-adj *value ≤ 0.05). Paralogs of each analyzed enzyme are represented in columns and grouped by their expression in immature (left boxes) and mature (right boxes) at each time point (hpi). Dashed lines indicated that some steps have been omitted. PM, plasmatic membrane; NM, nucleus membrane; ER, endoplasmic reticulum. Enzyme abbreviations and lists of paralogs genes for each protein are provided (Suppl. Table S7). c) Ethylene measurements of the nectarine - M. laxa pathosystem through time. Values represent the mean (n = 4) and the vertical bars, the standard error. Symbols (*) indicate significant differences according to Student's T-test (P ≤ 0.05). Uppercase and lowercase letters indicate significance differences (P ≤ 0.05, Tukey's test) in control and inoculated tissues, respectively.*

The steps committed to ET biosynthesis catalyzed by the 1-aminocyclopropane-1-carboxylate synthase (*ACS*) and the 1-aminocyclopropane- 1-carboxylate oxidase (*ACO*) genes were highly induced in response *to M. laxa* inoculations, particularly in mature fruit (Fig. 4b). The *ACS2* (*Prupe.5G106200.1*) and the *ACO3* (*Prupe.7G212000.1*) genes showed the highest up-regulation (*ACS2* in both tissues and *ACO3* in mature tissue). Ethylene signal transduction elements (*ETR*, *CTR*, *EIN2*, and *EIN3*) showed only moderate changes in gene expression in response to the pathogen. Interestingly, although the negative regulator *EBF1/2* was down-regulated at 14 and 48 hpi in both tissues, it was highly up-regulated in immature tissue at 6 and 24 hpi. However, all three paralogs of the ET Response Factor 1/2 (*ERF1/2*), which control multiple ET responses and are a point of signal integration for JA and ET signal transduction, were highly up-regulated in both tissues. The *ERF1/2* gene expression level of the second paralog (*Prupe.6G348700.1*) was significantly higher in mature inoculated than immature inoculated fruit at 14 hpi (data not shown).

Additionally, the ET produced by *M. laxa*-inoculated and control fruit was measured to complement the transcriptional data (Fig. 4c). Control nectarines followed the ET pattern of a climacteric fruit; low and steady levels of ET in immature fruit and high and significantly increasing levels in mature fruit until ripening. However, in inoculated immature fruit, ET production significantly peaked at 24 hpi, corresponding to the peak of transcriptional responses in this tissue, before returning to levels equivalent to the control fruit. In inoculated mature fruit, the ET production was significantly lower than control fruit at 6 hpi but then significantly increased. These results suggest that nectarine was performing a tightly regulated response of ET.

Monilinia laxa adapts its infection strategies according to the host environment conditions

To determine which fungal genes and functions are biologically relevant during *M. laxa* interactions with nectarine, we performed a functional analysis of the pathogen transcriptome. First, a total of 9,581 transcripts were *de novo* annotated for multiple functional categories, including carbohydrate-active enzymes (CAZymes), fungal peroxidases (fPox), genes involved in pathogen-host interactions (PHI), membrane transport proteins (TCBD), and proteins with signal peptides (SignalP), among others (Fig. 5a; Suppl. Table S4). Then, an enrichment analysis (Fisher, $P\text{-}adj \leq 0.05$) of these large functional categories in the up-regulated DEGs across infection was performed to obtain a general picture of specific gene categories induced by the pathogen in immature and mature fruit (Fig. 5b). In immature fruit, these large categories were

enriched in *M. laxa* up-regulated DEGs at least at one time point when compared to 6 hpi. Particularly at 24 hpi, a significant abundance of CAZymes and PHI genes was observed. Fungal peroxidases were only significantly enriched in immature fruit at 48 hpi. In contrast, enrichment of CAZymes and fungal peroxidases was not observed at any time point in mature tissues. Genes in involved in pathogen-host interactions and membrane transport remained enriched at relatively even levels from 14 to 48 hpi in mature fruit.

We identified GO terms related to pathogenicity, virulence, and fungal growth among the up-regulated DEGs for each host developmental stage (Fig. 5c). Among this subset of biologically relevant GO terms, 3-fold more up-regulated DEGs were detected when *M. laxa* was inoculated in mature fruit compared to immature fruit. Particularly, the number of *M. laxa* up-regulated DEGs in immature tissue increased progressively until 24 hpi and then decreased slightly at 48 hpi; whereas in mature tissue, the up-regulated DEGs increased along with infection time. Notably, these gene expression patterns resembled the transcriptional response of the host for each developmental stage (Fig. 3b). In both stages, *M. laxa* induced a high number of DEGs related to oxidative-reduction processes and transmembrane transport, although genes involved in protein translation and proteolysis were only abundantly expressed in mature tissue. However, genes involved in response to oxidative stress were mainly expressed in immature at 48 hpi, together with the enrichment of fungal peroxidases at this time point (Fig. 5b).

Lastly, enrichments of Pfam domains (*P-adj* ≤ 0.05) were also carried out using the *M. laxa* up-regulated DEGs (Fig. 5d and Suppl. Table S4). In agreement with previous results, Pfam categories were mainly enriched at 24 hpi in immature fruit, with the exception of proteins containing the fungal pathogenesis-related CFEM domain (PF05730), which were uniquely enriched earlier at 14 hpi. Additionally, Pfam domains related to fungal membrane transport (PF07690 and PF00083) were largely prominent in immature fruit, especially at 24 hpi, where up to 53 genes were induced. Less significantly enriched, fungal glycosyl hydrolases, dehydrogenases (DH) and catalases were found at 48 hpi in immature tissues.

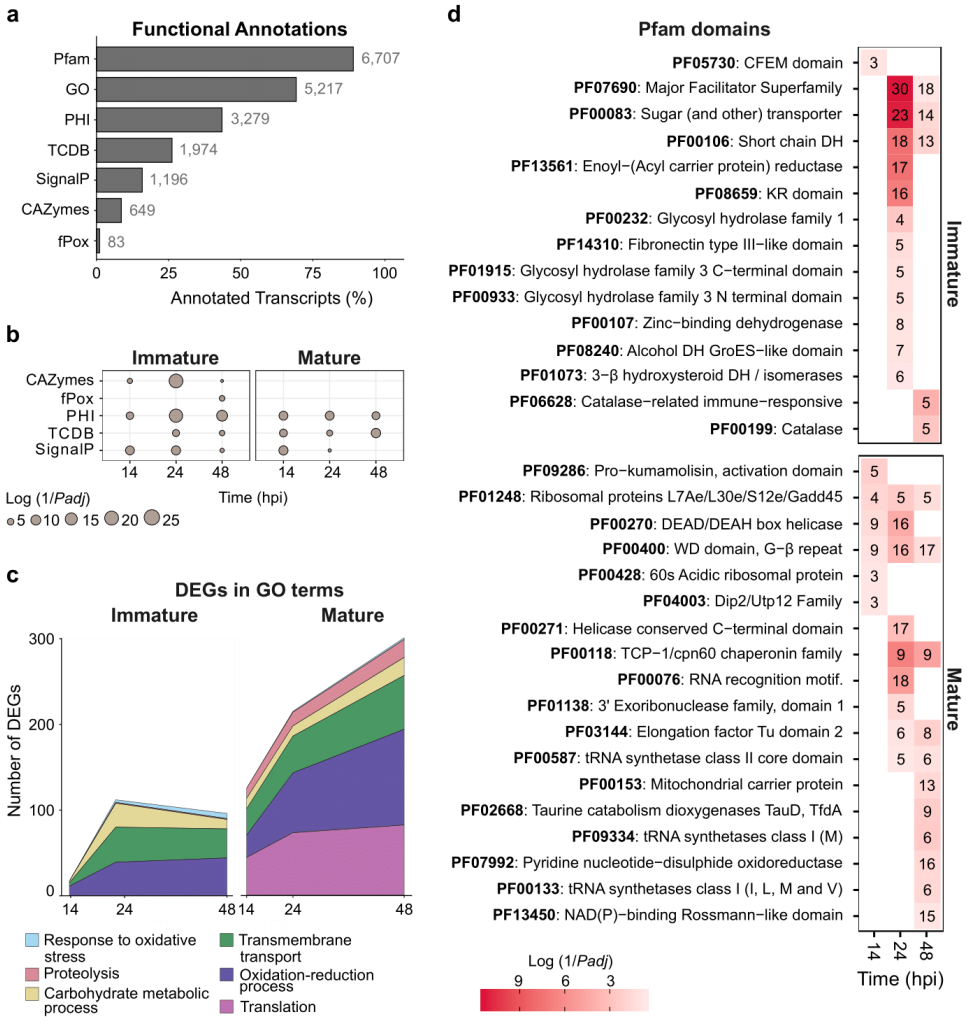

Figure 5. Summary of functional annotations and functional enrichments of* M. laxa.** ***a*) De novo functional annotations in all* M. laxa *transcripts obtained (9,581) (Suppl. table S8). Each category is represented by the proportion (%) of annotated transcripts across* M. laxa *transcriptome and the specific number of DEGs next to the bar. Pfam, Protein Family database; GO, Gene Ontology; PHI, Pathogen-Host Interaction; TCDB, Transporter Classification Database; SignalP, Presence of secretion signal peptides; CAZy, Carbohydrate-Active enzyme; fPox, fungal peroxidases.* ***b****) Enrichments of functional categories across all time points in both tissues. Pairwise comparisons were performed between 14, 24 or 48 hpi compared to 6 hpi, for each maturation stage. The dot size represents their significance (Log of the inverted P-adj value) obtained in Fisher tests.* ***c****) The magnitude of M. laxa response in terms of number of DEGs (P-adj ≤ 0.05) that have GO terms for some relevant terms in both stages along time. Each color represents one different GO term.* ***d****) Pfam enrichments of M. laxa genes that were overexpressed in 14, 24 and/or 48 hpi compared to 6 hpi, for each stage, obtained in DESeq2 (P-adj value ≤ 0.05) (Suppl. Table S8). The color scale of the heat maps represents the log of the inverted P-adj value. Number of DEGs in each Pfam are also shown.*

The number of enriched Pfam domains among *M. laxa* up-regulated DEGs in mature fruit, such as those related to transcription and translation (e.g., PF03144 and PF00587), increased throughout disease progression (Fig. 5d). However, other relevant domains, such as some related to proteolysis activity (e.g., PF09286 Pro−kumamolisin domain), uniquely peaked at 14 hpi. Notably, up-regulated DEGs annotated as ribosomal proteins and transcriptional factors (PF01248 and PF00400) involved in growth and cell cycle control were prevalent throughout infection of mature fruit. Later infection time points exhibited enrichments of protein domains belonging to membrane transport (e.g., mitochondrial carrier protein) and redox functions (e.g., an oxidoreductase).

Highly induced *M. laxa* genes during inoculation provide possible targets for disease control

To identify potential target genes for the control of *M. laxa*, a closer examination was conducted of the most highly *M. laxa* up-regulated DEGs (i.e., largest Log_2FC) from all time points and tissue comparisons (Table 1; Supplemental Table S4). The top five *M. laxa*-induced DEGs in immature and mature fruit were unique between the tissue types, reinforcing the evidence that the pathogen displays a different behavior according to the developmental stage of the host. Strongly induced DEGs at 14 hpi unique to early infections of immature fruit included fungal phosphate transporters, phospholipases, and oxidoreductases. A member of the glycosidase hydrolase family 31 (*Monilinia_056600*) was highly expressed at 24 hpi in immature fruit, alongside a transmembrane fructose transporter (*Monilinia_074660*) and histidine phosphatase (*Monilinia_002270*). The highest induced DEGs in immature fruit were detected at 48 hpi and corresponded to an oxidoreductase gene (*Monilinia_010850*), a homolog of the alcohol oxidase (*OAX1*) from *Cladosporium fulvum,* and the same transmembrane fructose transporter (*Monilinia_074660*) already found at 24 hpi. Interestingly, *M. laxa* DEGs with fungal peroxidase annotations, a catalase (*Monilinia_039930*) and a haloperoxidase (*Monilinia_049900*), were only detected at 48 hpi in immature fruit.

In mature fruit, a single protease gene (*Monilinia_077490*) was the highest up-regulated *M. laxa* DEG at all time points. Two polygalacturonases (Glycoside hydrolase family 28) were among the largest induced DEGs during infections of mature fruit; *Monilinia_000560* was highly up-regulated at 14 hpi whereas *Monilinia_041700* was highly expressed at 24 and 48 hpi. Another CAZyme (Glycoside hydrolase family 71, *Monilinia_037020*) was also highly enriched at 14 and 24 hpi. In mature tissue, transporters and hormone-related genes were among the highest expressed DEGs. An

amino acid transporter (*Monilinia_015240*) was significantly expressed at 14 hpi while a tryptophan 2-monooxygenase (*Monilinia_013220*) was induced at 48 hpi, known to be involved in virulence in another pathosystem (Cerboneschi et al., 2016). Altogether, these results suggest that targeting of specific genes involved in response to oxidative stress, nutrient transport, and carbohydrate catabolism may reduce quiescent infections, while specific proteolytic genes and additional CAZymes may help inhibit or reduce the severity of disease in susceptible fruit.

***Table 1. Top up-regulated genes of* M. laxa.** *Represented genes are the 5 most up-regulated genes, obtained in the pairwise comparisons generated by DESeq2. Values correspond to the expression (log_2FC) of each time point (14, 24 and 48 hpi) compared to 6 hpi of both immature and mature fruit. Accession number of genes and selected functional annotations for each gene are also shown. TCDB, Transporter Classification Database; PHI, Pathogen-Host Interaction; Pfam, Protein Family database; SignalP, Presence of secretion signal peptides; CAZy, Carbohydrate-Active enzyme; GO, Gene Ontology; fPox, fungal peroxidases.*

Accession	Log_2FC	Selected Functional Annotations
Immature – 14 HPI		
Monilinia_058830	5.90	**TCDB**: 2.A.1.9.2 (Inorganic phosphate transporter) | **PHI**: *PHO84* (*Cryptococcus neoformans*, reduced virulence)
Monilinia_028560	5.37	**PFAM**: PF04185.14 (Phosphoesterase family) | **PHI**: *plcC* (*Mycobacterium tuberculosis*, unaffected pathogenicity) | **SignalP**: 0.811
Monilinia_060140	3.51	**SignalP**: 0.686
Monilinia_079910	3.12	**PFAM**: PF01633.20 (Choline/ethanolamine kinase)
Monilinia_009770	2.99	**PFAM**: PF00264.20 (Common central domain of tyrosinase) | **SignalP**: 0.667
Immature – 24 HPI		
Monilinia_056600	8.45	**CAZy**: GH31 | **PHI**: *Gls2* (*Magnaporthe oryzae*, reduced virulence)
Monilinia_074660	8.09	**TCDB**: 2.A.1.1.69 (Sugar/H+ symporter) | **PHI:** *FRT1* (*Botrytis cinerea*, unaffected pathogenicity)
Monilinia_002270	7.72	**PFAM**: PF00300.22 (Histidine phosphatase superfamily (branch 1) | **PHI**: *FGSG_02549* (*Fusarium graminearum*, reduced virulence) | **SignalP**: 0.897
Monilinia_033100	7.68	**TCDB**: 2.A.1.1.119 (Putative uncharacterized protein *An14g04280*) | **PHI**: *MoST1* (*Magnaporthe oryzae*, unaffected pathogenicity)
Monilinia_016250	7.42	**TCDB**: 2.A.1.7.11 (Glucose/galactose transporter) | **PHI**: *PD0681* (*Xylella fastidiosa*, increased virulence) | **SignalP**: 0.632
Immature – 48 HPI		
Monilinia_010850	9.53	**GO**: GO:0055114 (oxidation-reduction process) | **PFAM**: PF00732.19 (GMC oxidoreductase) **CAZy**: AA3-3 | **PHI**: *AOX1* (*Passalora fulva*, reduced virulence)
Monilinia_074660	8.77	**TCDB**: 2.A.1.1.69 (Sugar/H+ symporter) | **PHI:** *FRT1* (*Botrytis cinerea*, unaffected pathogenicity)
Monilinia_022560	7.92	**SignalP**: 0.844
Monilinia_039930	7.91	**fPox**: Catalase | **PHI**: *CAT1* (*Candida albicans*, reduced virulence)
Monilinia_034450	7.79	**CAZy:** GH3 | **PHI**: Avenacinase (*Gaeumannomyces graminis*, loss of pathogenicity) **SignalP**: 0.718
Mature – 14 HPI		
Monilinia_077490	9.43	**GO**: GO:0006508 (proteolysis) | **PFAM**: PF01828.17 (Peptidase A4 family) | **SignalP**: 0.64
Monilinia_037020	6.77	**CAZy**: GH71 | **SignalP**: 0.886
Monilinia_015240	6.54	**TCDB**: 2.A.3.4.3 (GABA-specific permease) | **PHI**: *bcaP* (*Staphylococcus aureus*, unaffected pathogenicity / reduced virulence)
Monilinia_000560	6.10	**CAZy**: GH28 | **PHI**: *BcPG2* (*Botrytis cinerea*, reduced virulence) | **SignalP**: 0.837
Monilinia_050850	5.68	**GO**: GO:0006508 (proteolysis) | **PFAM**: PF09286.11 (Pro-kumamolisin, activation domain) **SignalP**: 0.84
Mature – 24 HPI		
Monilinia_077490	8.90	**GO**: GO:0006508 (proteolysis) | **PFAM**: PF01828.17 (Peptidase A4 family) | **SignalP**: 0.64
Monilinia_006190	7.37	**PFAM**: PF00107.26 (Zinc-binding dehydrogenase)
Monilinia_041700	7.18	**CAZy**: GH28 | **PHI**: *PGX1* (*Cochliobolus carbonum*, unaffected pathogenicity) **SignalP**: 0.913
Monilinia_041730	6.89	**TCDB**: 2.A.1.14.38 (Uncharacterized transporter YIL166C) | **PHI**: *GzMyb019* (*Fusarium graminearum*, unaffected pathogenicity)
Monilinia_073540	6.74	**CAZy**: AA7 | **PHI**: *ZEB1* (*Fusarium graminearum*, unaffected pathogenicity) | **SignalP**: 0.778
Mature – 48 HPI		
Monilinia_077490	9.25	**GO**: GO:0006508 (proteolysis) | **PFAM**: PF01828.17 (Peptidase A4 family) | **SignalP**: 0.64
Monilinia_041700	7.99	**CAZy**: GH28 | **PHI**: *PGX1* (*Cochliobolus carbonum*, unaffected pathogenicity) **SignalP**: 0.913
Monilinia_068440	7.64	None
Monilinia_013220	7.46	**GO**: GO:0055114 (oxidation-reduction process) | **PFAM**: PF00743.19 (Flavin-binding monooxygenase-like) | **PHI**: *iaaM* (*Pseudomonas savastanoi*, reduced virulence)
Monilinia_041730	7.42	**TCDB**: 2.A.1.14.38 (Uncharacterized transporter YIL166C) | **PHI**: *GzMyb019* (*Fusarium graminearum*, unaffected pathogenicity)

Discussion

The first line of plant defense that *M. laxa* has to overcome is the constitutive physical (e.g., cuticle and plant cell wall) and chemical barriers (e.g., preformed antifungal compounds) present in the fruit surface. The developmental process from immature to mature fruit is characterized by physical and chemical changes in fruit firmness leading to softening at the onset of ripening (Brummell et al., 2004). In fact, the flesh firmness of immature fruit was higher than mature fruit (Suppl. Table S1). *Monilinia laxa* appeared to produce more CWDE (e.g., CAZymes) in immature fruit, which suggests that the pathogen could be trying harder to overcome the host cell walls in these tissues. Nevertheless, the immature tissue had no visible disease symptoms. Other alterations occurring during fruit development include changes in plant cuticle, sugar accumulation, volatile compounds and secondary metabolites synthesis, which have been reviewed as promoting susceptibility to pathogens in ripening fruit. Hence, higher soluble solids content and lower titratable acidity on mature fruit (Suppl. Table S1) could favor pathogen colonization.

Plant-pathogen interactions take place when pathogen-associated molecular patterns (PAMP) are recognized by the plant's pattern recognition receptors (Zipfel, 2014), which ultimately triggers a defense response known as PAMP-triggered immunity (PTI)(Pandey et al., 2016). The chitin elicitor receptor kinase 1 (*CERK1*) (Kombrink et al., 2011) (*Prupe.3G213100.1*) was up-regulated in the mature tissue at 14 hpi. Also, the expression levels of the transcriptional activator *PTI5* (*Prupe.4G055500.1*) were up to 2.5- and 5-fold higher in mature fruit when compared to immature fruit, at 24 and 48 hpi, respectively. PTI responses can be suppressed by effector proteins secreted by the pathogen, which in turn, will elicit effector-triggered immunity (ETI) (Jones and Dangl, 2006). In our pathosystem, proteins with the CFEM domain (Pfam PF05730) and signal peptides were enriched in early infection stage (14 hpi) on immature tissue. Among the annotated genes with the CFEM domain, the *Monilinia_077410* is an homolog of *BcCFEM1* from *B. cinerea*, an effector shared by many *Botrytis* spp. (Valero-Jiménez et al., 2019) and described to be important for its virulence (Zhu et al., 2017). These results suggest that *M. laxa* may secrete some type of effector proteins in immature fruit.

Once the host-pathogen interaction began, both pathogen and host triggered their own transcriptional reprograming. In mature tissue, both nectarine and *M. laxa* abruptly changed their gene expression profile at 14 hpi, coinciding with the ability of the pathogen to grow and macerate the fruit tissues within 14 hours. From 14 hpi

onwards, the pathogen started to penetrate and switched towards an agressive necrotrophic phase, which was retained at later infection times. Functions related to transmembrane transport, oxidation-reduction process and translation were among the most abundant activities in mature fruit, denoting the growth and spread of the pathogen. In contrast, the number of nectarine and *M. laxa* DEGs in immature fruit remained somewhat steady through infection time, even when fungal biomass peaked at 24 hpi. Overall, these findings suggest that inoculated mature nectarines displayed an earlier and broader response to *M. laxa* than immature ones, likely due to the faster pathogen growth and virulence mechanisms activation in these tissues.

Both PTI and ETI are able to induce the host hormone signaling transduction pathway (Pandey et al., 2016) which was found to be enriched starting at 6 hpi in both tissues. Jasmonic acid and ET are known to be involved in defense responses against necrotrophs, such as mediating the host's responses against them (McDowell and Dangl, 2000), but ET is also required for fruit ripening and senescence processes, which are conducive to disease susceptibility (Van Der Ent and Pieterse, 2012; Blanco-Ulate et al., 2013; Pandey et al., 2016). Jasmonic acid can also mediate the disease resistance of fruit, by increasing the fruit antioxidant capacity (Zhu and Tian, 2012) but some fungi are able to hijack the JA signaling pathway to cause disease (Zhang et al., 2017). Although the early steps of JA biosynthesis were highly induced upon *M. laxa* inoculation, down-regulation of receptor genes was observed in mature fruit inoculated with *M. laxa* when compared to controls. These findings suggest that *M. laxa* could be somehow blocking the JA signaling pathway, although the mechanisms involved are unknown.

Ethylene biosynthesis increases during ripening of climacteric fruit (Oetiker and Yang, 1995), such as nectarines. In our study, the control immature fruit (system 1, associated with fruit development), produced basal ethylene levels whereas ethylene production in control mature fruit (system 2, involved in ripening) increased through time after harvest. In inoculated immature fruit, there was a significant peak of ET production as compared to the control at 24 hpi. This discrete induction of ET can be part of the fruit defense responses against *M. laxa*. Alternatively, the pathogen could be inducing fruit ethylene biosynthesis in immature fruit to accelerate ripening, in an attempt to promote fruit physicochemical changes that are conducive to disease (Blanco-Ulate et al., 2013). Along this line, *ACS2* and *ACO1*, involved in system 2 ET production (Tadiello et al., 2016), were overexpressed in inoculated immature tissues. Previous studies have reported on a similar modulation of ET biosynthesis by the pathogen (Baró-Montel et

al., 2019b). However, after 24 hpi, ethylene levels in inoculated immature fruit fell to control levels, and the fruit remained resistant. This may be in part due to the upregulation of the ethylene signaling inhibitors *EBF1/2*, which could mitigate the ethylene-induced ripening processes that contribute to susceptibility. In contrast, in inoculated mature fruit, ET production and signal transduction were lower at 6 hpi in inoculated fruit but grater from 24 hpi onward, following the autocatalytic system 2 ethylene biosynthesis. Overall, the results indicate the ability of *M. laxa* to differentially alter ET production to promote susceptibility and, in turn, the ability for immature fruit, but not mature fruit, to mitigate the consequences of this induction (Van Der Ent and Pieterse, 2012).

The above observations indicate that although *M. laxa* was deploying some strategies to infect the immature tissues, it was not able to overcome either the surface or the active defense responses deployed by the immature fruit. *Monilinia laxa* remained on the immature tissue, increasing its biomass and multiplying on the surface, until 14 hpi when it ceased to grow. It is known that *Monilinia* spp. can remain quiescent on fruit surfaces (Luo et al., 2005) and that they can employ appresoria as resting structures on immature nectarines(Lee and Bostock, 2006). After 14 hpi, *M. laxa* biomass and reads started to decrease, switching its transcriptional machinery by employing different sets of genes in order to deploy different strategies to survive on the fruit's surface. Some results point out that *M. laxa* could either be starting a quiescence period or moving towards an autolysis process, breaking cells to feed on its remains. Another possibility is that the remaining *M. laxa* cells on immature fruit were being attacked by the host defenses. This is supported by the expression of a *M. laxa* genes associated with response to oxidative stress at late time points, such as catalases, previously reported in detoxification during an infection of tomato leaves by *B. cinerea* (Schouten et al., 2002). Thus, it is likely that immature fruit were generating reactive oxygen species (ROS) during the interaction through an oxidative burst (Torres et al., 2006) to kill the pathogen.

Monilinia laxa could also be producing ROS for its development and as a pathogenicity mechanism to damage the host tissue. Particularly, the NADPH oxidase (Nox) complex is involved in both fungal ROS production and its use in sclerotia development and virulence (Kim et al., 2011; Li et al., 2016). Some genes encoding the Nox regulator R (NoxR) (e.g., *Monilinia_061250* and *Monilinia_079620*) were found to be upregulated at 24 hpi in both mature and immature tissue. At later stages, a highly induced alcohol oxidase expressed in immature tissue at 48 hpi could be another ROS

producer, previously described as an alternative ROS production system. Lin et al. (2019) demonstrated that *AOX1* was involved in pathogenicity and oxygen stress responses in *B. cinerea*. Concomitantly, nectarine counteracted the pathogen oxidative burst by expressing genes of antioxidant metabolism compounds such as glutathione and redox-related amino acids (Cys and Met).

Plant secondary metabolites such as terpenoids have been described to protect the fruit under biotic and abiotic stresses (Bartwal et al., 2013), although their role can be tissue-dependent. Overall, the enrichment of genes involved in secondary metabolite biosynthesis was higher in resistant immature than susceptible mature tissue, which suggests that either the host was producing terpenoids in the resistant immature tissue to prevent the attack or that *M. laxa* was inhibiting its biosynthesis on mature tissue. *M. laxa* could also be able to degrade and transform terpenoids as described for *B. cinerea* (Collado et al., 2007). The phenylpropanoid metabolism is also triggered in response to brown rot. In both immature and mature fruit, from 14 hpi to 48 hpi, phenylpropanoid-related pathways were highly induced. While on the immature tissue these pathways could be involved in reinforcing the cell wall through lignin production (Veloso and van Kan, 2018), the role in the mature fruit could be more focused on the detoxification of fungal ROS production (Bartwal et al., 2013). Nevertheless, these hypotheses need to be further tested.

On mature nectarines, *M. laxa* deployed other virulence factors in addition to ROS production and scavenging. The pathogen expressed up-regulated DEGs related to proteolytic activity, containing domains such as Pro−kumamolisin domain (PF09286). The list of genes summarized in Table 1 could be putative pathogen target genes as they were expressed only when *M. laxa* infected the mature tissues, as none of the top 5 up-regulated genes in mature tissue was found in the immature fruit. For instance, the highest expressed protease (*Monilinia_077490*) at in all time points is a homolog of a non-aspartyl protease (*ACP1*) found during pathogenesis in *Sclerotinia sclerotiorum* (Poussereau et al., 2001). Cell wall degrading enzymes are commonly produced by necrotrophic fungi as virulence factors and their secretion by *Monilinia* spp. on culture media has been previously reported (Garcia-Benitez et al., 2019). A rhamnogalacturonan hydrolase (Glycoside hydrolase family 28, *Monilinia_041700*), which was highly expressed at both 24 and 48 hpi, was already described as a putative virulence factor in *M. laxa* infecting peaches (Baró-Montel et al., 2019c).

Current information regarding the strategies utilized by either *Monilinia* spp. or stone fruit or during their interaction is mainly focused on specific metabolic pathways or

actions developed by one of the two players. As a novel feature of the present research, we demonstrated the synchronized responses from nectarine and *M. laxa*, by utilizing a resistant immature and susceptible mature fruit throughout a course of infection. Future research studies should be focused on delving into the host defense system for the ongoing development of nectarine cultivars with increased resistance to brown rot, as well as conducting in-depth fungal studies to alter the ability of *M. laxa* to cause disease.

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Conflict of interests

All authors declare that they have no conflicts of interest.

Contributions

JU, RT, AC and BBU conceived and designed the experiments. NV, RT, NT and MBL led the fruit inoculations, sampling, pathological studies, and ethylene measurements. NV and SSP did the RNA extractions, while CJS, SDMP and MBL prepared the cDNA libraries. CJS and BBU performed the bioinformatics analysis, differential expression analysis and functional analysis enrichments. SSP, NT and SDMP did the RNAseq validation by qPCR. MBL, CJS, NV, RT and BBU wrote the article and all remaining authors contributed in improving the final version of the manuscript.

References

De Miccolis Angelini, R. M., Abate, D., Rotolo, C., Gerin, D., Pollastro, S., and Faretra, F. (2018). De novo assembly and comparative transcriptome analysis of *Monilinia fructicola*, *Monilinia laxa* and *Monilinia fructigena*, the causal agents of brown rot on stone fruits. 19, 1–21. doi:10.1186/s12864-018-4817-4.

Baró-Montel, N., Torres, R., Casals, C., Teixidó, N., Segarra, J., and Usall, J. (2019a). Developing a methodology for identifying brown rot resistance in stone fruit. *Eur. J. Plant Pathol.* 154, 287–303. doi:10.1007/s10658-018-01655-1.

Baró-Montel, N., Vall-llaura, N., Giné-Bordonaba, J., Usall, J., Serrano-Prieto, S., Teixidó, N., et al. (2019b). Double-sided battle: The role of ethylene during *Monilinia* spp. infection in peach at different phenological stages. *Plant Physiol. Biochem.* 144, 324–333. doi:10.1016/j.plaphy.2019.09.048.

Baró-Montel, N., Vall-llaura, N., Usall, J., Teixidó, N., Naranjo-Ortíz, M. A., Gabaldón, T., et al. (2019c). Pectin methyl esterases and rhamnogalacturonan hydrolases: weapons for successful *Monilinia laxa* infection in stone fruit? *Plant Pathol.* 68, 1381–93. doi:10.1111/ppa.13039.

Bartwal, A., Mall, R., Lohani, P., Guru, S. K., and Arora, S. (2013). Role of secondary metabolites and brassinosteroids in plant defense against environmental stresses. *J. Plant Growth Regul.* 32, 216–232. doi:10.1007/s00344-012-9272-x.

Blanco-Ulate, B., Vincenti, E., Powell, A. L. T., and Cantu, D. (2013). Tomato transcriptome and mutant analyses suggest a role for plant stress hormones in the interaction between fruit and *Botrytis cinerea*. *Front. Plant Sci.* 4, 1–16. doi:10.3389/fpls.2013.00142.

Bolger, A. M., Lohse, M., and Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114–2120. doi:10.1093/bioinformatics/btu170.

Brummell, D. A., Dal Cin, V., Crisosto, C. H., and Labavitch, J. M. (2004). Cell wall metabolism during maturation, ripening and senescence of peach fruit. *J. Exp. Bot.* 55, 2029–2039. doi:10.1093/jxb/erh227.

Byrne, D. H. (2012). "Trends in fruit Breeding," in *Fruit Breeding*, eds. M. L. Badenes and D. H. Byrne (Boston, MA: Springer US), 3–36. doi:10.1007/978-1-4419-0763-9.

Cerboneschi, M., Decorosi, F., Biancalani, C., Ortenzi, M. V., Macconi, S., Giovannetti, L., et al. (2016). Indole-3-acetic acid in plant–pathogen interactions: a key molecule for in planta bacterial virulence and fitness. *Res. Microbiol.* 167, 774–787. doi:10.1016/j.resmic.2016.09.002.

Collado, I. G., Sánchez, A. J. M., and Hanson, J. R. (2007). Fungal terpene metabolites: Biosynthetic relationships and the control of the phytopathogenic fungus *Botrytis cinerea*. *Nat. Prod. Rep.* 24, 674–686. doi:10.1039/b603085h.

Garcia-Benitez, C., Melgarejo, P., De Cal, A., and Fontaniella, B. (2016). Microscopic analyses of latent and visible *Monilinia fructicola* infections in nectarines. *PLoS One* 11, e0160675. doi:10.1371/journal.pone.0160675.

Garcia-Benitez, C., Melgarejo, P., Sandin-España, P., Sevilla-Morán, B., and De Cal, A. (2019). Degrading enzymes and phytotoxins in *Monilinia* spp. *Eur. J. Plant Pathol.* 154, 305–318. doi:10.1007/s10658-018-01657-z.

Gununu, P. R., Munhuweyi, K., Obianom, P. C., and Sivakumar, D. (2019). Assessment of eleven South African peach cultivars for susceptibility to brown rot and blue mould. *Sci. Hortic. (Amsterdam).* 254, 1–6. doi:10.1016/j.scienta.2019.04.067.

Jones, J. D. G., and Dangl, J. L. (2006). The plant immune system. *Nature* 444, 323–329. doi:10.1038/nature05286.

Kim, H. jin, Chen, C., Kabbage, M., and Dickman, M. B. (2011). Identification and characterization of *Sclerotinia sclerotiorum* NADPH oxidases. *Appl. Environ. Microbiol.* 77, 7721–7729. doi:10.1128/AEM.05472-11.

Kombrink, A., Sánchez-Vallet, A., and Thomma, B. P. H. J. (2011). The role of chitin detection in plant-pathogen interactions. *Microbes Infect.* 13, 1168–1176. doi:10.1016/j.micinf.2011.07.010.

Langmead, B., and Slazberg, S. L. (2012). Fast gapped-read alignmnet with Bowtie 2. *Nat. Methods* 9, 357–359. doi:10.1038/nmeth.1923.Fast.

Lee, M.-H., and Bostock, R. M. (2006). Induction, regulation, and role in pathogenesis of appressoria in *Monilinia fructicola*. *Phytopathology* 96, 1072–1080. doi:10.1094/phyto-96-1072.

Lee, M. H., and Bostock, R. M. (2007). Fruit exocarp phenols in relation to quiescence and development of *Monilinia fructicola* infections in *Prunus* spp.: A role for cellular redox? *Phytopathology* 97, 269–277. doi:10.1094/PHYTO-97-3-0269.

Li, H., Zhang, Z., He, C., Qin, G., and Tian, S. (2016). Comparative proteomics reveals the potential targets of BcNoxR, a putative regulatory subunit of NADPH oxidase of Botrytis cinerea. *Mol. Plant-Microbe Interact.* 29, 990–1003. doi:10.1094/MPMI-11-16-0227-R.

Lin, Z., Wu, J., Jamieson, P. A., and Zhang, C. (2019). Alternative oxidase is involved in the pathogenicity, development, and oxygen stress response of *Botrytis cinerea*. *Phytopathology*, PHYTO-01-19-001. doi:10.1094/phyto-01-19-0012-r.

Love, M. I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 15, 550. doi:10.1186/s13059-014-0550-8.

Luo, Y., Michailides, T. J., Morgan, D. P., Krueger, W. H., and Buchner, R. P. (2005). Inoculum dynamics, fruit infection, and development of brown rot in prune orchards in California. *Phytopathology* 95, 1132–1136. doi:10.1094/PHYTO-95-1132.

Ma, Z., Yoshimura, M. A., Holtz, B. A., and Michailides, T. J. (2005). Characterization and PCR-based detection of benzimidazole-resistant isolates of *Monilinia laxa* in California. *Pest Manag. Sci.* 61, 449–457. doi:10.1002/ps.982.

Mari, M., Casalini, L., Baraldi, E., Bertolini, P., and Pratella, G. C. (2003). Susceptibility of apricot and peach fruit to *Monilinia laxa* during phenological stages. *Postharvest Biol. Technol.* 30, 105–109. doi:10.1016/S0925-5214(03)00138-8.

Mari, M., Spadaro, D., Casals, C., Collina, M., De Cal, A., and Usall, J. (2019). "Stone Fruits," in *Postharvest Pathology of Fresh Horticultural Produce*, eds. L. Palou and J. L. Smilanick (CRC Press), 111–140.

Martini, C., and Mari, M. (2014). "*Monilinia fructicola*, *Monilinia laxa* (Monilinia Rot, Brown Rot)," in *Postharvest Decay: Control Strategies*, ed. Silvia Bautista-Baños (Elsevier), 233–265. doi:10.1016/B978-0-12-411552-1.00007-7.

McDowell, J. M., and Dangl, J. L. (2000). Signal transduction in the plant immune response. *Trends Biochem. Sci.* 25, 79–82. doi:10.1016/S0968-0004(99)01532-7.

Naranjo-Ortíz, M. A., Rodríguez-Píres, S., Torres, R., Cal, A. De, Usall, J., and Gabaldón, T. (2018). Genome sequence of the brown rot fungal pathogen *Monilinia laxa*. *BMC Res. Notes* 11, 1–2. doi:10.1186/s13104-018-3854-z.

Oetiker, J. H., and Yang, S. F. (1995). The role of ethylene in fruit ripening. *Acta Hortic.* 398, 167–178. doi:10.17660/actahortic.1995.398.17.

Pandey, D., Rajendran, S. R. C. K., Gaur, M., Sajeesh, P. K., and Kumar, A. (2016). Plant defense signaling and responses against necrotrophic fungal pathogens. *J. Plant Growth Regul.* 35, 1159–1174. doi:10.1007/s00344-016-9600-7.

Petersen, T. N., Brunak, S., Von Heijne, G., and Nielsen, H. (2011). SignalP 4.0: Discriminating signal peptides from transmembrane regions. *Nat. Methods* 8, 785–786. doi:10.1038/nmeth.1701.

Poussereau, N., Creton, S., Billon-Grand, G., Rascle, C., and Fevre, M. (2001). Regulation of acp1, encoding a non-aspartyl acid protease expressed during pathogenesis of Sclerotinia sclerotiorum. *Microbiology* 147, 717–726. doi:10.1099/00221287-147-3-717.

RosBREED RosBREED Peach Brown Rot. Available at: https://www.rosbreed.org/node/424 [Accessed August 10, 2021].

Rungjindamai, N., Jeffries, P., and Xu, X.-M. (2014). Epidemiology and management of brown rot on stone fruit caused by *Monilinia laxa*. *Eur. J. Plant Pathol.* 140, 1–17. doi:10.1007/s10658-014-0452-3.

Schouten, A., Tenberge, K. B., Vermeer, J., Stewart, J., Wagemakers, L., Williamson, B., et al. (2002). Functional analysis of an extracellular catalase of Botrytis cinerea. *Mol. Plant Pathol.* 3, 227–238. doi:10.1046/j.1364-3703.2002.00114.x.

Tadiello, A., Ziosi, V., Negri, A. S., Noferini, M., Fiori, G., Busatto, N., et al. (2016). On the role of ethylene, auxin and a GOLVEN-like peptide hormone in the regulation of peach ripening. *BMC Plant Biol.* 16, 1–17. doi:10.1186/s12870-016-0730-7.

Torres, M. A., Jones, J. D. G., and Dangl, J. L. (2006). Reactive oxygen species signaling in response to pathogens. 141, 373–378. doi:10.1104/pp.106.079467.

Usall, J., Casals, C., Sisquella, M., Palou, L., and De Cal, A. (2015). Alternative technologies to control postharvest diseases of stone fruits. *Stewart Postharvest Rev.* 11, 1–6. doi:10.2212/spr.2015.4.2.

Valero-Jiménez, C. A., Veloso, J., Staats, M., and Van Kan, J. A. L. (2019). Comparative genomics of plant pathogenic *Botrytis* species with distinct host specificity. *BMC Genomics* 20. doi:10.1186/s12864-019-5580-x.

Van Der Ent, S., and Pieterse, C. M. J. (2012). "Ethylene: multi-tasker in plant-attacker interactions," in *Annual Plant Reviews*, ed. Michael T. McManus (Blackwell Publishing Ltd), 343–377. doi:10.1002/9781118223086.ch13.

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

Verde, I., Abbott, A. G., Scalabrin, S., Jung, S., Shu, S., Marroni, F., et al. (2013). The high-quality draft genome of peach (*Prunus persica*) identifies unique patterns of genetic diversity, domestication and genome evolution. *Nat. Genet.* 45, 487–494. doi:10.1038/ng.2586.

Verde, I., Jenkins, J., Dondini, L., Micali, S., Pagliarani, G., Vendramin, E., et al. (2017). The Peach v2.0 release: High-resolution linkage mapping and deep resequencing improve chromosome-scale assembly and contiguity. *BMC Genomics* 18, 1–18. doi:10.1186/s12864-017-3606-9.

Villarino, M., Sandín-España, P., Melgarejo, P., and De Cal, A. (2011). High chlorogenic and neochlorogenic acid levels in immature peaches reduce *Monilinia laxa* infection by interfering with fungal melanin biosynthesis. *J. Agric. Food Chem.* 59, 3205–3213. doi:10.1021/jf104251z.

Zhang, L., Zhang, F., Melotto, M., Yao, J., and He, S. Y. (2017). Jasmonate signaling and manipulation by pathogens and insects. *J. Exp. Bot.* 68, 1371–1385. doi:10.1093/jxb/erw478.

Zhu, W., Wei, W., Wu, Y., Zhou, Y., Peng, F., Zhang, S., et al. (2017). BcCFEM1, a CFEM domain-containing protein with putative GPI-anchored site, is involved in pathogenicity, conidial production, and stress tolerance in Botrytis cinerea. *Front. Microbiol.* 8. doi:10.3389/fmicb.2017.01807.

Zhu, Z., and Tian, S. (2012). Resistant responses of tomato fruit treated with exogenous methyl jasmonate to *Botrytis cinerea* infection. *Sci. Hortic. (Amsterdam).* 142, 38–43. doi:10.1016/j.scienta.2012.05.002.

Zipfel, C. (2014). Plant pattern-recognition receptors. *Trends Immunol.* 35, 345–351. doi:10.1016/j.it.2014.05.004.

Supplementary Material

All supplementary information can be found in:

https://doi.org/10.1038/s41438-020-00387-w

Supplementary Table S1. Summary of fruit quality parameters: harvest date, minimum and maximum values of single index of absorbance difference (I_{AD}), weight, cheek diameter (CD), flesh firmness (FF), soluble solids content (SSC) and titratable acidity (TA) of 'Venus' nectarines. Data represent the mean (n = 20) ± the Standard Error.

Supplementary Table S2. Raw, parsed and mapped reads of mRNA of all 48 samples. Three biological replicates were analyzed from each inoculated and control tissue of immature and mature stages at 4 time points (6, 14, 24 and 48 hpi). Reads and genes of nectarine and *M. laxa* are shown in number and percentage (%). Abbreviations: CK (control), Inoc (inoculated with *M. laxa*), IM (immature), M (mature). The last number (1, 2, 3 or 4) in column E represents the biological replicate that was taken for the analysis.

Supplementary Table S3. Differential expression and functional analysis of nectarine. Tab 2) Differentially expressed genes, where the first column denotes the transcript ID, and the following columns show the annotated function, and each of the following pairs of columns indicate the DESeq2 results for each pairwise comparison. Values represent the Log_2FC expression of the comparison between tissues at each time point and the P-*adj* value of the statistic. Abbreviations: IMC (immature control), IMI (immature inoculated), MC (mature control), MI (mature inoculated). Abbreviations for the following tabs: IM (immature), M (mature). Tab 3) Fruit KEGG Enrichments. From left to right, the following are shown: the KEGG term, the description, the metabolic pathway and the Gene Expression (in terms of Log (1/P*adj*) in inoculated tissue compared to control at each time point for both tissues. Tab 4) Heatmap of jasmonic pathway. From left to right: the gene accession, the abbreviation, the KEGG orthologue, the KEGG pathway and the Gene Expression (in terms of Log_2FC) in inoculated tissue compared to control at each time point for both tissues. Tab 5) Heatmap of ethylene pathway. From left to right: the gene accession, the abbreviation, the KEGG orthologue, the KEGG pathway and the Gene Expression (in terms of Log_2FC) in inoculated tissue compared to control at each time point for both tissues.

Supplementary Table S4. Differential expression and functional analysis of *M. laxa*. Differentially expressed genes, where the first column denotes the transcript ID, the following columns show the annotated function, and each of the following group of columns indicates the DESeq2 results for each pairwise comparison. Values represent the Log_2FC expression and the P-*adj* value of the statistic of the comparisons between 14, 24 and 48 hpi compared to 6 hpi for both tissues. Abbreviations: IM (immature), M (mature).

Supplementary Table S5. RNA-Seq validation by RT-qPCR of 8 selected nectarine and *M. laxa* genes. Values of RT-qPCR are the Log_2FC expression obtained in the RT-qPCR. Values of RNA-Seq are the Log_2FC expression of the DE analyses of nectarine and the DE analyses of *M. laxa*. Person correlations are shown among all the genes for both *M. laxa* and nectarine. Gene abbreviations can be found in Suppl. Table S8.

Supplementary Table S6. Read count matrices of nectarine. The second tab contains the raw read counts of nectarine transcripts in each of the 48 samples retrieved from the RNA-seq output. Abbreviations: CK (control), Inoc (inoculated with *M. laxa*), IM (immature), M (mature). The following number represents the time point (6, 14, 24 or 48h). The last number (1, 2, 3 or 4) represents the biological replicate that was taken for the analysis.

Supplementary Table S7. Read count matrices of *M. laxa*. The second tab contains the raw read counts of *M. laxa* transcripts in the inoculated samples (24 samples) from the RNA-seq output. Abbreviations: Inoc (inoculated with *M. laxa*), IM (immature), M (mature). The following number represents the time point (6, 14, 24 or 48h). The last number (1, 2, 3 or 4) represents the biological replicate that was taken for the analysis.

Supplementary Table S8. List of the primers used for RT-qPCR. From left to right: Organism, Pathway, Target Gene, Gene Abbreviation, Transcript Accession, Reference, Type, Primer Sequence (5´-3'), Amplicon length (bp) and Primer efficiency (%). Reference or *de novo* design is also specified.

Chapter 4. Transcriptional profiling of the terpenoid biosynthesis pathway reveals putative roles of linalool and farnesal in nectarine resistance against brown rot

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Plant Science (submitted on 12th January 2022)

Abstract

The most devastating fungal disease of peaches and nectarines is brown rot, caused by *Monilinia* spp. Among the many plant responses against biotic stress, plant terpenoids play essential protective functions, including antioxidant activities, and inhibition of pathogen growth. Herein, we aimed to characterize the expression of terpenoid biosynthetic genes in fruit tissues that presented different susceptibility to brown rot. For that, we performed artificial inoculations with *Monilinia laxa* at two developmental stages (immature and mature fruit) of two nectarine cultivars ('Venus' and 'Albared'). All tissues were susceptible to *M. laxa* except for immature 'Venus' nectarines. In response to the pathogen, the mevalonic acid (MVA) pathway of the 'Venus' cultivar was highly induced in both stages rather than the methylerythritol phosphate (MEP) pathway, being the expression of some terpenoid biosynthetic genes (e.g., *PpPFT,* and *PpLIS*) different between stages. In 'Albared', both stages presented similar responses to *M. laxa*. Comparisons between cultivars showed that *PpHMGR1* expression was common in susceptible tissues. Whitin all the terpenoid biosynthetic pathway, farnesal and linalool-related pathways stood out for being upregulated only in resistant tissues, which suggest their role in mediating the resistance to *M. laxa.* Understanding the different responses between resistant and susceptible tissues could be further considered for breeding or developing new strategies to control brown rot in stone fruit.

Keywords: *Monilinia*, stone fruit disease, fruit developmental stage, secondary metabolism, terpenoids, postharvest

Introduction

Stone fruit comprise species of the *Prunus* genus, which includes over 400 to 430 species (Biswajit Das, 2011), such as apricots, cherries, peaches, nectarines, and plums. Peaches and nectarines are the fifth most important fruit crop within the Rosaceae family (Shahbandeh, 2019), with a global production of 25.7 million tons in 2019 (FAO, 2021). During harvest and postharvest, stone fruit are generally susceptible to fungal diseases, particularly infections caused by *Monilinia* spp., the etiological agent of brown rot (Mustafa et al., 2021). The main pathogenic *Monilinia* species in stone fruit are *M. laxa*, found worldwide (Obi et al., 2018), and *M. fructicola,* which is more virulent (Kreidl et al., 2015) but only restricted to Australasia, North and South America (Rungjindamai et al., 2014), and in Europe since 2001 (EPPO 2002). *Monilinia* spp. can infect fruit without naturally occurring entry points (Garcia-Benitez et al., 2016) at any developmental stage, although brown rot susceptibility increases with maturation (Guidarelli et al., 2014). Meanwhile, the fungus can establish latent or quiescent infections until optimal conditions trigger the disease cycle (Luo et al., 2005).

In response to fungal attack, nectarine fruit activate different signaling pathways (e.g., oxidative burst and hormone signaling) leading to the expression of pathogenesis-related proteins and accumulation of secondary metabolites, among others (Balsells-Llauradó et al., 2020). Secondary metabolites are involved in fruit defenses as constitutive or inducible responses (Alkan and Fortes, 2015). Among them, terpenoids represent the largest and most diverse class of secondary metabolites, known to play defense roles against abiotic stress (e.g., UV-B light) (Liu et al., 2017) and various biotic interactions (Khare et al., 2020). For instance, monoterpenes, triterpenes, sesquiterpenes, and terpene glycosides are accumulated at all stages of noble rot caused by *Botrytis cinerea* in ripe grape berries (Blanco-Ulate et al., 2015). Quilot-Turion et al. (2020) *[congress communication*] found that up to 30 phenolic and terpenoid compounds of peach were released in response to wounding and inoculation with *M. laxa.* Nevertheless, there are no studies aiming to decipher the regulation of the terpenoid biosynthetic pathway during the interaction between *Monilinia* spp. and unwounded nectarines.

All terpenoids are derived from the five-carbon (C5) precursor isopentenyl diphosphate (IPP) and its double-bond isomer dimethylallyl diphosphate (DMAPP) (Tholl, 2015). Their biosynthesis mainly comes from two pathways; the cytosolic mevalonic acid (MVA) pathway, which predominantly provides the precursors for sesquiterpenoids, steroids, and triterpenoids, and the plastidial methylerythritol

phosphate (MEP) pathway, which supplies precursors for hemiterpenoids, monoterpenoids, diterpenoids and carotenoids (Tholl, 2015). The 3-hydroxy-3-methylglutaryl-CoA (HMG) synthase (HMGS) and HMG reductase (HMGR) are the rate-limiting steps of the MVA pathway (Hemmerlin et al., 2012; Chang et al., 2015), whereas the 1-deoxy-D-xylulose 5-phosphate synthase (DXS) is considered the regulator of the MEP pathway (Lois et al., 2000). Knowledge about the regulation and dynamics of the MVA and MEP pathways during fruit-pathogen interactions will help resolve the relevance of particular terpenoids in fruit resistance or susceptibility to fungal disease.

In a recent publication, we observed that several genes involved in terpenoid metabolism were significantly induced after *M. laxa* inoculation of resistant immature nectarines (cv. 'Venus') when compared to susceptible mature fruit (cv. 'Venus') (Balsells-Llauradó et al., 2020). Here, we assessed disease development followed by a detailed transcriptional analysis of the terpenoid biosynthetic pathway in healthy and *M. laxa*-inoculated tissues of two nectarine cultivars, 'Venus' and 'Albared,' which present differences in susceptibility to brown rot according to their developmental stage. We then focused on specific terpenoid biosynthesis genes that displayed differential expression between cultivars and developmental stages and could explain the resistance or susceptibility outcomes observed. These genes should be further considered for functional analyses and targets for future breeding or management strategies against brown rot.

Materials and methods

Plant material and fungal culture

Two cultivars of nectarine (*P. persica* var. *nucipersica* (Borkh.) Schneider) were used for the experiments. 'Venus' and 'Albared' nectarines were obtained from organic orchards located in Lleida (Catalonia, Spain). To avoid the presence of natural occurring inoculum, fruit were bagged at least 6 weeks before the commercial harvest. Fruit was harvested at two different developmental stages, based on grower's recommendations: "immature" (184 and 219 Julian days for 'Venus' and 'Albared' cultivars, respectively) and "mature" (211 and 246 Julian days for 'Venus' and 'Albared' cultivars, respectively). For each sampling, fruit were homogenized using a DA-Meter (TR-Turoni, Forli, Italy), based on the single index of absorbance difference (I_{AD} = 1.99–2.26 and 1.00–2.06 for immature fruit and I_{AD} = 0.25–1.60 and 0.16–1.32 for mature fruit, for 'Venus' and 'Albared' cultivars, respectively). Flesh firmness was measured on

20 randomly fruit on harvest day, following the previously described protocol (Baró-Montel et al., 2019a). The fungal strain used for all experiments was the *M. laxa* single-spore strain 8L (ML8L, Spanish Culture Type Collection number CECT 21100) and conidial suspensions were prepared as described previously by Baró-Montel et al. (2019b).

Fruit inoculations and disease evaluation

For disease evaluation, one drop of 30 µL of conidial suspension (10^6 conidia mL^{-1}) was applied on the fruit surface. The same inoculation methodology using sterile water with 0.01% (w/v) Tween-80 was conducted for mock-inoculated fruit. Fruit was incubated in containers in darkness and with high relative humidity (97 ± 3% and 20 ± 1 °C). Disease development was examined daily as disease incidence (% of brown rot) and severity (lesion diameter, cm) were calculated for each stage and cultivar (n = 20) across time for 72 hours post inoculation (hpi). Immature 'Venus' nectarines were incubated until 8 days post inoculation to confirm the absence of disease symptoms.

For gene expression analyses of 'Albared' nectarines, six drops of 30 µL of conidial suspension (10^6 conidia mL^{-1}) were applied on each fruit. Sterile water containing 0.01% (w/v) Tween-80 was used for mock-inoculated fruit (control). Fruit was incubated at the same conditions previously described for disease evaluation. Three replicates consisting of five fruit per treatment were obtained at each sampling point (6, 14, 24, 48 and 72 hpi). Gene expression analysis for terpenoid biosynthetic genes of 'Venus' cultivar was conducted using the data of normalized read counts from previous studies of the group (Balsells-Llauradó et al., 2020). In that case, fruit was sampled, inoculated and RNA-extracted like 'Albared'.

RNA extraction and gene expression analysis of 'Albared' samples

Tissue sampling, extraction of total RNA, elimination of contaminant DNA, RNA concentration and quality assessment, synthesis of first-strand cDNA, primer efficiency and quantification of gene expression through Real-Time Quantitative PCR (RT-qPCR) were conducted following the same methodology described for 'Venus' cultivar (Balsells-Llauradó et al., 2020). Fungal biomass determination was calculated based on the relative gene expression of the *M. laxa* reference gene (*MlACT*) normalized to the nectarine reference gene expression (*PpTEF2*). Gene expression levels of each gene of interest were normalized to *PpTEF2* (Tong et al., 2009), using the formula $2^{(\text{reference gene Ct} - \text{gene of interest Ct})}$. Primers (Supplemental Table S1) were retrieved from the literature (Tong et al., 2009; Cao et al., 2017; Liu et al., 2017) or designed *de novo.* The RNA-Seq

expression profiles of nine terpenoid genes previously reported in the 'Venus' cultivar (Balsells-Llauradó et al., 2020) were validated by RT-qPCR using the same tissues. The Person correlation values between RNA-Seq and RT-qPCR data was *R*: 0.75, *P* value = 2.58 x 10^{-20} (Suppl. Table S2).

Statistical analysis

Data were statistically analyzed with JMP® software version 14.2.0 (SAS Institute Inc., Cary, NC, USA). Brown rot incidence was analyzed using the generalized linear model (GLM) based on a binomial distribution and logit-link function. When the analysis was statistically significant, orthogonal contrasts ($P \leq 0.05$) were performed for means separation among stages and cultivars. Lesion diameter length and relative gene expression were subjected to analysis of variance (ANOVA). When comparisons were conducted between two means (control *vs* inoculated), Student's *T* test ($P \leq 0.05$) was used. For means comparison between stages and cultivars (lesion diameter length), or across time for each control and inoculated fruit (normalized read counts or relative gene expression), Tukey's HSD test ($P \leq 0.05$) was conducted.

Results

Fruit developmental stage and cultivar determine susceptibility to brown rot

Evaluation of the fungal disease in two nectarine cultivars at two different developmental stages revealed that 'Albared' was susceptible to *M. laxa* in both stages after 72 hpi (Figure 1). In 'Venus' nectarines, *M. laxa* was only able to cause significant incidence and severity in mature fruit, whereas no disease symptoms were observed in immature fruit. Contrary to 'Venus', the pathogen caused evident incidence (80%) and moderate lesions (0.8 ± 0.18 cm) in immature 'Albared' nectarines, although significantly lower than the high incidence (100%) and lesions in mature tissues (3.4 ± 0.07). Such differences between immature stages could not rely on fruit quality attributes since both cultivars were comparable between developmental stages for each cultivar in terms of flesh firmness (N), in which values were 108.8 ± 1.9 ('Venus') and 105.6 ± 1.7 ('Albared') for immature and 74.4 ± 2.7 ('Venus') and 73.5 ± 2.3 ('Albared') for mature fruit.

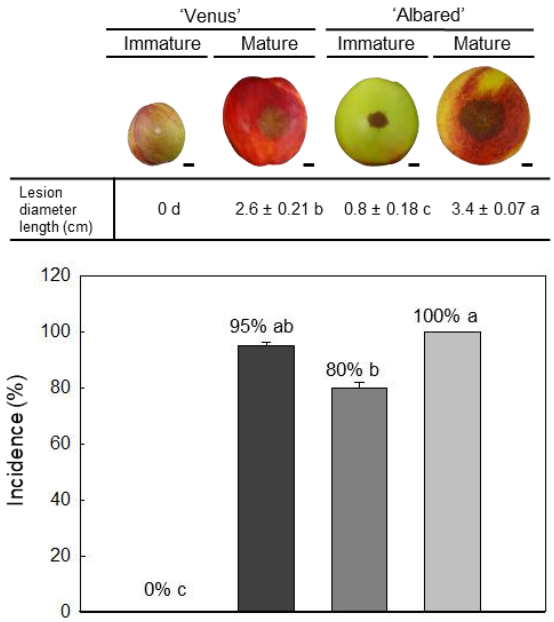


Figure 1. Brown rot evaluation in 'Venus' and 'Albared' nectarines at immature and mature stages. *Lesion diameter length (cm of rotted fruit) and incidence (% of brown rot) and of* M. laxa *at 72 hpi. The black line indicates the scale (1 cm). Different letters indicate significant differences (P ≤ 0.05) of incidence and lesion diameter length, among tissues. Values represent the mean and error bars represent the standard error of the means (n = 20).*

The analysis of the fungal biomass (Figure 2) revealed that in the immature tissue of 'Albared' nectarines, fungal biomass of the inoculated tissues significantly peaked at 72 hpi, moment in which the lesion spread was completely visible. In inoculated mature fruit, the fungal biomass significantly increased exponentially ($y = 0.6947e^{0.0636t}$, $R^2 = 0.8719$), paralleling the rotting of the pericarp. Remarkably, the fungal biomass in mature tissue was significantly higher than in the immature fruit at all time points (Suppl. Figure S1). In control tissues, traces of fungal biomass (2.04×10^{-3} and 5.46×10^{-2} normalized mean expression, NME) were detected across all time points in immature and mature stages, respectively (Figure 2).

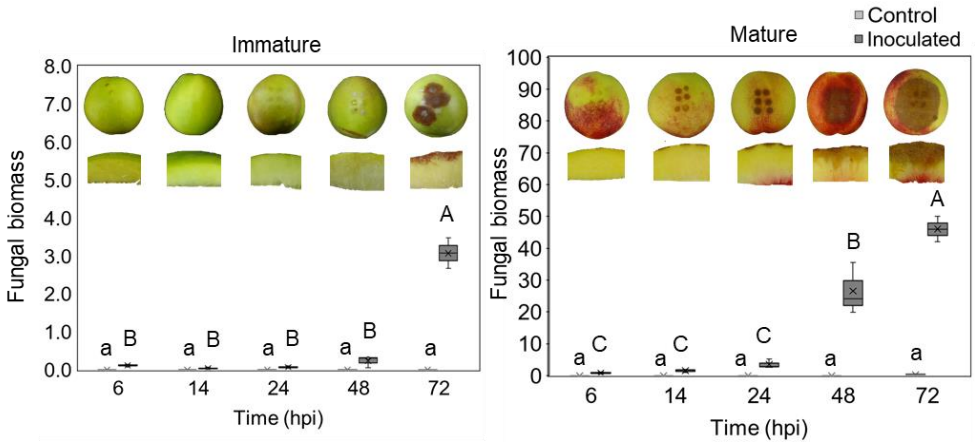


Figure 2. Brown rot disease progression and* M. laxa *biomass in 'Albared' nectarines. *Images (entire fruit and perpendicular section) display brown rot development in immature (left) and mature (right) tissues across time after inoculation (hours post inoculation, hpi). Assessment of* M. laxa *biomass by relative gene expression of* M. laxa *reference gene (MlACT), normalized to nectarine reference gene (PpTEF2) in both stages (immature and mature) of both control (light grey) and inoculated (dark grey) tissues. The box plot represents the mean of three biological replicates consisting of five fruit each with its interquartile range. Lowercase and uppercase letters indicate significant differences across time (P ≤ 0.05, Tukey's test) in control and* M. laxa*-inoculated tissues, respectively, for each developmental stage.*

Terpenoid biosynthetic genes were differentially expressed in the 'Venus' cultivar in response to *M. laxa*

Normalized read counts from our previous RNA-Seq study (Balsells-Llauradó et al., 2020), were used to depict the expression patterns of the terpenoid biosynthetic genes in both control- and *M. laxa*-inoculated 'Venus' fruit at two developmental stages (Figure 3). In control tissues, both immature and mature stages presented a similar gene expression pattern, with no significant differences among developmental stages. Most of the genes significantly change (i.e., increase, decrease or only fluctuated) their expression along the incubation time course. The RNA-Seq data revealed an evident response to *M. laxa* inoculation at both fruit developmental stages.

Specifically, in the first steps of the terpenoid backbone biosynthesis (from *PpAACT* to *PpMDS*), the presence of the pathogen significantly upregulated the expression of the MVA pathway compared to control tissues in both stages, whereas the MEP pathway was largely downregulated (Figure 3). The average expression across time of *PpHMGS* and *PpHMGR1* in *M. laxa*-inoculated fruit compared to control was 2.22 and 1.44-fold higher in immature tissues, whereas both genes were up to 19.23 and 24.87-fold higher, respectively, at 48 hpi on mature tissues. In contrast, *PpDXS1* was 1.27-fold

less expressed (average of 14 and 48 hpi) in immature fruit, and *PpDXS1* was 1.38-fold less expressed (average of 14 and 24 hpi) in mature tissues.

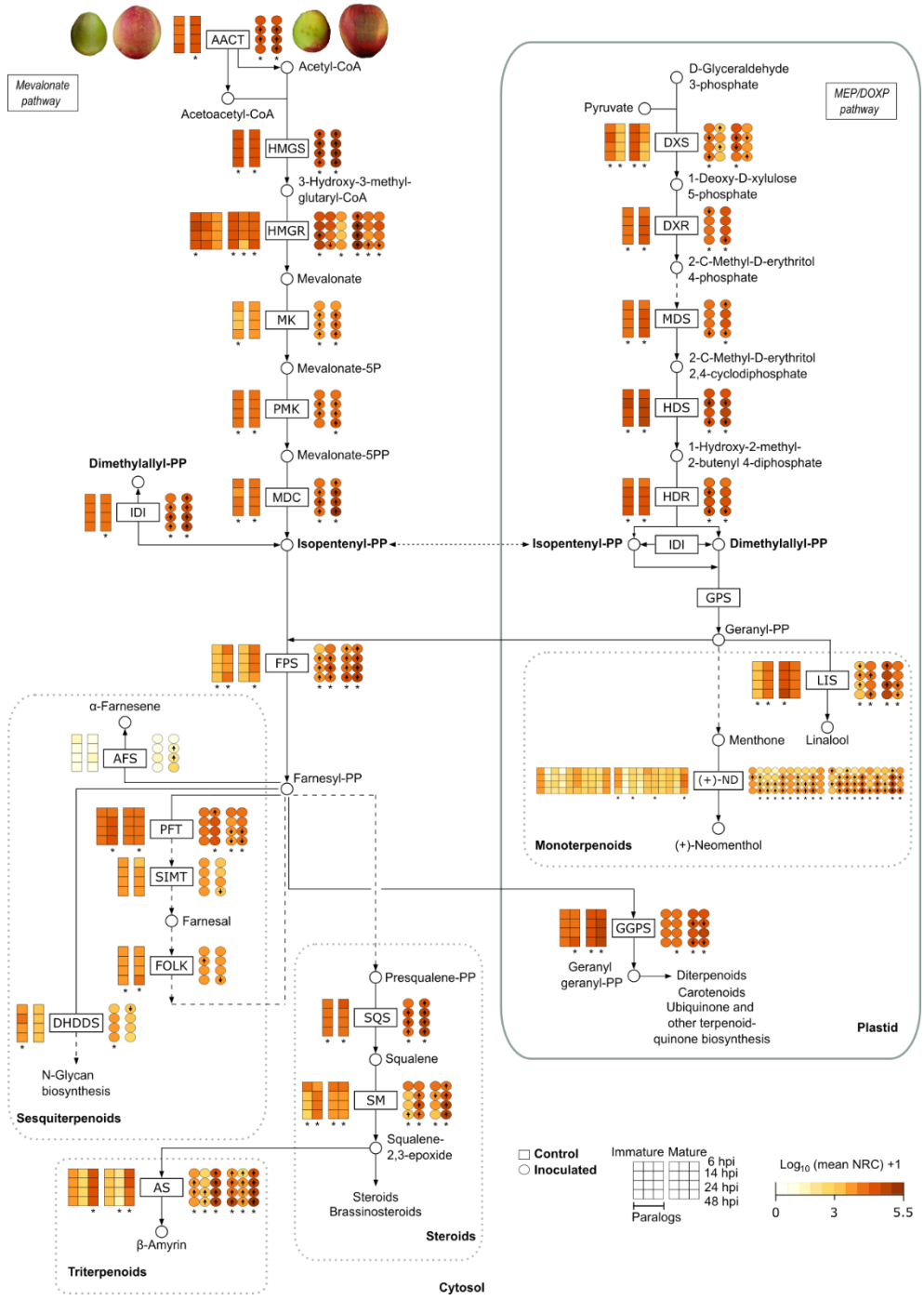


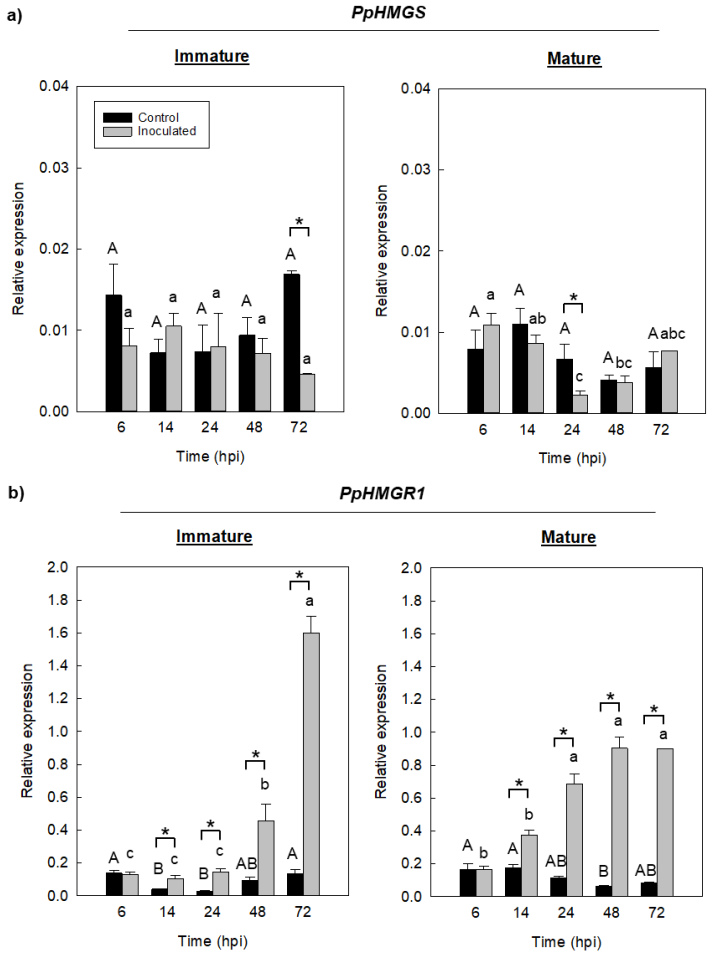
Figure 3. Expression of terpenoid pathway in control and* M. laxa*-inoculated 'Venus' nectarine at immature and mature stages. *The terpenoid biosynthetic pathway is shown with substrates (cercles) and enzymes (enzyme name inside boxes) and includes 42 differentially expressed genes based on previous studies (Balsells-Llauradó et al., 2020). The scale color of the heat map represents the intensity of the mean of normalized read counts, expressed as* $Log_{10} + 1$*. The normalized read counts expression is represented for control (□) and inoculated (○) tissues for each immature (left) and mature (right) stage at each time point after inoculation (hpi). Multiple columns of the same gene represent different paralogs. Dashed lines indicated that some steps had been omitted. Up or down black arrows on circles represent significantly higher or lower normalized read counts for the inoculated tissues compared to control fruit for each time point, stage, and gene (*$P \leq 0.05$*, Student's T test). Asterisks indicate significant differences across time for each gene, tissue, and stage (*$P \leq 0.05$*, Tukey's test). Enzyme abbreviations, corresponding gene accessions and details of statistical analysis are provided in Suppl. Table S3. Fruit images correspond to immature and mature stages of control (left) and inoculated (right) tissues at 48 hpi (Balsells-Llauradó et al., 2020).*

The activation of the MVA pathway occurred faster in mature than immature fruit, as observed by the induction of genes involved in MVA terpenoid backbone biosynthesis (e.g., *PpIDI, PpFPS, PpSQS*). Most of the biosynthetic genes that are downstream to farnesyl-PP and geranyl-PP were largely upregulated in *M. laxa*-inoculated fruit compared to control at both stages. Hence, results seemed to point out that final targets of 'Venus' nectarines in response to *M. laxa* were steroids (e.g., *PpSQS* and *PpSM*), monoterpenoids (*PpLIS* and *PpND*), and triterpenoids *(PpAS*). Overall, paralogs within each gene family behaved similar, except for *LIS*, in which *PpLIS1* and *PpLIS2* paralogs remained downregulated to increase thereafter in immature fruit. On the contrary, in mature fruit, *PpLIS1* expression was, in average, 1.39-fold higher whereas *PpLIS2* was 1.85-fold less expressed (average of 24 and 48 hpi) in response to *M. laxa* compared to control. On the other side, sesquiterpenoid biosynthetic genes (*PpPFT* and *PpFOLK*) in inoculated tissues were significantly downregulated comparted to controls in mature fruit and only upregulated at early time points (6 or 14 hpi) in immature fruit.

Monilinia laxa induces the expression of the terpenoid backbone and steroid biosynthetic genes in the 'Albared' cultivar

Potential candidate genes of terpenoid biosynthesis in 'Venus' cultivar, which were largely induced in response to *M. laxa* in both developmental stages (MVA pathway and downstream genes), were selected for expression analysis in the 'Albared' cultivar. The expression levels of two genes of the MVA pathway (*PpHMGS* and *PpHMGR1*) and two other backbone terpenoid biosynthetic genes (*PpIDI* and *PpFPS2*) were lower

(< 0.3 relative expression) in controls compared to *M. laxa*-inoculated fruit, and overall similar between immature and mature tissues (Figure 4). Although the expression of *PpHMGR1* did not show a clear pattern in both stages across time, the expression of *PpFPS2* in immature fruit tended to decrease through time. Other genes in this pathway (*PpHMGS* and *PpIDI* in both stages and *PpFPS2* in mature fruit) showed a steadily expression across the time points.



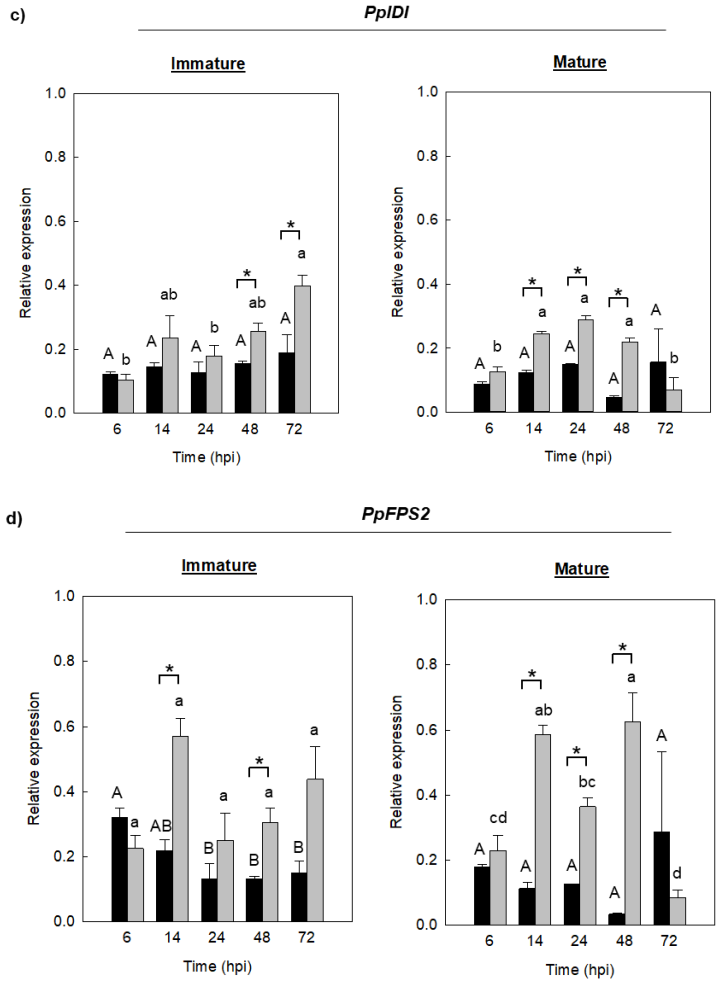


Figure 4. Relative expression of four genes of the terpenoid backbone pathway in the 'Albared' cultivar. *a)* PpHMGS *(Hydroxymethylglutaryl-CoA synthase); b)* PpHMGR1 *(Hydroxymethylglutaryl-CoA reductase); c)* PpIDI *(Isopentenyl-diphosphate delta-isomerase); d) PpFPS2 (Farnesyl diphosphate synthase / farnesyl pyrophosphate synthase). Asterisks indicate significant differences between control and inoculated tissues for each developmental stage (immature and mature) at each time point (P ≤ 0.05, Student's T test). Different uppercase (A-D) and lowercase (a-d) letters indicate significant differences across time (P ≤ 0.05, Tukey's test) for each control and inoculated immature or mature tissues, respectively. Values represent the mean and error bars represent the standard error of the means (n = 3).*

In *M. laxa*-inoculated immature fruit, *PpHMGS* expression remained steadily across most time points and was later significantly downregulated (3.7-fold less) at 72 hpi compared to control *PpHMGR1* was significantly activated by the pathogen since 14 hpi onwards, displaying an upregulation of 11.9-fold at 72 hpi (Figure 4). *PpIDI* was significantly upregulated later in time in immature tissues (1.6 and 2.1-fold higher at 48 and 72 hpi, respectively compared to the control), whereas *PpFPS2* was significantly induced by the pathogen at some time points (2.6 and 2.3-fold higher at 14 and 48 hpi, respectively, compared to control). The relative gene expression in *M. laxa*-inoculated mature fruit revealed a similar pattern to that in immature fruit. The unique significant downregulation of *PpHMGS* in inoculated tissues compared to control (3-fold less) occurred earlier than that in immature (at 24 hpi), whereas *M. laxa* inoculation significantly increased *PpHMGR1* expression (up to 14.8 and 11.1-fold higher at 48 and 72 hpi, respectively) compared to control since 14 hpi onwards. The relative expression of *PpIDI* and *PpFPS2* in mature inoculated fruit were significantly higher than control fruit through time (from 14 to 48 hpi), being in average, 2.9 and 8.9-fold more expressed, respectively.

Several groups of compounds can be derived from the terpenoid backbone. Relative expression levels of steroid biosynthetic genes (*PpSQS* and *PpSM2*) in control 'Albared' fruit were scarce (< 0.17 relative expression) compared to *M. laxa*-inoculated tissues, and non-statistically significant between developmental stages (Figure 5). Besides, the relative expression fluctuated across time in both tissues. In contrast, in *M. laxa*-inoculated immature fruit, the presence of the pathogen significantly induced the expression of *PpSQS* later in time (1.9 and 2.7-fold change at 48 and 72 hpi, respectively, compared to controls), paralleling the spread of the disease. *Monilinia laxa* inoculation also induced the expression of *PpSM2* since 24 hpi onwards (an average of 4.9-fold change until 72 hpi). In mature tissues, the induction of the expression of *PpSQS* and *PpSM2* by *M. laxa* inoculation occurred earlier (since 14 hpi), similarly to *PpIDI* and *PpFPS2*, both displaying a significantly higher expression in inoculated compared to control tissues, being 12 and 44.7-fold higher for *PpSQS* and *PpSM2*, respectively, at 48 hpi.

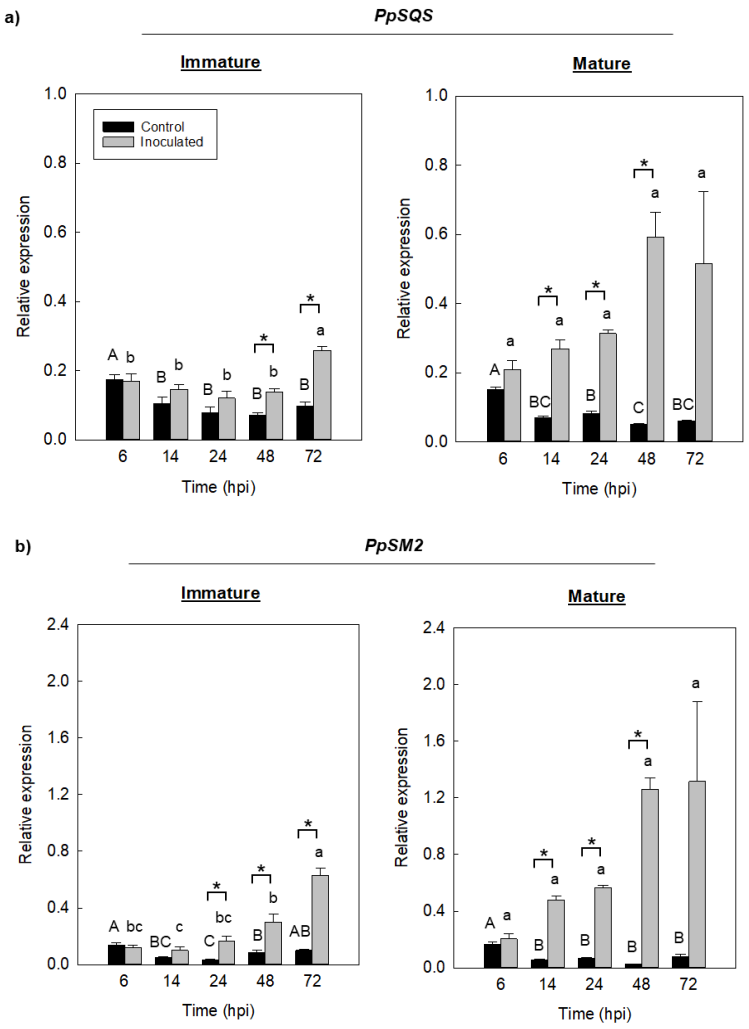


Figure 5. Relative expression of steroid biosynthetic genes in the 'Albared' cultivar. *a)* PpSQS *(Squalene synthase / Farnesyl-diphosphate farnesyltransferase); b)* PpSM2 *(Squalene monooxygenase). Asterisks indicate significant differences between control and inoculated tissues for each developmental stage (immature and mature) at each time point (*P ≤ *0.05, Student's T test). Different uppercase (A-D) and lowercase (a-d) letters indicate significant differences across time (*P ≤ *0.05, Tukey's test) for each control and inoculated immature or mature tissues, respectively. Values represent the mean and error bars represent the standard error of the means (n = 3).*

Expression of genes in the sesquiterpenoid and monoterpenoid pathways is differentially induced by *M. laxa* in the 'Albared' cultivar

Within the sesquiterpenoids family, gene expression of farnesal biosynthetic genes were downregulated across time and due to *M. laxa* inoculation (Figure 6). Relative expression of *PpPFT1*, *PpSIMT* and *PpFOLK* genes in control 'Albared' nectarines were low (< 0.16 relative expression) compared to the other genes analyzed and their expression patterns differed across time. In detail, *PpPFT1* and *PpFOLK* expression in immature control fruit significantly increased from 14 to 72 hpi (4.7 and 3.3-fold, respectively). In contrast, levels of *PpSIMT* of control fruit remained steady across time in both stages.

In *M. laxa*-inoculated immature fruit, the relative expression of *PpPFT1* at the beginning (6 hpi) and at the end (72 hpi) of the infection course was significantly reduced (up to 3.1-fold less) compared to control fruit (Figure 6). The expression of *PpFOLK* was downregulated (up to 6.9-fold) at 72 hpi compared to controls, coinciding with the spread of the tissue maceration. In *M. laxa*-inoculated mature fruit, both *PpPFT1* and *PpFOLK* expressions were also largely downregulated compared to controls although such reduction occurred from 24 to 48 hpi (in average, 2.6 and 2.4-fold less for each gene, respectively). Remarkably, *M. laxa*-inoculation caused no significant effect in the expression levels of *PpSIMT* compared to controls across time.

Regarding the monoterpenoid biosynthesis, the expression pattern of genes codifying for 3S-linalool synthase (*PpLIS1* and *PpLIS2*) was dependent on the developmental stage analyzed (Figure 7). Overall, in control fruit, expression levels of the paralog *PpLIS1* were higher (up to 1.2 NME) than *PpLIS2* (up to 0.05 NME). The relative expression of *PpLIS1* was significantly higher in mature fruit (average of 0.753 ± 0.14 NME) than immature fruit (average of 0.097 ± 0.02 NME). Remarkably, whereas the expression of *PpLIS1* in control immature fruit significantly peaked at 72 hpi, *PpLIS2* expression significantly changed throughout time, although with a non-clear pattern. In fruit inoculated with *M. laxa, PpLIS1* also displayed a higher gene expression level in both stages than *PpLIS2*; however, *PpLIS2* expression was more impacted by *M. laxa* inoculation across time (Figure 7). The presence of the pathogen in the immature fruit significantly reduced by 1.8-fold *PpLIS1* expression compared to control fruit only at 72 hpi, while significantly increased by 2.7-fold the expression in mature fruit at 6 hpi when compared to the control. *Monilinia laxa* inoculation significantly induced the expression of *PpLIS2* in the immature tissues at 14 hpi and then caused a significant reduction in expression levels up to 6.4-fold at 72 hpi compared to controls. In contrast, in *M. laxa*-inoculated mature fruit, *PpLIS2* was already significantly reduced by 3.3-fold at 24 hpi compared to control.

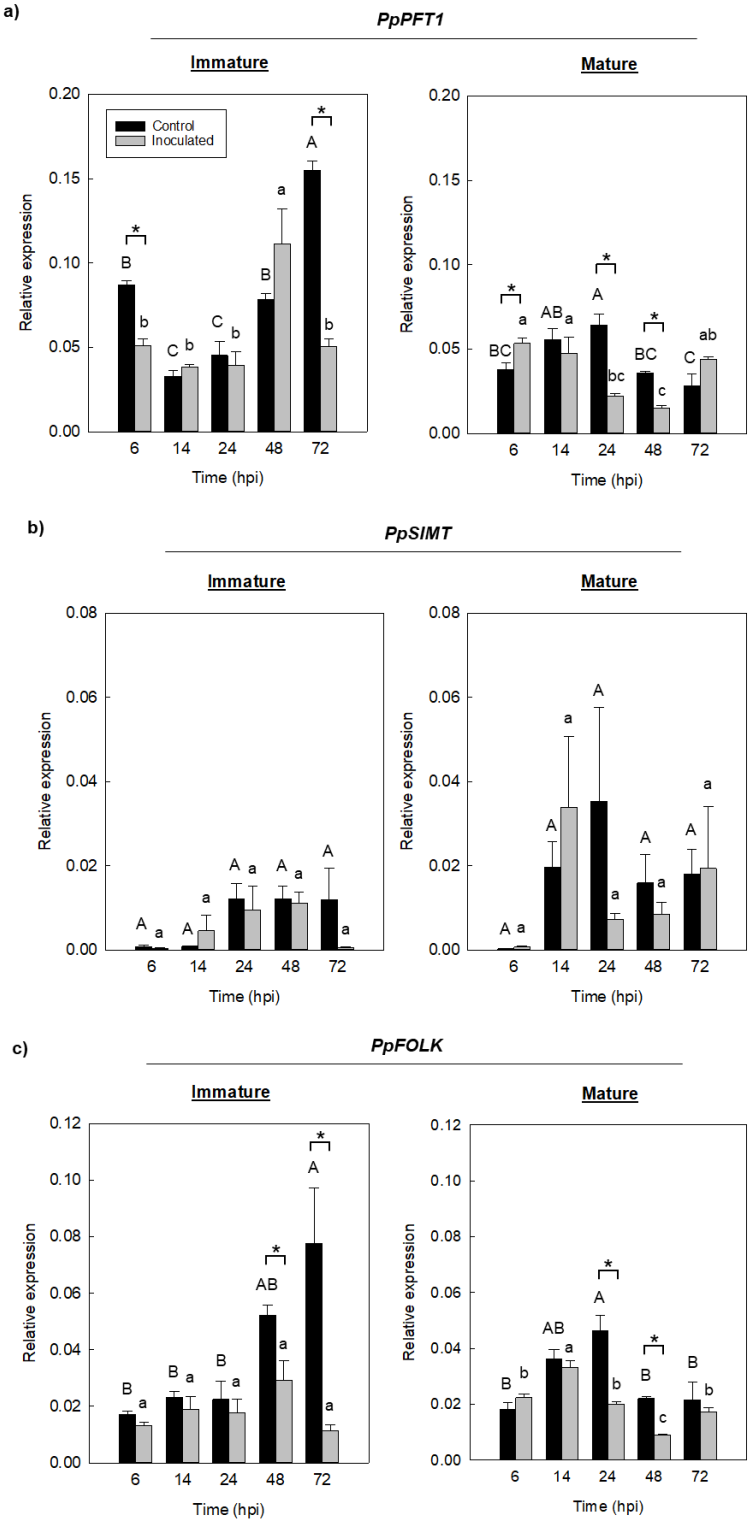

a)
PpPFT1
Immature
Mature
Control
Inoculated
Relative expression
Time (hpi)
b)
PpSIMT
Immature
Mature
Relative expression
Time (hpi)
c)
PpFOLK
Immature
Mature
Relative expression
Time (hpi)

Figure 6. Relative expression of farnesal biosynthetic genes in the 'Albared' cultivar. *a)* PpPFT *(Protein farnesyltransferase subunit beta); b)* PpSIMT *(Protein-S-isoprenylcysteine O-methyltransferase); c)* PpFOLK *(Farnesol kinase). Asterisks indicate significant differences between control and inoculated tissues for each developmental stage (immature and mature) at each time point (P ≤ 0.05, Student's T test). Different uppercase (A-D) and lowercase (a-d) letters indicate significant differences across time (P ≤ 0.05, Tukey's test) for each control and inoculated immature or mature tissues, respectively. Values represent the mean and error bars represent the standard error of the means (n = 3).*

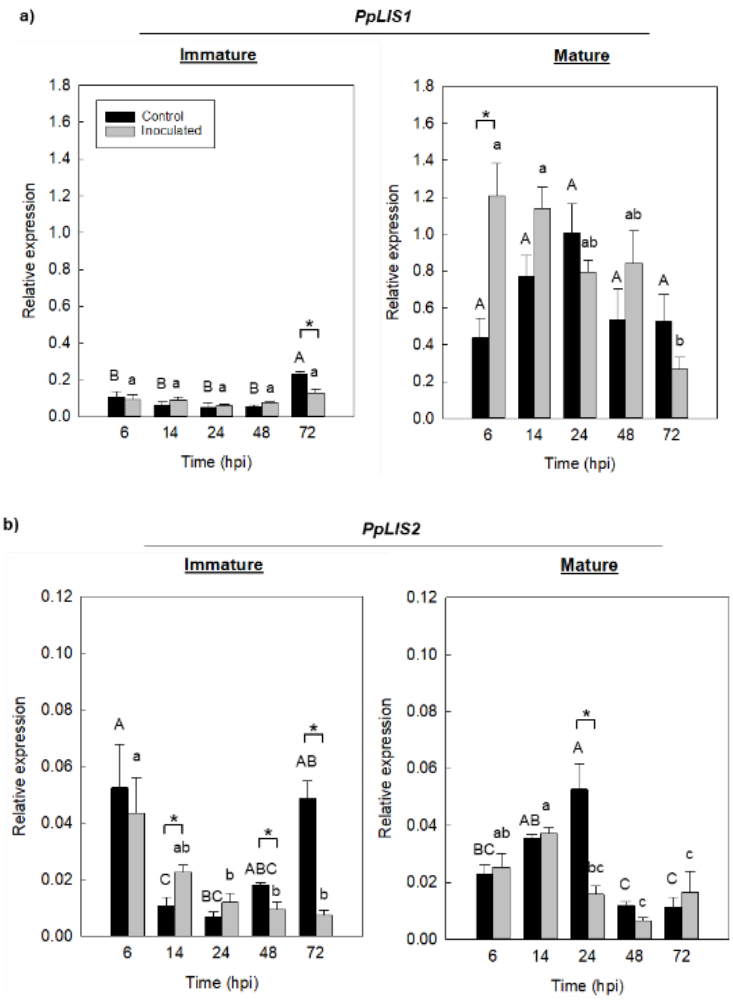


Figure 7. Expression of linalool biosynthetic genes in the 'Albared' cultivar. *Two paralogs of the 3S-linalool synthase gene,* PpLIS1 *(a) and* PpLIS2 *(b). Asterisks indicate significant differences between control and inoculated tissues for each developmental stage (immature and mature) at each time point (P ≤ 0.05, Student's T test). Different uppercase (A-D) and lowercase (a-d) letters indicate significant differences across time (P ≤ 0.05, Tukey's test) for each control and inoculated immature or mature tissues, respectively. Values represent the mean and error bars represent the standard error of the means (n = 3).*

Discussion

The fruit host defense responses and the virulence strategies displayed by the pathogen during the nectarine-*M. laxa* interaction are starting to be unveiled. Our previous RNA-Seq study pointed out the possible involvement of nectarine terpenoids metabolism in response to *M. laxa*. However, to the best of our knowledge, there are no studies reporting the role of nectarine terpenoids in resistance or susceptibility to brown rot. Recently, Muto et al. (2020) reported the terpenoid profiles of eight nectarine and peach cultivars (without fungal infection), at both gene (e.g., *PpLIS2* and *PpAFS*) and metabolite levels. In our work, the expression levels of the terpenoid biosynthetic genes in healthy tissues (controls) of both nectarine cultivars were like those reported by Muto et al. 2020.

Monilinia laxa infections behaved differently between cultivars at the immature stage. Although is generally accepted that the pathogen can infect fruit at any growth stage (Guidarelli et al., 2014), in 'Venus' immature fruit, the disease did not progressed. *Monilinia laxa* was still active in the immature 'Venus' fruit since a peak on the fungal biomass occurred at 14 hpi, deaccelerating afterwards probably due to a shift to a quiescent or autolytic state (Balsells-Llauradó et al., 2020). In this work and contrary to the 'Venus' cultivar, *M. laxa* managed to infect immature 'Albared' nectarines. Such disease progression was also evident when analyzing the fungal biomass since it progressively increased across time and significantly peaked at 72 hpi, when the disease symptoms where most visible.

Many factors, including those climatological or intrinsic of the host itself, can influence brown rot progression. In fact, conidia of *Monilinia* spp. can remain quiescent until favorable factors trigger the disease (Garcia-Benitez et al., 2020). Although fruit physical attributes could influence fruit susceptibility to pathogens and hence, should not be obviated, the results from our study demonstrated that there were no significant differences among cultivars within each developmental stage for flesh firmness (e.g., an important parameter associated with fruit quality). Thus, differences on brown rot susceptibility among 'Venus' and 'Albared' should be derived from additional fruit genetic and compositional characteristics. Hence, identifying molecular pathways and genes that differ between resistant tissues (i.e., immature 'Venus') and susceptible ones (both tissues of 'Albared' or mature 'Venus' nectarines) can provide clues about the main host factors driving resistance and susceptibility to brown rot.

'Venus' fruit, irrespective of the developmental stage, mainly activated the MVA and not the MEP pathway to respond against *M. laxa*. In fact, the plastidial pathway was downregulated in response to the pathogen. Which pathway is activated depends on the stimuli to which the plants are submitted and the need for specific end-compounds to properly face the stress. Under pathogen attack, plant cells can induce the MVA pathway to direct the flux toward the production of sesquiterpenes, known to exhibit antifungal activities (Tholl, 2015). For instance, some sesquiterpenes (β-elemene from rice) exhibit antifungal activity against *Magnaporthe oryzae* (Taniguchi et al., 2014). Both *HMGS* and *HMGR* are considered key regulatory genes of the MVA pathway (Hemmerlin et al., 2012; Chang et al., 2015). The expression of HMGR family members depends on several factors including the developmental stage, plant tissue, and external stimuli (e.g., pest and pathogen attack) (Hemmerlin et al., 2012; Haile et al., 2019). Many studies have reported the overall control of *HMGR* genes to the steroid pathway which often depend on individual genes of HMGR families, i.e., *HMGR1* and *HMGR2* differentially regulate the phytosterols and sesquiterpenoids production, respectively (Hemmerlin et al., 2012). However, under biotic stress, individual *HMGR* genes direct the flux towards the production of stress-induced compounds. For instance, the fungal elicitor arachidonic acid induces the *SlHMGR2* expression and carotenoid production (lycopene) in young and mature tomato (Rodríguez-Concepción and Gruissem, 1999). In our work, the upregulation of *PpHMGR1* in susceptible tissues (both fruit stages of 'Albared' and mature 'Venus' fruit) could be directing the flux towards steroid synthesis. In addition to the primary function of steroids as membrane structure compounds and regulators of growth and development (Tholl, 2015), steroids such phytosterol stigmasterol (the end-product of the steroid pathway) are involved in plant–pathogen interactions, as reported for *A. thaliana–Pseudomonas syringae* (Griebel and Zeier, 2010). Besides, its precursor (β-sitosterol) is increased in infected berries with *B. cinerea* (Agudelo-Romero et al., 2015). In our work, the expression of steroid biosynthetic genes was induced by the pathogen in all inoculated tissues (both stages of both cultivars) along with the infection progression.

Overall, terpenoid metabolism was induced in susceptible and resistant nectarine tissues; however, some specific pathways (i.e., farnesal-related genes) were almost not activated in susceptible fruit. The overall downregulation of farnesal-related pathway (e.g., *PpPFT* and *PpFOLK*) in susceptible tissues (i.e., mature 'Venus' and both tissues of 'Albared' cultivar) suggest that these genes may be repressed by the pathogen in the susceptible tissues. In this line, the upregulation at the beginning of the infection

in resistant immature 'Venus', coinciding with the highest fungal biomass on the fruit surface (Balsells-Llauradó et al., 2020), pointed out a putative role towards plant protection, since in these tissues, *M. laxa* did not succeed in causing disease. Although farnesal has only shown antimicrobial activity against human pathogens (Nagaki et al., 2011; Biva et al., 2019), the application of farnesol (a structurally related compound to farnesal) to pepper leaf discs has been shown to reduce the aphid populations (Cantó-Tejero et al., 2021). Overall, we could hypothesize that the slight upregulation of farnesal biosynthesis allowed the resistant tissues (immature 'Venus') to control the pathogen while the susceptible tissues were probably unprotected. Further studies to unravel de production of farnesal and other related compounds under these conditions are encouraged to finally decipher the potential of this terpenoid to control brown rot development.

The upregulation of *PpLIS* paralogs in resistant tissues after 14 hpi and downregulation in susceptible tissues at some time points, suggested that linalool synthase expression could be implicated in protective functions, either through signaling or direct implication of the linalool product. Under various conditions, the cytosolic MVA and plastidial MEP pathways exchange metabolites (Hemmerlin et al., 2012) and hence, linalool could be exclusively synthesized by the MVA pathway as does in strawberry fruit (Hampel et al., 2006). The production of linalool, the major terpenoid in peach fruit (Wang et al., 2009), varies across time in *Monilinia fructicola*-inoculated peaches, i.e., higher production followed by lower production compared to control fruit along time (Liu et al., 2018). In particular, the application of linalool in culture media reduced to around a half the *in vitro* growth of three *Monilinia* spp., including *M. laxa* (Elshafie et al., 2015). Our results show that *PpLIS1* expression in immature resistant 'Venus' fruit was first suppressed in response to *M. laxa* and later activated, probably acting as a defense mechanism. In immature susceptible 'Albared' fruit, one paralog, *PpLIS2*, was first activated probably as a rapid response to cope against the aggressive pathogen; however, both *PpLIS* decreased thereafter, coinciding with the onset of disease symptoms.

Results presented herein demonstrated that the different gene expression patterns of the terpenoid biosynthetic pathways among nectarine cultivars with different susceptibility levels to *M. laxa*, are dependent on the capability of the fruit to activate inducible defenses, potentially, the farnesal and linalool-related biosynthetic pathways. The *M. laxa* inoculation clearly activated the MVA pathway and possible key genes (*PpHMGR1*) were responsive to *M. laxa*. The flux-direction functions of *HMGR*

paralogs associated with the MVA pathway may explain the upregulation of stress-induced genes (e.g., steroids biosynthetic genes) that are implicated in biotic stress response, which in turn, can alter other terpenoid pathways (e.g., farnesal-related). The impaired farnesal-related and linalool biosynthetic gene expression, which have antimicrobial properties, also seemed to be clue in determining the susceptibility to *M. laxa*. This knowledge provides new information regarding the essential terpenoid pathways involved in resistance to *M. laxa*. Further approaches aiming to functionally determine the role of specific terpenoid compounds are encouraged to finally develop new strategies to control brown rot in stone fruit.

Author contributions

Marta Balsells-Llauradó: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Data curation. Núria Vall-llaura: Conceptualization, Methodology, Investigation, Writing- Reviewing and Editing. Josep Usall: Supervision, Funding acquisition, Writing- Reviewing and Editing. Christian J. Silva: Formal analysis, Data curation, Writing- Reviewing and Editing. Barbara Blanco-Ulate: Conceptualization, Methodology, Funding acquisition, Writing- Reviewing and Editing. Neus Teixidó: Investigation, Resources, Writing- Reviewing and Editing. Maria Caballol: Methodology, Investigation, Writing- Reviewing and Editing. Rosario Torres: Conceptualization, Supervision, Project administration, Writing- Reviewing and Editing.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Agudelo-Romero, P., Erban, A., Rego, C., Carbonell-Bejerano, P., Nascimento, T., Sousa, L., et al. (2015). Transcriptome and metabolome reprogramming in *Vitis vinifera* cv. Trincadeira berries upon infection with *Botrytis cinerea*. *J. Exp. Bot.* 66, 1769–1785. doi:10.1093/jxb/eru517.

Alkan, N., and Fortes, A. M. (2015). Insights into molecular and metabolic events associated with fruit response to post-harvest fungal pathogens. *Front. Plant Sci.* 6, 889. doi:10.3389/fpls.2015.00889.

Balsells-Llauradó, M., Silva, C. J., Usall, J., Vall-llaura, N., Serrano-Prieto, S., Teixidó, N., et al. (2020). Depicting the battle between nectarine and *Monilinia laxa*: the fruit developmental stage dictates the effectiveness of the host defenses and the pathogen's infection strategies. *Hortic. Res.* 7, 1–15. doi:10.1038/s41438-020-00387-w.

Baró-Montel, N., Torres, R., Casals, C., Teixidó, N., Segarra, J., and Usall, J. (2019a). Developing a methodology for identifying brown rot resistance in stone fruit. *Eur. J. Plant Pathol.* 154, 287–303. doi:10.1007/s10658-018-01655-1.

Baró-Montel, N., Vall-llaura, N., Usall, J., Teixidó, N., Naranjo-Ortíz, M. A., Gabaldón, T., et al. (2019b). Pectin methyl esterases and rhamnogalacturonan hydrolases: weapons for successful *Monilinia laxa* infection in stone fruit? *Plant Pathol.* 68, 1381–93. doi:10.1111/ppa.13039.

Biswajit Das (2011). *Prunus* diversity- early and present development: A review. *Int. J. Biodivers. Conserv.* 3, 721–734. doi:10.5897/ijbcx11.003.

Biva, I. J., Ndi, C. P., Semple, S. J., and Griesser, H. J. (2019). Antibacterial performance of terpenoids from the australian plant *Eremophila lucida*. *Antibiotics* 8, 6–11. doi:10.3390/antibiotics8020063.

Blanco-Ulate, B., Amrine, K. C., Collins, T. S., Rivero, R. M., Vicente, A. R., Morales-Cruz, A., et al. (2015). Developmental and metabolic plasticity of white-skinned grape berries in response to *Botrytis cinerea* during noble rot. *Plant Physiol.* 169, pp.00852.2015. doi:10.1104/pp.15.00852.

Cantó-Tejero, M., Casas, J. L., Marcos-García, M. Á., Pascual-Villalobos, M. J., Florencio-Ortiz, V., and Guirao, P. (2021). Essential oils-based repellents for the management of *Myzus persica*e and *Macrosiphum euphorbiae*. *J. Pest Sci. (2004).* 1, 1–15. doi:10.1007/s10340-021-01380-5.

Cao, S., Liang, M., Shi, L., Shao, J., Song, C., Bian, K., et al. (2017). Accumulation of carotenoids and expression of carotenogenic genes in peach fruit. *Food Chem.* 214, 137–146. doi:10.1016/j.foodchem.2016.07.085.

Chang, J., Ning, Y., Xu, F., Cheng, S., and Li, X. (2015). Research advance of 3-hydroxy-3-methylglutaryl-coenzyme a synthase in plant isoprenoid biosynthesis. *J. Anim. Plant Sci* 25, 1441–1450.

Elshafie, H. S., Mancini, E., Sakr, S., De Martino, L., Mattia, C. A., De Feo, V., et al. (2015). Antifungal activity of some constituents of *Origanum vulgare* L. essential oil against postharvest disease of peach fruit. *J. Med. Food* 18, 929–934. doi:10.1089/jmf.2014.0167.

FAO (2021). Database of Food and Agriculture Organization of the United Nations. Available at: http://www.fao.org/faostat/en/#data/QC/visualize [Accessed April 29, 2021].

Garcia-Benitez, C., Casals, C., Usall, J., Sánchez-Ramos, I., Melgarejo, P., and De Cal, A. (2020). Impact of postharvest handling on preharvest latent infections caused by *Monilinia* spp. In nectarines. *J. Fungi* 6, 1–14. doi:10.3390/jof6040266.

Garcia-Benitez, C., Melgarejo, P., De Cal, A., and Fontaniella, B. (2016). Microscopic analyses of latent and visible *Monilinia fructicola* infections in nectarines. *PLoS One* 11, e0160675. doi:10.1371/journal.pone.0160675.

Griebel, T., and Zeier, J. (2010). A role for β-sitosterol to stigmasterol conversion in plant-pathogen interactions. *Plant J.* 63, 254–268. doi:10.1111/j.1365-313X.2010.04235.x.

Guidarelli, M., Zubini, P., Nanni, V., Bonghi, C., Rasori, A., Bertolini, P., et al. (2014). Gene expression analysis of peach fruit at different growth stages and with different susceptibility to *Monilinia laxa*. *Eur. J. Plant Pathol.* 140, 503–513. doi:10.1007/s10658-014-0484-8.

Haile, Z. M., Nagpala-De Guzman, E. G., Moretto, M., Sonego, P., Engelen, K., Zoli, L., et al. (2019). Transcriptome profiles of strawberry (*Fragaria vesca*) fruit interacting with *Botrytis cinerea* at different ripening stages. *Front. Plant Sci.* 10. doi:10.3389/fpls.2019.01131.

Hampel, D., Mosandl, A., and Wüst, M. (2006). Biosynthesis of mono- and sesquiterpenes in strawberry fruits and foliage: ^{2}H labeling studies. *J. Agric. Food Chem.* 54, 1473–1478. doi:10.1021/jf0523972.

Hemmerlin, A., Harwood, J. L., and Bach, T. J. (2012). A raison d'être for two distinct pathways in the early steps of plant isoprenoid biosynthesis? *Prog. Lipid Res.* 51, 95–148. doi:10.1016/j.plipres.2011.12.001.

Khare, S., Singh, N. B., Singh, A., Hussain, I., Niharika, K., Yadav, V., et al. (2020). Plant secondary metabolites synthesis and their regulations under biotic and abiotic constraints. *J. Plant Biol.* 63, 203–216. doi:10.1007/s12374-020-09245-7.

Kreidl, S., Edwards, J., and Villalta, O. N. (2015). Assessment of pathogenicity and infection requirements of *Monilinia* species causing brown rot of stone fruit in Australian orchards. *Australas. Plant Pathol.* 44, 419–430. doi:10.1007/s13313-015-0362-7.

Liu, H., Cao, X., Liu, X., Xin, R., Wang, J., Gao, J., et al. (2017). UV-B irradiation differentially regulates terpene synthases and terpene content of peach. *Plant Cell Environ.* 40, 2261–2275. doi:10.1111/pce.13029.

Liu, Q., Zhao, N., Zhou, D., Sun, Y., Sun, K., Pan, L., et al. (2018). Discrimination and growth tracking of fungi contamination in peaches using electronic nose. *Food Chem.* 262, 226–234. doi:10.1016/j.foodchem.2018.04.100.

Lois, L. M., Rodríguez-Concepción, M., Gallego, F., Campos, N., and Boronat, A. (2000). Carotenoid biosynthesis during tomato fruit development: Regulatory role of 1-deoxy-D-xylulose 5-phosphate synthase. *Plant J.* 22, 503–513. doi:10.1046/j.1365-313X.2000.00764.x.

Luo, Y., Michailides, T. J., Morgan, D. P., Krueger, W. H., and Buchner, R. P. (2005). Inoculum dynamics, fruit infection, and development of brown rot in prune orchards in California. *Phytopathology* 95, 1132–1136. doi:10.1094/PHYTO-95-1132.

Mustafa, M. H., Bassi, D., Corre, M.-N., Lino, L. O., Signoret, V., Quilot-Turion, B., et al. (2021). Phenotyping brown rot susceptibility in stone fruit: A literature review with emphasis on peach. *Horticulturae* 7, 115. doi:10.3390/horticulturae7050115.

Muto, A., Müller, C. T., Bruno, L., McGregor, L., Ferrante, A., Chiappetta, A. A. C., et al. (2020). Fruit volatilome profiling through GC × GC-ToF-MS and gene expression analyses reveal differences amongst peach cultivars in their response to cold storage. *Sci. Reports 2020 101* 10, 1–16. doi:10.1038/s41598-020-75322-z.

Nagaki, M., Narita, T., Ichikawa, H., Kawakami, J., and Nakane, A. (2011). Antibacterial and antifungal activities of isoprenoids. *Trans. Mater. Res. Soc. Japan* 36, 55–58.

Obi, V. I., Barriuso, J. J., and Gogorcena, Y. (2018). Peach brown rot: Still in search of an ideal management option. *Agriculture* 8, 1–34. doi:10.3390/agriculture8080125.

Quilot-Turion, B., Corre, M.-N., Costagliola, G., Heurtevin, L., Signoret, V., Raseira, M. D. C., et al. (2020). Wounding nectarine fruit disrupts *Monilinia laxa* infection: deciphering fruit gene pathway involved and the role of phenolic and volatile compounds. in *10th Rosaceae Genomics Conference* (Barcelona, Spain). Available at: hal-03267785%0AHAL [Accessed July 5, 2021].

Rodríguez-Concepción, M., and Gruissem, W. (1999). Arachidonic acid alters tomato *HMG* expression and fruit growth and induces 3-hydroxy-3-methylglutaryl coenzyme A reductase-independent lycopene accumulation. *Plant Physiol.* 119, 41–48. doi:10.1104/pp.119.1.41.

Rungjindamai, N., Jeffries, P., and Xu, X.-M. (2014). Epidemiology and management of brown rot on stone fruit caused by *Monilinia laxa*. *Eur. J. Plant Pathol.* 140, 1–17. doi:10.1007/s10658-014-0452-3.

Shahbandeh, M. (2019). Global fruit production in 2017, by variety. Available at: https://www.statista.com/statistics/264001/worldwide-production-of-fruit-by-variety/ [Accessed December 16, 2019].

Taniguchi, S., Miyoshi, S., Tamaoki, D., Yamada, S., Tanaka, K., Uji, Y., et al. (2014). Isolation of jasmonate-induced sesquiterpene synthase of rice: Product of which has an antifungal activity against *Magnaporthe oryzae*. *J. Plant Physiol.* 171, 625–632. doi:10.1016/J.JPLPH.2014.01.007.

Tholl, D. (2015). Biosynthesis and biological functions of terpenoids in plants. *Adv. Biochem. Eng. Biotechnol.* 148, 63–106. doi:10.1007/10_2014_295.

Tong, Z., Gao, Z., Wang, F., Zhou, J., and Zhang, Z. (2009). Selection of reliable reference genes for gene expression studies in peach using real-time PCR. *BMC Mol. Biol.* 10, 1–13. doi:10.1186/1471-2199-10-71.

Wang, Y. J., Yang, C. X., Li, S. H., Yang, L., Wang, Y. N., Zhao, J. B., et al. (2009). Volatile characteristics of 50 peaches and nectarines evaluated by HP-SPME with GC-MS. *Food Chem.* 116, 356–364. doi:10.1016/j.foodchem.2009.02.004.

Supplementary Material

Supplementary Table S1: List of the primers used for RT-qPCR. From left to right: Target Gene, Gene Abbreviation, Transcript Accession, Reference, Type, Primer Sequence (5´-3') and Primer efficiency (%). Reference or de novo design is also specified.

Target Gene	Gene (Abbreviation)	Transcript Acession	Type	Primer Sequence (5´-3)	Primer Efficiency (%)	Reference
Elongation factor 2	*PpTEF2*	Prupe.4G138900	Fw	GGTGTGACGATGAAGAGTGATG	97.34	Tong et al., 2009
			Rv	TGAAGGAGAGGGAAGGTGAAAG		
Squalene/phytoene synthase / Farnesyl-diphosphate farnesyltransferase	*PpSQS*	Prupe.8G087000.1	Fw	TGTGCCATCCCTCAGATCAT	91.26	*de novo*
			Rv	AGGCCCCTCCTCATTTTGAC		
Squalene monooxygenase	*PpSM*	Prupe.8G156800.1	Fw	GGAGGCATCTGCTCGTATGG	95.31	*de novo*
			Rv	TGATTCCTGACGCACCCAAGA		
Protein farnesyltransferase subunit beta	*PpPFT1*	Prupe.3G284100.1	FW	TACTCCTTTTGGCAGGGAGG	113.90	*de novo*
			RV	AGTAGAGTGTCCTCCAGCGT		
Protein-S-isoprenylcysteine O-methyltransferase	*PpSIMT*	Prupe.1G348900.1	FW	GAGGCCGGAGCTAAACAACA	126.17	*de novo*
			RV	ACTGTAAGGAGCCCTGGAAAA		
Hydroxymethylglutaryl-CoA reductase (NADPH)	*PpHMGR1*	Prupe.8G182300.1	Fw	TGGCATGGAGAGGGAGGATT	101.95	*Balsells-Llauradó et al., 2020*
			Rv	CATGTTGGTAGCATTTCCGGC		
Hydroxymethylglutaryl-CoA synthase	*PpHMGS*	Prupe.5G088900.1	Fw	GCACGAAGCTCACGAAGTCT	122.40	*de novo*
			Rv	GTGCTTTACGTTTGGGAGGC		
(3S)-linalool synthase	*PpLIS1*	Prupe.4G030300.1	Fw	ACAGCAGCAGTTGAGAGATGG	110.21	*de novo*
			Rv	CACCCATGCCTTTTGTACGC		
(3S)-linalool synthase	*PpLIS2*	Prupe.4G030400.1	Fw	TCAACGGCTGGGTATTGACC	100.43	*Liu et al., 2017*
			Rv	TGAGCAGTCGAAAGCGAACT		
Isopentenyl-diphosphate delta-isomerase (IDI or IPI)	*PpIDI*	Prupe.6G361700.1	FW	ACCTGTTGTAGCCATCACTG	99.90	*de novo*
			RV	GCAGCATTTCTTACCCCAAGAG		
Farnesyl diphosphate synthase / Farnesyl pyrophosphate synthase 2 (FPS2 / FPPS2)	*PpFPS2*	Prupe.4G002700.1	FW	GGCGGATCTGAAGTCAAAG	99.72	*Cao et al., 2017*
			RV	CTCCAGGAACATTGTAGTC		
Farnesol kinase / probable phytol kinase 3, chloroplastic	*PpFOLK*	Prupe.6G052700.1	Fw	GTGGGTTCTTCGACCAGAAAC	96.54	*de novo*
			Rv	CCAGAACTGAATAGCGGCCA		

Supplementary Table S2: Pearson's correlation between RNA-Seq (normalized read counts) and relative expression (RT-qPCR) of 'Venus' cultivar.

	RT-qPCR	RNA-Seq (normalized read counts)
PpHMGR	12.063	4323.720
	3.485	3047.618
	7.600	2938.647
	0.746	1598.158
	2.245	1432.919
	1.101	1310.814
	11.055	4668.311
	6.926	6135.954
	13.872	4229.257
	4.001	13533.135
	9.854	22507.212
	94.373	32604.011
PpSQS	0.149	654.205
	0.101	968.411
	0.136	754.868
	0.048	796.844
	0.164	740.765
	0.076	593.491
	0.113	1349.087
	0.152	2140.242
	0.120	1114.850
	0.176	4068.123
	0.454	5165.579
	0.451	10654.299
PpSM	0.152	435.800
	0.124	557.390
	0.229	516.317
	0.025	265.883
	0.057	298.819
	0.019	195.123
	0.177	832.858
	0.254	1589.359
	0.192	755.159
	0.325	3810.175
	0.748	5979.668
	1.178	12017.923
PpPFT	0.101	613.062
	0.068	606.190
	0.081	535.301
	0.024	445.998
	0.072	475.283
	0.028	450.660
	0.048	480.287
	0.039	563.854
	0.050	535.153
	0.020	370.738
	0.017	338.377
	0.013	278.953
PpFPS2	0.109	302.354
	0.112	358.552
	0.182	432.671
	0.086	647.178
	0.123	447.472
	0.044	305.738
	0.136	846.661
	0.257	1631.910
	0.255	679.163
	0.352	3773.634
	0.468	4149.544
	0.756	9029.636

	RT-qPCR	RNA-Seq (normalized read counts)
PpLIS1	0.011	121.955
	0.008	57.712
	0.034	80.993
	0.170	3939.568
	0.478	3574.603
	0.310	5521.363
	0.018	204.870
	0.005	79.888
	0.015	110.844
	0.185	4451.030
	0.222	5685.098
	0.283	6614.120
PpLIS2	0.021	443.731
	0.086	984.558
	0.061	544.347
	0.027	634.463
	0.084	795.224
	0.026	648.204
	0.041	543.966
	0.043	903.652
	0.044	771.860
	0.019	678.869
	0.017	501.434
	0.009	289.355
PpIDI	0.115	820.027
	0.147	675.715
	0.278	731.577
	0.073	1077.277
	0.191	991.109
	0.072	945.053
	0.167	1207.597
	0.152	1379.796
	0.162	1064.808
	0.192	3283.385
	0.289	4324.736
	0.379	6027.532
PpFOLK	0.030	256.936
	0.044	325.721
	0.070	306.504
	0.018	286.720
	0.053	327.032
	0.031	409.139
	0.025	330.515
	0.023	256.344
	0.047	325.544
	0.016	296.018
	0.019	268.990
	0.013	268.706

Pearson's correlation	
R	0.7657
P valor	2.58E-20

Supplementary Table S3: Gene accession, enzyme name and abbreviation of genes related to terpenoid pathway (tab 1) and details of statistical analysis of normalized read counts of 'Venus' cultivar (tab 2).

Tab 1)

Group	Gene accession*	Enzyme name and EC	Enzyme abbreviation
Mevalonate pathway	Prupe.3G196800.1	acetyl-CoA C-acetyltransferase [EC:2.3.1.9]	AACT
	Prupe.5G088900.1	**hydroxymethylglutaryl-CoA synthase [EC:2.3.3.10]**	**HMGS**
	Prupe.8G182300.1	**hydroxymethylglutaryl-CoA reductase (NADPH)**	**HMGR1**
	Prupe.7G187000.1	hydroxymethylglutaryl-CoA reductase (NADPH)	HMGR2
	Prupe.7G187500.1	hydroxymethylglutaryl-CoA reductase (NADPH)	HMGR3
	Prupe.6G327600.1	mevalonate kinase [EC:2.7.1.36]	MK
	Prupe.5G026300.1	phosphomevalonate kinase [EC:2.7.4.2]	PMK
	Prupe.2G078900.1	diphosphomevalonate decarboxylase [EC:4.1.1.33]	MDC
MEP/DOXP pathway	Prupe.1G144100.1	1-deoxy-D-xylulose-5-phosphate synthase [EC:2.2.1.7]	DXS1
	Prupe.6G204700.1	1-deoxy-D-xylulose-5-phosphate synthase [EC:2.2.1.7]	DXS2
	Prupe.5G174000.1	1-deoxy-D-xylulose-5-phosphate reductoisomerase [EC:1.1.1.267]	DXR
	Prupe.5G106300.1	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase [EC:4.6.1.1	MDS
	Prupe.6G101000.1	E-4-hydroxy-3-methylbut-2-enyl-diphosphate synthase [EC:1.17.7.	HDS
	Prupe.8G105800.1	4-hydroxy-3-methylbut-2-enyl diphosphate reductase [EC:1.17.7.4	HDR
Precursors	**Prupe.6G361700.1**	**isopentenyl-diphosphate delta-isomerase [EC:5.3.3.2]**	**IDI**
	Prupe.6G028100.1	farnesyl diphosphate synthase [EC:2.5.1.1 2.5.1.10]	FPS1
	Prupe.4G002700.1	**farnesyl diphosphate synthase [EC:2.5.1.1 2.5.1.10]**	**FPS2**
	Prupe.8G170800.1	geranylgeranyl diphosphate synthase, type II [EC:2.5.1.1 2.5.1.10 2	GGPS1
	Prupe.7G148600.1	geranylgeranyl diphosphate synthase, type II [EC:2.5.1.1 2.5.1.10 2	GGPS2
Sesquiterpenoids	**Prupe.6G052700.1**	**farnesol kinase [EC:2.7.1.216]**	**FOLK**
	Prupe.3G284100.1	**protein farnesyltransferase subunit beta [EC:2.5.1.58]**	**PFT1**
	Prupe.1G191500.1	protein farnesyltransferase/geranylgeranyltransferase type-1 subu	PFT/GGT-I
	Prupe.1G348900.1	**protein-S-isoprenylcysteine O-methyltransferase [EC:2.1.1.100]**	**SIMT**
	Prupe.7G181400.1	ditrans,polycis-polyprenyl diphosphate synthase [EC:2.5.1.87]	DHDDS
	Prupe.4G029900.1	alpha-farnesene synthase [EC:4.2.3.46]	AFS
Triterpenoids	Prupe.3G026800.1	beta-amyrin synthase [EC:5.4.99.39]	AS1
	Prupe.3G026200.1	beta-amyrin synthase [EC:5.4.99.39]	AS2
	Prupe.3G025900.1	beta-amyrin synthase [EC:5.4.99.39]	AS3
Monoterpenoids	**Prupe.4G030300.1**	**3S-linalool synthase [EC:4.2.3.25]**	**LIS1**
	Prupe.4G030400.1	**3S-linalool synthase [EC:4.2.3.25]**	**LIS2**
	Prupe.2G161600.1	(+)-neomenthol dehydrogenase [EC:1.1.1.208]	(+)-ND1
	Prupe.2G162200.1	(+)-neomenthol dehydrogenase [EC:1.1.1.208]	(+)-ND2
	Prupe.2G161500.1	(+)-neomenthol dehydrogenase [EC:1.1.1.208]	(+)-ND3
	Prupe.2G161400.1	(+)-neomenthol dehydrogenase [EC:1.1.1.208]	(+)-ND4
	Prupe.2G161300.1	(+)-neomenthol dehydrogenase [EC:1.1.1.208]	(+)-ND5
	Prupe.2G161100.1	(+)-neomenthol dehydrogenase [EC:1.1.1.208]	(+)-ND6
	Prupe.2G161000.1	(+)-neomenthol dehydrogenase [EC:1.1.1.208]	(+)-ND7
	Prupe.2G160500.1	(+)-neomenthol dehydrogenase [EC:1.1.1.208]	(+)-ND8
	Prupe.2G160400.1	(+)-neomenthol dehydrogenase [EC:1.1.1.208]	(+)-ND9
	Prupe.2G160100.1	(+)-neomenthol dehydrogenase [EC:1.1.1.208]	(+)-ND10
Steroids	**Prupe.8G087000.1**	**squalene/phytoene synthase / farnesyl-diphosphate farnesyltrans**	**SQS**
	Prupe.1G418100.1	squalene monooxygenase [EC:1.14.14.17]	SM1
	Prupe.8G156800.1	**squalene monooxygenase [EC:1.14.14.17]**	**SM2**

*Genes used for gene expression in the 'Albared' cultivar are highlighted in bold.

Tab 2) Details of statistical analysis of normalized read counts of 'Venus' cultivar for each gene. For each gene, two statistical analyses were conducted: (1) Differences of normalized read counts between mock (C) and inoculated (I) tissues for each time point (from 6 to 48 hpi), stage (IM, immature; M, mature) and gene ($P \leq 0.05$, Student's T test). Upregulation in inoculated tissues is indicated in red; downregulation is indicated in green. (2) Differences of normalized read counts along 4 time points for each tissue (C, mock; I, inoculated), stage and gene ($P \leq 0.05$, Tukey's test). When at least the normalized read count in one time point is significantly different from the others, letters are indicated in blue.

	PpAACT	(1)	(2)	***PpHMGS***	(1)	(2)	***PpHMGR1***	(1)	(2)	*PpHMGR2*	(1)	(2)	*PpHMGR3*	(1)	(2)
IM_C_6	1234.7	a	A	1528.5	b	B	1919.8	a	B	1184.0	a	A	157.2	a	A
IM_C_14	1182.6	b	A	1609.9	b	AB	4323.7	a	A	1774.4	a	A	138.9	a	A
IM_C_24	1191.9	b	A	1866.0	b	AB	3047.6	b	B	1198.0	a	A	131.7	a	A
IM_C_48	1319.1	a	A	2010.7	b	A	2938.6	a	B	1534.6	a	A	151.6	a	A
IM_I_6	1638.9	a	B	1911.7	a	C	2353.8	a	C	1369.9	a	A	164.1	a	AB
IM_I_14	1824.1	a	AB	4286.3	a	B	4668.3	a	B	1398.2	a	A	97.0	a	B
IM_I_24	2426.3	a	A	6379.8	a	A	6136.0	a	A	846.5	a	A	109.3	a	AB
IM_I_48	1725.9	a	AB	3146.1	a	BC	4229.3	a	B	1235.1	b	A	218.6	a	A
M_C_6	1732.4	a	A	2464.9	b	A	2275.2	b	A	986.8	a	A	768.8	a	B
M_C_14	1701.9	b	AB	2089.5	b	AB	1598.2	b	B	530.2	a	B	984.1	a	AB
M_C_24	1376.0	b	AB	1933.7	b	B	1432.9	b	B	530.5	a	B	1043.8	a	AB
M_C_48	1279.1	b	B	1861.3	b	B	1310.8	b	B	112.0	b	C	1363.0	a	A
M_I_6	2102.9	a	C	4031.8	a	C	3433.2	a	D	988.3	a	A	693.8	a	B
M_I_14	4657.7	a	B	16071.8	a	B	13533.1	a	C	729.9	a	A	1150.6	a	A
M_I_24	5294.4	a	B	19514.6	a	B	22507.2	a	B	293.4	a	A	1055.0	a	A
M_I_48	8774.5	a	A	35787.1	a	A	32604.0	a	A	751.6	a	B	785.0	b	B

	PpMK	(1)	(2)	*PpPMK*	(1)	(2)	*PpMDC*	(1)	(2)	*PpDXS1*	(1)	(2)	*PpDXS2*	(1)	(2)
IM_C_6	157.4	a	A	648.5	a	A	422.7	a	A	954.9	a	B	76.3	b	B
IM_C_14	89.1	b	B	509.7	b	B	276.3	b	B	1289.4	a	A	117.6	a	A
IM_C_24	117.8	b	AB	609.9	b	A	399.4	b	AB	903.3	a	B	58.3	b	B
IM_C_48	139.9	b	AB	613.3	a	A	400.7	b	AB	952.8	a	B	72.6	a	B
IM_I_6	155.1	a	B	685.0	a	A	514.1	a	C	1059.1	a	AB	123.0	a	A
IM_I_14	213.0	a	AB	610.5	a	A	808.2	a	B	994.1	b	AB	134.3	a	A
IM_I_24	253.3	a	A	733.3	a	A	1603.3	a	A	1102.6	a	A	106.2	a	A
IM_I_48	240.2	a	AB	663.7	a	A	715.3	a	BC	768.5	b	B	75.0	a	A
M_C_6	178.8	a	A	635.9	a	A	734.7	b	A	1431.2	a	C	175.1	a	B
M_C_14	182.0	b	A	513.6	b	BC	636.2	b	AB	2687.1	a	A	261.6	a	A
M_C_24	175.5	b	A	580.6	b	AB	481.2	b	B	1685.0	a	C	119.9	a	B
M_C_48	171.8	b	A	487.7	b	C	535.3	b	AB	2033.8	a	B	111.5	b	B
M_I_6	219.7	a	C	685.9	a	C	1135.7	a	D	1326.3	a	B	174.1	a	A
M_I_14	553.9	a	B	1008.9	a	B	5128.6	a	C	1851.3	b	A	137.8	b	A
M_I_24	625.7	a	B	1166.3	a	B	6724.6	a	B	1321.4	b	B	129.6	a	A
M_I_48	1151.2	a	A	1829.5	a	A	13803.9	a	A	1005.0	b	B	173.0	a	A

	PpDXR	(1)	(2)	*PpMDS*	(1)	(2)	*PpHDS*	(1)	(2)	*PpHDR*	(1)	(2)	*PpIDI*	(1)	(2)
IM_C_6	642.9	a	C	485.2	a	B	2104.4	a	C	888.1	a	C	616.8	a	A
IM_C_14	1093.2	a	A	726.2	a	A	5857.5	a	A	2205.1	a	AB	820.0	b	A
IM_C_24	889.1	a	B	600.3	a	AB	3267.1	a	B	1818.4	a	B	675.7	b	A
IM_C_48	1044.3	a	AB	616.2	a	AB	3702.3	a	B	2257.9	a	A	731.6	b	A
IM_I_6	535.9	b	D	502.3	a	A	2105.5	a	C	943.1	a	C	755.2	a	C
IM_I_14	1223.8	a	A	607.0	a	A	4050.7	b	A	1787.0	b	AB	1207.6	a	AB
IM_I_24	876.9	a	C	541.3	a	A	3385.5	a	AB	1645.5	a	B	1379.8	a	A
IM_I_48	1025.4	a	B	489.8	b	A	2784.6	b	BC	1986.4	b	A	1064.8	a	B
M_C_6	1225.3	a	C	965.4	b	C	3567.5	a	D	1124.9	a	D	1084.4	b	A
M_C_14	1651.0	a	B	1316.4	a	B	6714.8	a	B	1884.8	a	C	1077.3	b	A
M_C_24	1606.7	a	B	1385.4	a	B	5199.2	a	C	2317.6	a	B	991.1	b	AB
M_C_48	2139.5	a	A	1818.2	a	A	8921.3	a	A	3069.7	a	A	945.1	b	B
M_I_6	1279.5	a	B	1088.0	a	AB	3799.0	a	B	1167.2	a	C	1608.8	a	D
M_I_14	1625.6	a	AB	1364.5	a	AB	5536.4	b	A	1657.6	a	B	3283.4	a	C
M_I_24	1810.9	a	A	1431.2	a	A	5896.3	a	A	1833.5	a	B	4324.7	a	B
M_I_48	1617.2	b	AB	1077.1	b	B	4893.8	b	AB	2069.1	b	A	6027.5	a	A

	PpFPS1	(1)	(2)	*PpFPS2*	(1)	(2)	*PpGGPS1*	(1)	(2)	*PpGGPS2*	(1)	(2)	*PpFOLK*	(1)	(2)
IM_C_6	59.3	a	AB	443.4	a	A	736.3	a	A	634.1	a	B	304.5	a	AB
IM_C_14	40.9	b	B	302.4	b	B	960.1	a	A	1223.4	a	A	256.9	b	B
IM_C_24	51.2	b	AB	358.6	b	AB	841.3	a	A	870.3	a	B	325.7	a	A
IM_C_48	69.0	b	A	432.7	b	AB	876.1	a	A	1470.0	a	A	306.5	a	AB
IM_I_6	59.6	a	C	577.2	a	B	931.8	a	A	512.9	a	C	270.0	a	A
IM_I_14	151.1	a	B	846.7	a	B	850.0	a	A	1246.7	a	A	330.5	a	A
IM_I_24	275.1	a	A	1631.9	a	A	827.9	a	A	945.7	a	B	256.3	a	A
IM_I_48	136.2	a	B	679.2	a	B	849.0	a	A	1234.9	a	A	325.5	a	A
M_C_6	84.0	b	A	763.9	b	A	1797.1	a	C	2300.2	b	B	255.9	a	B
M_C_14	116.9	b	A	647.2	b	AB	2208.0	a	AB	4297.7	a	A	286.7	a	B
M_C_24	87.0	b	A	447.5	b	AB	2024.9	a	BC	4286.1	a	A	327.0	a	B
M_C_48	73.8	b	A	305.7	b	C	2472.7	a	A	4347.8	a	A	409.1	a	A
M_I_6	175.9	a	C	1133.4	a	C	2034.9	a	A	2653.6	a	C	265.5	a	A
M_I_14	812.2	a	B	3773.6	a	B	1907.0	b	A	3548.0	b	B	296.0	a	A
M_I_24	946.3	a	B	4149.5	a	B	1894.3	a	A	4350.8	a	A	269.0	a	A
M_I_48	2018.6	a	A	9029.6	a	A	1443.9	b	B	3226.4	b	BC	268.7	b	A

	PpPFT1	(1)	(2)	*PpPFT2/G(*	(1)	(2)	*PpSIMT*	(1)	(2)	*PpDHDDS*	(1)	(2)	*PpAFS*	(1)	(2)
IM_C_6	522.2	a	A	923.8	b	C	160.1	a	A	139.5	a	B	2.3	a	A
IM_C_14	613.1	a	A	1495.7	a	AB	132.5	a	A	452.1	a	A	0.0	a	A
IM_C_24	606.2	a	A	1344.4	a	B	161.5	a	A	132.6	a	B	1.0	a	A
IM_C_48	535.3	a	A	1647.0	a	A	149.8	a	A	151.9	a	B	0.0	a	A
IM_I_6	572.0	a	A	1036.4	a	B	147.9	a	A	122.7	a	B	0.0	a	A
IM_I_14	480.3	a	A	1484.6	a	A	154.7	a	A	342.8	a	A	0.7	a	A
IM_I_24	563.9	a	A	1312.6	a	AB	155.7	a	A	143.0	a	B	1.3	a	A
IM_I_48	535.2	a	A	1408.0	a	A	146.4	a	A	128.3	a	B	0.7	a	A
M_C_6	504.5	a	A	775.8	a	C	114.4	a	A	120.4	a	A	0.5	a	A
M_C_14	446.0	a	A	818.8	a	BC	134.5	a	A	91.0	a	A	0.0	b	A
M_C_24	475.3	a	A	1028.2	a	A	141.9	a	A	58.1	a	A	2.4	b	A
M_C_48	450.7	a	A	940.6	a	AB	157.2	a	A	61.5	a	A	0.0	a	A
M_I_6	503.8	a	A	774.7	a	AB	112.3	a	A	65.8	b	A	0.5	a	A
M_I_14	370.7	a	B	794.5	a	A	124.7	a	A	44.1	a	A	3.7	a	A
M_I_24	338.4	b	B	717.7	b	AB	128.5	a	A	40.9	a	A	9.3	a	A
M_I_48	279.0	b	B	689.2	b	B	94.3	b	A	26.1	a	A	19.8	a	A

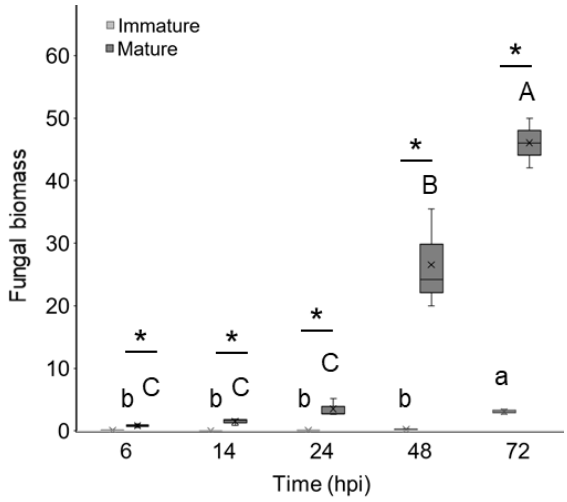
	PpAS1	(1)	(2)	*PpAS2*	(1)	(2)	*PpAS3*	(1)	(2)	*PpLIS1*	(1)	(2)	*PpLIS2*	(1)	(2)
IM_C_6	160.2	a	A	17.4	a	A	2466.1	b	B	124.8	a	A	781.7	a	B
IM_C_14	160.2	b	A	16.0	a	A	3088.6	b	AB	122.0	b	A	443.7	b	C
IM_C_24	175.7	b	A	17.1	b	A	3720.2	b	A	57.7	b	B	984.6	a	A
IM_C_48	134.8	a	A	18.0	b	A	2813.1	b	B	81.0	a	AB	544.3	b	C
IM_I_6	157.6	a	B	19.4	a	B	3543.9	a	B	57.0	b	B	514.3	b	B
IM_I_14	289.0	a	A	48.9	a	AB	5035.9	a	B	204.9	a	A	544.0	a	B
IM_I_24	340.7	a	A	71.4	a	A	9414.6	a	A	79.9	a	B	903.7	a	A
IM_I_48	132.1	a	B	46.9	a	AB	4004.2	a	B	110.8	a	AB	771.9	a	AB
M_C_6	121.4	b	A	7.9	b	AB	2562.9	b	A	1429.4	b	B	682.7	a	A
M_C_14	96.2	b	A	11.5	b	A	1485.0	b	B	3939.6	a	A	634.5	a	A
M_C_24	85.4	b	A	4.9	b	AB	1099.5	b	BC	3574.6	b	AB	795.2	a	A
M_C_48	56.7	b	A	3.6	b	B	897.2	b	C	5521.4	a	A	648.2	a	A
M_I_6	280.2	a	C	13.6	a	B	3289.2	a	D	2319.4	a	B	718.7	a	A
M_I_14	938.3	a	B	133.2	a	A	8008.3	a	C	4451.0	a	AB	678.9	a	A
M_I_24	1010.5	a	B	195.2	a	A	11615.6	a	B	5685.1	a	AB	501.4	b	AB
M_I_48	4252.7	a	A	188.0	a	A	17828.2	a	A	6614.1	a	A	289.4	b	B

	PpSQS	(1)	(2)	*PpSM1*	(1)	(2)	*PpSM2*	(1)	(2)	*PpND1*	(1)	(2)	*PpND2*	(1)	(2)
IM_C_6	1120.8	a	A	492.8	a	A	348.0	a	B	170.1	a	A	0.5	a	A
IM_C_14	654.2	b	B	121.8	a	B	435.8	b	AB	177.0	b	A	3.9	b	A
IM_C_24	968.4	b	A	66.9	a	C	557.4	b	A	184.7	a	A	4.1	b	A
IM_C_48	754.9	b	B	28.8	b	C	516.3	b	AB	220.3	a	A	7.0	b	A
IM_I_6	1211.4	a	B	531.8	a	A	407.0	a	B	195.1	a	A	5.8	a	C
IM_I_14	1349.1	a	B	108.0	a	B	832.9	a	B	218.5	a	A	22.0	a	C
IM_I_24	2140.2	a	A	62.4	a	B	1589.4	a	A	177.3	a	A	77.6	a	B
IM_I_48	1114.8	a	B	50.2	a	B	755.2	a	B	231.8	a	A	175.6	a	A
M_C_6	1318.7	b	A	510.9	a	A	440.3	b	A	156.7	a	A	1.7	a	A
M_C_14	796.8	b	B	345.6	a	B	265.9	b	B	132.1	a	AB	2.0	b	A
M_C_24	740.8	b	B	152.9	a	C	298.8	b	AB	158.6	a	A	0.6	b	A
M_C_48	593.5	b	C	126.6	a	C	195.1	b	B	100.7	a	B	1.0	b	A
M_I_6	1731.6	a	D	490.5	a	A	1005.5	a	D	135.6	b	A	4.6	a	C
M_I_14	4068.1	a	C	251.8	b	B	3810.2	a	C	124.2	a	AB	125.0	a	BC
M_I_24	5165.6	a	B	197.7	a	B	5979.7	a	B	94.7	b	AB	410.4	a	A
M_I_48	10654.3	a	A	89.9	a	C	12017.9	a	A	88.9	a	B	337.8	a	AB

	PpND3	(1)	(2)	*PpND4*	(1)	(2)	*PpND5*	(1)	(2)	*PpND6*	(1)	(2)	*PpND7*	(1)	(2)
IM_C_6	20.6	a	A	1.2	a	A	60.9	a	A	129.5	a	A	15.9	a	A
IM_C_14	6.6	a	A	4.7	a	A	57.4	b	A	175.3	a	A	21.1	b	A
IM_C_24	20.1	b	A	2.7	b	A	62.2	b	A	145.3	a	A	18.9	b	A
IM_C_48	9.8	b	A	8.1	b	A	67.4	b	A	110.3	a	A	23.9	b	A
IM_I_6	17.3	a	B	2.0	a	B	66.5	a	C	106.4	a	B	18.9	a	B
IM_I_14	32.1	a	B	20.8	a	B	127.6	a	BC	167.3	a	A	50.4	a	B
IM_I_24	47.3	a	AB	44.6	a	A	181.5	a	AB	170.4	a	A	86.8	a	B
IM_I_48	78.6	a	A	48.5	a	A	250.4	a	A	157.6	a	AB	163.3	a	A
M_C_6	21.7	b	B	5.7	a	A	112.1	a	A	159.4	a	A	28.3	a	A
M_C_14	14.9	b	B	4.9	b	A	91.5	b	A	106.9	a	B	16.6	b	A
M_C_24	24.2	b	AB	1.8	b	A	86.8	b	A	121.7	a	AB	20.6	b	A
M_C_48	33.2	b	A	2.9	b	A	81.8	b	A	97.6	b	B	13.0	b	A
M_I_6	35.0	a	B	7.5	a	C	103.1	a	C	135.5	a	AB	35.8	a	C
M_I_14	176.5	a	B	118.1	a	B	489.2	a	BC	104.4	a	B	259.5	a	BC
M_I_24	244.7	a	AB	240.7	a	A	975.5	a	B	124.4	a	AB	510.9	a	AB
M_I_48	467.2	a	A	196.1	a	AB	1934.4	a	A	221.5	a	A	948.2	a	A

	PpND8	(1)	(2)	*PpND9*	(1)	(2)	*PpND10*	(1)	(2)
IM_C_6	32.3	a	A	4.7	a	A	249.5	a	A
IM_C_14	31.4	a	A	14.2	b	A	249.1	a	A
IM_C_24	37.3	b	A	5.0	b	A	260.1	a	A
IM_C_48	57.8	b	A	8.6	b	A	303.6	a	A
IM_I_6	39.6	a	B	23.6	a	B	219.5	a	B
IM_I_14	77.9	a	B	211.8	a	AB	310.1	a	AB
IM_I_24	76.4	a	B	395.1	a	A	275.5	a	AB
IM_I_48	137.6	a	A	415.7	a	A	359.1	a	A
M_C_6	58.1	a	A	20.3	b	A	295.6	a	B
M_C_14	51.3	a	A	38.1	b	A	421.5	a	A
M_C_24	49.4	b	A	8.7	b	A	419.1	a	A
M_C_48	41.3	b	A	9.2	b	A	286.1	b	B
M_I_6	51.3	a	A	76.7	a	C	258.6	a	B
M_I_14	85.8	a	A	1821.4	a	BC	313.8	b	B
M_I_24	123.8	a	A	3899.2	a	B	390.5	a	AB
M_I_48	134.4	a	A	7904.7	a	A	505.1	a	A

Supplementary Figure S1. Fungal biomass in *M. laxa*-inoculated nectarines in the 'Albared' cultivar. Assessment of *M. laxa* biomass by relative gene expression of *M. laxa* reference gene (*MlACT*), normalized to nectarine reference gene (*PpTEF2*) in both stages (immature and mature) of *M. laxa*-inoculated fruit. The box plot represents the mean of three biological replicates with its interquartile range. Lowercase and uppercase letters indicate significant differences through time ($P \leq 0.05$, Tukey's test) in immature and mature fruit, respectively. Asterisks indicate significant differences between stages at each time point ($P \leq 0.05$, Student's *T* test).



Chapter 5. Emission of volatile organic compounds during nectarine-*Monilinia laxa* interaction and its relationship with fruit susceptibility to brown rot

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Abstract

Fruit volatile organic compounds (VOCs) can be emitted by stone fruit in response to biotic stress. One of the main fungal diseases of stone fruit is brown rot, caused by species of *Monilinia* spp. Hence, we aimed to reveal the most relevant VOCs that participate either in resistance or susceptibility to *Monilinia laxa* in nectarines. For that, we analyzed the VOCs emitted by two developmental stages of two nectarine cultivars with different susceptibility to *M. laxa*. Besides, we also determined the VOCs profile of *M. laxa* grown in *in vitro* culture media based on peach juice. Results elucidated 34 VOCs whose production pattern was different among samples (control and inoculated of both stages and cultivars), being 13 VOCs also emitted by *M. laxa* culture. A hierarchical analysis and a multivariate analysis exhibited the variations in the VOCs profile of all samples according to their susceptibility to *M. laxa*, and the suitability of the model to predict the *M. laxa* disease (91.94 % of the total variation). In general, results highlighted i) a group of VOCs, positively correlated with *M. laxa* disease, that were emitted by visual *M. laxa* symptomatic tissues (e.g., aldehyde (E,E)-2,6-nonadienal) and also by *M. laxa* itself (e.g., terpenoids alpha-muurolene and (E)-beta-ionone), and ii) a group of VOCs, negatively correlated with brown rot disease, that were emitted by visual *M. laxa* symptomatic tissues (e.g., ketone butyrolactone and aldehyde (E)-2-decenal) and also by *M. laxa* itself (e.g., aldehyde decanal), suggesting an antifungal role of these compounds. Therefore, this study provides putative potential VOCs that not only will help to improve the knowledge of brown rot development on nectarines, but also provides target volatiles that may serve as potential brown rot control compounds.

Keywords: postharvest, storage, developmental stages, stone fruit, fruit volatiles, fungal volatiles

Introduction

Fruit is continuously exposed to a variety of biotic and abiotic stresses. All these stresses cause, among others, an oxidative stress, metabolic imbalances, alteration of hormone responsive pathways and programmed cell death (Vickers et al., 2009; Alkan and Fortes, 2015). The most common biotic stress affecting fruit, in particular stone fruit, is caused by phytopathogenic fungi, being *Monilinia* spp., the causal agent of brown rot, one of the main fungal diseases of this fruit (Mustafa et al., 2021). To protect themselves against these biotic stresses, fruit can produce a plethora of secondary metabolites, including the emission of volatile organic compounds (VOCs) (Baldwin et al., 2006). Volatiles are classified in several chemical categories, among which the most common in peach are alcohols, aldehydes, C6 compounds, C9 compounds, C13 norisoprenoid, esters, lactones, ketones, phenylalanine derived compounds and terpenoids (Wang et al., 2009; Montero-Prado et al., 2013; Xi et al., 2017). Terpenoid-derived VOCs are of special interest since they are considered the largest class of plant VOCs (Abbas et al., 2017). In fact, in a recent study, Balsells-Llauradó, et al. (unpublished) showed the importance of this metabolism in determining the resistance/susceptibility of nectarines against brown rot.

In general, VOCs have direct defensive functions by acting on the pathogen, such as inhibiting the germination, the *in vitro* growth and development, or changing the activity of specific enzymes, among others (Mari et al., 2016). Alternatively, VOCs can act by activating the defensive response of the plant (induced resistance), and thus are also considered a sustainable strategy to control postharvest decays (Romanazzi et al., 2016). For instance, treatments with volatile esters in tomato resulted in stomatal closure, induction of pathogenesis-related genes, and enhanced resistance to *Pseudomonas syringae* pv. *tomato* (López-Gresa et al., 2018). In the specific case of brown rot, several VOCs have shown antimicrobial activity against *Monilinia* spp. in *in vitro* and *in vivo* conditions, controlling the postharvest brown rot decay (Mari et al., 2016; Gotor-Vila et al., 2017). For instance, thyme oil vapor (with monoterpene thymol as active ingredient) increases the activity of defense-related enzymes (e.g., chitinase) and total phenolic content, which results in a reduction of *Monilinia laxa* incidence in peaches (Cindi et al., 2016). Previous studies have demonstrated that peaches inoculated with major postharvest fungi of stone fruit (*Botrytis cinerea, Monilinia fructicola* and *Rhizopus stolonifer*) significantly emitted up to eight VOCs different to mock-inoculated fruit (Liu et al., 2018). The cited study was conducted to test the use of volatiles as marker molecules to detect early fungal infections in postharvest

chambers. Until now, just only one study evaluated the emission of plant volatiles in fruit tissues with different susceptibility to *M. laxa*. In this study, Dini (2019) studied the VOCS emitted in immature nectarines compared with wounded immature nectarines in which the disease progressed slowly compared to ripe fruit, although they did not find a promising relation.

Herein, we aimed to elucidate the most relevant VOCs emitted by unwounded nectarine tissues with different susceptibility to *M. laxa*. Besides, as *Monilinia* spp. are also able to emit VOCs in *in vitro* conditions (Mang et al., 2015), which can also interfere during the plant-pathogen interactions, we also aimed to analyze the volatile profile of *M. laxa* during *in vitro* growth on media based on peach juice in order to try to discern among volatiles emitted by either the pathosystem or the pathogen itself. Findings from this study would lead to identify volatiles emitted by nectarines in response to brown rot infection but also, those volatiles that may be helpful to further define nectarine defense mechanisms against *M. laxa*, and thus, useful in brown rot control strategies development.

Materials and methods

Plant material, fruit quality and fungal cultures

Two organically grown cultivars ('Venus' and 'Albared') of nectarine [*Prunus persica* var. *nucipersica* (Borkh.) Schneider] were used for the experiments. Nectarines were obtained from an orchard located in Lleida (Catalonia, Spain). White paper bags, impregnated with paraffin wax, were used to bag fruit at least 6 weeks before harvest to avoid the presence of natural occurring inoculum. Fruit was harvested at two different fruit developmental stages: "immature" and "mature" fruit. Mature stage corresponded to commercially harvest date, established according to grower's recommendations, and immature stage was harvested 3 and 4 weeks before the mature stage for 'Venus' and 'Albared', respectively. Fruit was homogenized by using a portable DA-Meter (TR-Turoni, Forli, Italy), based on the single index of absorbance difference. Fruit quality between stages were further confirmed by assessing the flesh firmness (FF), total soluble solids content (SSC), and titratable acidity (TA), following previously described protocols (Baró-Montel et al., 2019a) (Table 1).

Table 1. Fruit quality parameters of immature and mature 'Venus' and 'Albared' cultivars on harvest day. *Maturity date, minimum and maximum values of single index of absorbance difference (I_{AD}), flesh firmness (FF), soluble solids content (SSC) and titratable acidity (TA) of 'Venus' and 'Albared' nectarine cultivars. Data represent the mean (n = 20 fruit) ± Standard Error.*

Cultivar	Maturity date[1]	I_{AD}	FF (N)	SSC (%)	TA (g malic acid L^{-1})
'Venus' Immature	189	1.6 - 2.1	83.3 ± 3.4	9.0 ± 0.2	5.2 ± 0.1
'Venus' Mature	206	0.4 - 1.3	63.6 ± 2.9	10.4 ± 0.3	9.6 ± 0.1
'Albared' Immature	209	1.9 - 2.2	94.5 ± 1.8	12.9 ± 0.3	5.3 ± 0.1
'Albared' Mature	241	0.2 - 1.4	84.4 ± 2.4	16.0 ± 0.3	11.1 ± 0.28

[1] *Maturity date is expressed as Julian days (e.g., January 1st is considered as day 1).*

Monilinia laxa single-spore strain 8L (ML8L, Spanish Culture Type Collection number CECT 21100) was used and conidial suspensions were maintained and prepared as previously described by Baró-Montel et al. (2019b). Potato dextrose agar (PDA; Biokar Diagnostics, 39 g L^{-1}) supplemented with 25 % tomato pulp was used for culture media and incubation was conducted under photoperiod conditions (12 h light at 25 ºC/12 h dark at 18 ºC). Conidial suspensions were obtained by rubbing the surface of a 7-day-old culture with sterile water containing 0.01 % (w/v) Tween-80 and filtered conidia suspensions were diluted to the desired concentration using an hemocytometer.

Fruit inoculations and sampling

Inoculations, incubation and sampling for volatile organic compounds (VOCs) profile analyses were conducted as previously described by Balsells-Llauradó et al. (2020). Briefly, six drops of 30 µL of *M. laxa* conidial suspension (10^6 conidia mL^{-1}) or sterile water containing 0.01 % (w/v) Tween-80 (control) were applied on each fruit. Fruit was incubated in containers with a relative humidity of 97 % ± 3 and 20 ºC ± 1 temperature under darkness conditions. The assay was conducted with three replicates consisting of seven fruit each per treatment. Sampling was carried out at 3 days post inoculation (dpi) by freezing in liquid nitrogen six cylinders of peel and pulp tissue (1 cm) encompassing the inoculation sites. Frozen samples were ground into powder and stored at -80 ºC until further analysis.

In vitro growth of *M. laxa* and sampling

To assess the VOCs emitted by *M. laxa*, 50 mL-flasks containing 30 mL of peach juice based-medium (100 % of organic peach juice, pH = 4.0) were inoculated with conidial suspensions to a final concentration of 2 x 10^4 conidia mL^{-1}. Flasks were incubated at 20 ºC ± 1 under complete darkness. Sampling was conducted at 3 and 7 dpi by extracting the mycelium from the top of the liquid media and rinsing with sterile water to remove the medium residues. Mycelia were immediately flash-frozen in liquid nitrogen, ground into powder, and stored at −80 °C until further analysis. Three biological replicates were conducted.

Analyses of VOCs

Sample preparation and headspace solid-phase microextraction (HS-SPME)

Headspace solid-phase microextraction (HS-SPME) was performed for extracting and determining the VOCs emitted both by the nectarine-*M. laxa* study and *M. laxa in vitro* study. SPME fiber coated with a 50/30 µm layer of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (Supelco Co., Bellefonte, PA, USA) was used after being activated, according to the manufacturer's instructions. For each extraction, 5 or 1.5 g of frozen homogenized plant tissue or *M. laxa* mycelium were mixed with 5 mL or 1.5 mL, respectively, with 20 % (w/v) NaCl into a 28 mL screw-capped glass vial (previously cooled) to facilitate the release of volatile compounds. A volume (2 µL) of 3-nonanone (0.82 g L^{-1}) was added as an internal standard, whose absence was previously checked in all samples. Vials were immediately sealed with a magnetic screw cap provided with a PTFE/silicone septum. To undergo the same temperature treatment, once prepared, samples were stored at -20 ºC until use. Slowly thawing was performed one hour before the incubation at room temperature. For volatile extraction and determination, each sample was incubated for 20 min at 40 ºC with stirring (600 rpm) and then, the SPME fiber was exposed to the headspace of the sample for 30 min under the same conditions for volatiles absorption.

Gas chromatography/mass spectrometry (GC-MS)

The compounds were separated, identified, and quantified with a 7890A gas chromatograph in conjunction with 5977A MSD mass spectrometry (GC-MS) (Agilent Technologies, Inc.). The volatile compounds were desorbed from the fiber for 5 min at 220 °C into the injection port (spitless mode) of the chromatograph, which had a cross-linked polyethylene glycol-TPA (FFAP) (50 m × 200 µm × 0.33 µm) as the capillary column. Helium at 1.0 mL min^{-1} was used as the carrier gas. Solvent delay was 5 min. Temperatures of source and quadrupole were 230 and 150 ºC, respectively. The oven program was 60 ºC for 1 min, then the temperature rose at 3 °C min^{-1} to 135 °C, followed by another constant ramp of 4 °C min^{-1} to 225 °C, and held at that temperature for 15 min. The total run time was 63.25 min. Mass spectra for each compound were obtained by electron impact ionization at 70 eV. The scan mode was used to detect all the compounds from 30 to 300 m/z. Compounds were identified by comparing the mass spectral data obtained with those from standards from the National Institute of Standards and Technology (NIST) Mass Spectral Library (NIST11.L). Data of VOCs for each sample were relativized using the concentration of the internal standard (3-nonanone).

Statistical analysis

Data were analyzed with JMP® software version 16.0.0 (SAS Institute Inc., Cary, NC, USA). As a pre-treatment, data were adjusted for the relation between dry fresh weight/fresh weight aiming to obviate the changes in water content occurring during fruit development. A hierarchical cluster analysis (HCA) dendrogram was conducted based on Ward's method. The dendrogram graph of the HCA and heat maps were conducted to establish a relationship between all analyzed VOCs (n=34) among cultivars, developmental stages, and treatments (8 samples). As a pre-treatment, data were centered and weighted by the inverse of the standard deviation for each variable. A partial least square (PLS) analysis was conducted to correlate all 34 VOCs (X variables or explanatory variables) with brown rot incidence and severity (Y variable or response). Values of incidence and severity were taken from previous works (Balsells-Llauradó, unpublished). The non-linear iterative partial least squares (NIPALS) algorithm with two factors was used for estimating the model parameters. Data for selected VOCs were subjected to analysis of variance (ANOVA). Tukey's HSD test ($P \leq 0.05$) was performed for means separation among all 8 fruit samples. Student's T-test ($P \leq 0.05$) was conducted between 3 and 7 dpi *M. laxa* samples.

Results

The distribution of VOCs is associated with the degree of brown rot symptoms

To evaluate the volatile profile of tissues with different susceptibility to *M. laxa*, two developmental stages of two different cultivars were artificially inoculated with *M. laxa*. The *M. laxa*-inoculated immature 'Venus' fruit did not show brown rot symptoms, whereas the pathogen caused a maceration and rotting in immature 'Albared' fruit and mature nectarines of both cultivars (Supplementary Figure S1). Brown rot disease evaluation showed that *M. laxa*-inoculated 'Albared' mature nectarines exhibited the highest incidence (100 %) and severity (3.4 cm ± 0.07), followed by 'Venus' mature (95 % and 2.6 cm ± 0.21) and 'Albared' immature (80 % and 0.8 cm ± 0.18), and that no visual disease symptoms were observed in *M. laxa*-inoculated 'Venus' immature nectarines (data from Balsells-Llauradó, unpublished). In the VOCs analysis of the nectarine-*M. laxa* study, a total of 34 VOCs were finally identified and quantified among all groups of samples. These VOCs included 10 aldehydes, 7 ketones, 5 acids, 4 alcohols, 3 benzenoids, 3 terpenoids, 1 ester, and 1 furan (Table 2). To explore the variations in the VOCs profile of both cultivars harvested at two different developmental stages and tissue (control or inoculated), an HCA was performed integrating all VOCs data (Figure 1). The hierarchical graph showed that samples can be grouped in two main clusters, *i*) *M. laxa*-inoculated mature fruit (P1), and *ii*) the rest of samples subdivided into control mature 'Venus' samples (P2), and control mature 'Albared' samples and all immature tissues (P3).

Table 2. *VOCs and their chemical categories detected in the nectarine-*M. laxa *study at 3 dpi.*

Organic compound families	Volatile compound
Aldehydes	(E,E)-2,4-Heptadienal
	2,5-Furandicarboxaldehyde
	(E,E)-2,6-Nonadienal
	(E)-2-Decenal
	(E)-2-Octenal
	3-Furaldehyde
	5-Hydroxymethylfurfural
	Benzaldehyde
	2-Hydroxy-benzaldehyde
	Decanal
Ketones	3-methyl-2-pent-2-enylcyclopent-2-en-1-one
	4-acetyl-2,3,4,5,5-pentamethyl-2-cyclopenten-1-one
	2H-Pyran-2,6(3H)-dione
	6-Pentyl-2H-pyran-2-one
	2-Hydroxy-gamma-butyrolactone
	4-Cyclopentene-1,3-dione
	Butyrolactone
Acids	3-Furancarboxylic acid
	Acetic acid
	n-Decanoic acid
	Nonanoic acid
	Octanoic acid
Alcohols	2-Ethyl-1-hexanol
	(E)-2-Hexen-1-ol
	2/3-Furanmethanol
	(Z)-3-Nonen-1-ol
Benzenoids	1,3-bis(1,1-dimethylethyl)-Benzene
	Benzyl nitrile
	Phenoxybenzene
Terpenoids	(E)-beta-ionone
	alpha-Muurolene
	3,7-Dimethyl-1,6-octadien-3-ol (linalool)
Ester	(3E,6E)-Nonadien-1-yl-acetate
Furan	2-Ethyl furan

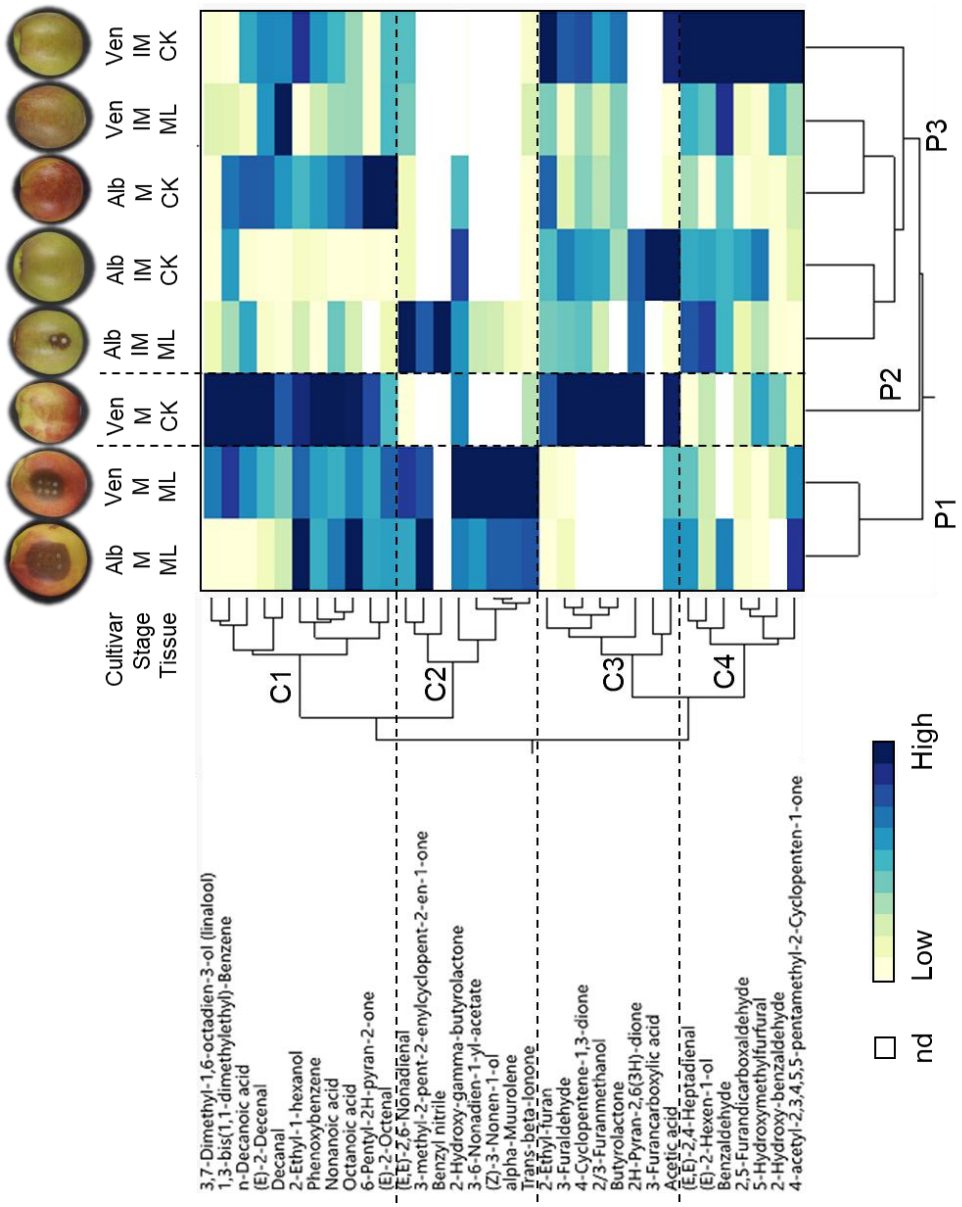

Figure 1. A two-way hierarchical cluster analysis (HCA) and heat map of the VOCs identified in each cultivar, stage, and tissue. *For each group of replicates (n=3), the cultivar ('Venus', Ven; 'Albared', Alb), the developmental stage (immature, IM; mature, M) and tissue (control, CK; M. laxa-inoculated, ML) are specified. Fruit images correspond to each sample at 3 dpi. (Suppl. Figure S1). Clusters for compounds (Lines; C1 to C4) and for samples (Columns; P1 to P3) are indicated. Colors indicate the relative quantity to the internal standard (3-nonanone) for each VOCs, where yellow represents low concentration and blue depicts high concentration. Empty cells (white) indicate non-detected compound ("nd"). Detailed data is available in Suppl. Table S1.*

To further explore the relationship between the VOCs emitted during the *M. laxa*-nectarine interaction and the susceptibility to the pathogen, data were integrated on a multivariate analysis, correlating the VOCs produced during the interaction (X explanatory variables) with the incidence and severity of *M. laxa* (Y variables) (Figure 2). The PLS model showed that the two PLS factors accounted for 91.94 % of the variation observed in the total *M. laxa* incidence and severity (Figure 2A). In particular, the first factor of the PLS correlation loading plot explained 83.72 % of the incidence and severity of *M. laxa,* and clearly separated the tissues with *M. laxa* symptoms from the visual asymptomatic or control samples. Besides, the correlation between measured and predicted incidence and severity demonstrated the effectiveness of the model (R^2 = 0.8803 and R^2 = 0.9585, respectively) for predicting brown rot incidence and severity, respectively. The variable importance plot (VIP) of the PLS model revealed 17 VOCs whose values were equal to or higher than 0.8 (Figure 2B), and hence considered the most influential volatiles determining the PLS projection model and explaining the variable susceptibility to *M. laxa* among the different samples analyzed.

VOCs can be grouped by clusters and by their correlation with brown rot disease

Analyzing in detail Figure 1, 4 main clusters were deployed according to the relationship between VOCs (Figure 1). Cluster 1 (C1) groups VOCs that, in general, were abundantly emitted by mature tissues (for both control and inoculated fruit). Among them, (E)-2-decenal and decanal had a significant VIP value (VIP ≥ 0.8) and were negatively correlated with the incidence and severity of *M. laxa*, whereas 2-ethyl-1-hexanol (VIP ≥ 0.8) was positively correlated with *M. laxa* disease (Figure 2). However, their relative quantification to the internal standard (3-nonanone) was almost similar across all samples (Tables 3 and 4).

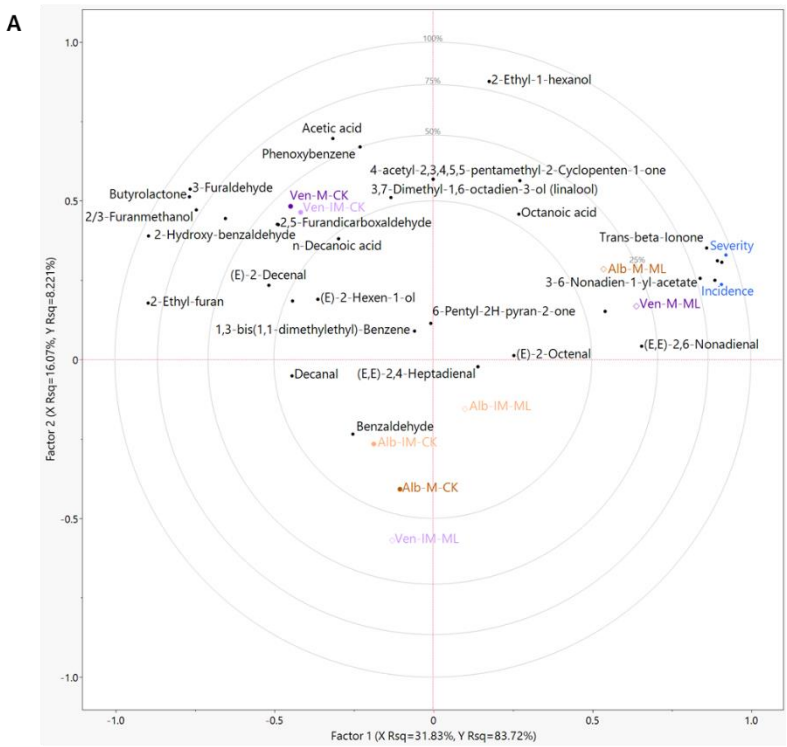
A
2-Ethyl-1-hexanol
Acetic acid
Phenoxybenzene
4-acetyl-2,3,4,5,5-pentamethyl-2-Cyclopenten-1-one
3-Furaldehyde
Butyrolactone
Ven-M-CK
Ven-IM-CK
3,7-Dimethyl-1,6-octadien-3-ol (linalool)
2/3-Furanmethanol
2,5-Furandicarboxaldehyde
Octanoic acid
2-Hydroxy-benzaldehyde
n-Decanoic acid
Trans-beta-Ionone
Severity
Alb-M-ML
(E)-2-Decenal
3-6-Nonadien-1-yl-acetate
Incidence
2-Ethyl-furan
(E)-2-Hexen-1-ol
Ven-M-ML
6-Pentyl-2H-pyran-2-one
1,3-bis(1,1-dimethylethyl)-Benzene
(E,E)-2,6-Nonadienal
(E)-2-Octenal
Decanal
(E,E)-2,4-Heptadienal
Alb-IM-ML
Benzaldehyde
Alb-IM-CK
Alb-M-CK
Ven-IM-ML
Factor 2 (X Rsq=16.07%, Y Rsq=8.221%)
Factor 1 (X Rsq=31.83%, Y Rsq=83.72%)
B
VIP
0.98
1.51
1.28
1.85
0.83
0.93
0.98
1.44
1.11
1.11
1.64
1.37
0.97
1.61
1.58
1.15
0.92
3,7-Dimethyl-1,6-octadien-3-ol (linalool)
2-Ethyl-1-hexanol
(E,E)-2,4-Heptadienal
2-Ethyl furan
2,5-Furandicarboxaldehyde
(E,E)-2,6-Nonadienal
3-methyl-2-pent-2-enylcyclopent-2-en-1-one
4-acetyl-2,3,4,5,5-pentamethyl-2-Cyclopenten-1-one
(E)-2-Decenal
(E)-2-Hexen-1-ol
2H-Pyran-2,6(3H)-dione
6-Pentyl-2H-pyran-2-one
2-Hydroxy-gamma-butyrolactone
(3E,6E)-Nonadien-1-yl-acetate
(E)-2-Octenal
3-Furaldehyde
2/3-Furanmethanol
(Z)-3-Nonen-1-ol
4-Cyclopentene-1,3-dione
5-Hydroxymethylfurfural
Acetic acid
alpha-Muurolene
Benzaldehyde
2-Hydroxy-benzaldehyde
1,3-bis(1,1-dimethylethyl)-Benzene
(E)-beta-Ionone
Butyrolactone
Decanal
Phenoxybenzene
n-Decanoic acid
Nonanoic acid
Octanoic acid

Figure 2. A) Partial Least Squares (PLS) correlation loading plot showing the contribution of each volatile to* M. laxa *incidence and severity. *Black labels indicate VOCs (explanatory variables). Blue labels indicate incidence and severity of M. laxa (Y variables). Color dots (●) and diamonds (◊) indicate control and* M. laxa*-inoculated samples, respectively, of 'Venus' (purple) and 'Albared' (orange) cultivars. Their labels indicate the cultivar ('Venus', Ven; 'Albared', Alb), the developmental stage (mature, M; immature, IM) and tissue (control, CK; M. laxa-inoculated, ML).* ***B) Variable importance plot (VIP) of the PLS model.*** *The number of VIP ≥ 0.8 (red dashed line) indicates which predictors are important in explaining the Y variables (*M. laxa *incidence and severity) used in the PLS model. VIP values of the VOCs that have VIP ≥ 0.8, are indicated.*

Cluster 2 (C2) shows those VOCs that, overall, were produced by *M. laxa* symptomatic tissues (Figure 1), indicating that they could be produced either by the host, the pathogen or both. The alpha-muurolene and (E)-beta-Ionone (both with VIP ≥ 0.8) were positively correlated with *M. laxa* disease (Figure 2). Their emission in tissues with high *M. laxa* incidence (mature fruit of both cultivars) were significantly higher, being 24.0- and 4.5-fold higher (in average) than the other tissue with less disease symptoms (*M. laxa*-inoculated immature 'Albared' nectarines) (Table 4). Besides, alpha-muurolene was not detected either in the asymptomatic tissue or control samples. Other compounds (3-methyl-2-pent-2-enylcyclopent-2-en-1-one, (3E,6E)-nonadien-1-yl-acetate and (Z)-3-nonen-1-ol) had also significant VIP values (VIP ≥ 0.8), were positively correlated with *M. laxa* disease, and exclusive emitted by tissues with visible *M. laxa* symptoms (Figure 2, Table 4). Remarkably, benzyl nitrile was only detected in *M. laxa*-inoculated immature 'Albared' nectarines (Figure 1).

VOCs from the cluster 3 (C3) of the HCA were, in general, more produced in control mature and immature tissues with low or no visual *M. laxa* symptoms than in tissues with advanced disease symptoms (Figure 1). Among them, 5 VOCs had significant VIP values (VIP ≥ 0.8) and were negatively correlated with *M. laxa* disease (Figure 2). The quantity of these compounds tends to be higher in control than in *M. laxa*-inoculated fruit, and only few significant differences were detected among all samples. For instance, the emission values of 3-furaldehyde for control immature and mature 'Venus' nectarines were 4.4- and 7.3-fold significantly higher, respectively, than those for *M. laxa*-inoculated 'Venus' fruit (Table 3). In turn, in 'Albared' cultivar, this compound showed a similar pattern among samples, although not statistically significant. For other VOCs such as 2/3-furanmethanol, 4-cyclopentene-1,3-dione, and butyrolactone, their production was lower in inoculated tissues with low or no *M. laxa* disease symptoms compared to control samples, and even not detected in tissues with high disease incidence. These results showed a clear pattern caused by the emission of these VOCs mainly due to the presence of pathogen on nectarine tissue rather than developmental stages and/or cultivar.

Taula 3. Relative quantification of VOCs (VIP ≥ 0.8) negatively correlated with *M. laxa* incidence and severity. Data is presented relative to the internal standard (3-nonanone). Each value represents the mean (n = 3) ± Standard Error. Different letters indicate significant differences (P ≤ 0.05) among all samples for each VOC. Non-detected compound are indicated as "nd".

		Immature				Mature			
		'Venus'		'Albared'		'Venus'		'Albared'	
Cluster	Compound	Control	*M. laxa* -inoc.	Control	*M. laxa* -inoc.	Control	*M. laxa*- inoc.	Control	*M. laxa* -inoc.
C1	(E)-2-Decenal	1.26 ± 0.34 ab	1.18 ± 0.21 ab	0.37 ± 0.09 b	0.43 ± 0.03 b	1.86 ± 0.72 a	0.99 ± 0.04 ab	1.45 ± 0.21 ab	0.48 ± 0.06 ab
C1	Decanal	1.09 ± 0.23 a	1.55 ± 0.01 a	0.43 ± 0 a	0.44 ± 0 a	1.26 ± 0.06 a	0.80 ± 0.05 a	1.04 ± 0.27 a	0.64 ± 0.2 a
C3	2-Ethyl furan	2.33 ± 0.52 a	1.01 ± 0.07 ab	1.16 ± 0.29 ab	1.03 ± 0.11 ab	1.83 ± 0.63 ab	0.40 ± 0.06 b	1.76 ± 0.41 ab	0.32 ± 0.09 b
C3	3-Furaldehyde	3.80 ± 1.12 a	0.87 ± 0.06 b	3.23 ± 1.01 ab	1.97 ± 0.73 ab	5.10 ± 0 a	0.70 ± 0.12 b	1.19 ± 0.11 ab	0.97 ± 0.14 ab
C3	2/3-Furanmethanol	1.27 ± 0 b	0.33 ± 0.02 b	1.09 ± 0.38 b	0.35 ± 0.07 b	3.10 ± 0.46 a	nd	0.45 ± 0.02 b	nd
C3	4-Cyclopentene-1,3-dion	0.90 ± 0 a	0.31 ± 0.01 a	0.58 ± 0.18 a	0.41 ± 0.01 a	1.18 ± 0.49 a	nd	0.34 ± 0.02 a	nd
C3	Butyrolactone	0.52 ± 0.12 ab	0.18 ± 0 ab	0.15 ± 0.03 b	nd	0.92 ± 0 a	nd	0.18 ± 0 ab	nd
C4	5-Hydroxymethylfurfural	20.69 ± 2.38 a	1.11 ± 0.1 c	11.41 ± 3.46 b	3.67 ± 0.58 c	7.71 ± 0 bc	0.83 ± 0.19 c	1.37 ± 0.2 c	0.85 ± 0.13 c

Taula 4. Relative quantification of VOCs (VIP ≥ 0.8) positively correlated with *M. laxa* incidence and severity. Data is presented relative to the internal standard (3-nonanone). Each value represents the mean (n = 3) ± Standard Error. Different letters indicate significant differences (P ≤ 0.05) among all samples for each VOC. Non-detected compound are indicated as "nd".

		Immature				Mature			
		'Venus'		'Albared'		'Venus'		'Albared'	
Cluster	Compound	Control	*M. laxa*-inoc.	Control	*M. laxa*-inoc.	Control	*M. laxa*-inoc.	Control	*M. laxa*-inoc.
C1	2-Ethyl-1-hexanol	0.89 ± 0.03 A	0.46 ± 0.10 A	0.49 ± 0.08 A	0.57 ± 0.06 A	0.91 ± 0 A	0.83 ± 0.20 A	0.72 ± 0.07 A	0.94 ± 0.17 A
C2	(E,E)-2,6-Nonadienal	0.22 ± 0.04 AB	0.20 ± 0.04 AB	0.11 ± 0.04 B	0.48 ± 0.09 A	0.08 ± 0 AB	0.41 ± 0.13 AB	0.11 ± 0.01 B	0.26 ± 0.07 AB
C2	3-methyl-2-pent-2-enylc	nd	nd	nd	1.23 ± 0.17 A	nd	1.29 ± 0.36 A	nd	1.82 ± 0.33 A
C2	2-Hydroxy-gamma-butyr	nd	nd	1.48 ± 0.26 A	1.13 ± 0.33 A	1.19 ± 0 A	1.73 ± 0 A	0.90 ± 0.32 A	1.22 ± 0.30 A
C2	(3E,6E)-Nonadien-1-yl-ac	nd	nd	nd	0.32 ± 0.08 B	nd	3.63 ± 0.61 A	nd	1.29 ± 0.42 B
C2	(Z)-3-Nonen-1-ol	nd	nd	nd	0.74 ± 0.12 B	nd	8.05 ± 2.35 A	nd	5.09 ± 1.06 AB
C2	alpha-Muurolene	nd	nd	nd	0.74 ± 0.27 B	nd	21.83 ± 5.12 A	nd	13.64 ± 0.14 A
C2	(E)-beta-Ionone	0.93 ± 0.4 B	1.20 ± 0.21 B	0.84 ± 0.19 B	0.91 ± 0.06 B	1.35 ± 0.10 B	4.67 ± 0.71 A	0.87 ± 0.15 B	3.46 ± 0.57 A
C4	4-acetyl-2,3,4,5,5-pentan	0.80 ± 0.33 A	0.46 ± 0.10 A	0.41 ± 0.14 A	0.36 ± 0.01 A	0.39 ± 0 A	0.60 ± 0.18 A	0.43 ± 0.06 A	0.73 ± 0.17 A

Finally, in cluster C4 (C4), VOCs such as (E,E)-2,4-heptadienal, (E)-2-hexen-1-ol, 2,5-furandicarboxaldehyde, 5-hydroxymethylfurfural and 4-acetyl-2,3,4,5,5-pentamethyl-2-cyclopenten-1-one were widely distributed throughout all samples, although emitted at low quantities in some samples (like mature ones) (Figure 1). Among them, 5-hydroxymethylfurfural and 4-acetyl-2,3,4,5,5-pentamethyl-2-cyclopenten-1-one had significant VIP values and were negatively and positively correlated with *M. laxa* disease, respectively (Figure 2). Specifically, 5-hydroxymethylfurfural was overall significantly higher in control immature fruit of both cultivars than the other samples (Table 3).

Monilinia laxa VOCs profile reveals shared compounds with control and *M. laxa*-inoculated nectarines

In attempt to investigate whether the VOCs detected in the *M. laxa*-nectarine pathosystem were exclusively produced by nectarines as a host response or could be produced by *M. laxa* itself, a VOCs analyze of *M. laxa* grown in an *in vitro* peach-based medium was conducted. Sampling was performed at 3 dpi (the same sampling time point of the *M. laxa*-nectarine interaction study) and at 7 dpi (time in which the pathogen reached its maximum mycelium growth) (Figure 3A). A total of 72 VOCs were finally identified and quantified, being 13 of them, also detected in the nectarine-*M. laxa* interaction study (Figure 3B and 3C). Three VOCs were only detected at 3 dpi (decanal, 2-ethyl-1-hexanol, and (E)-beta-Ionone) and two were similarly emitted at both 3 and 7 dpi (e.g., phenoxybenzene and linalool). Remarkably, 6 VOCs detected at 7dpi were significantly higher than at 3 dpi, including (E)-2-octenal, acetic acid, 3-furaldehyde, 2/3-furanmethanol, alpha-muurolene, and 3-methyl-2-pent-2-enylcyclopent-2-en-1-one, ranging from 1.8 to 26.9-fold higher. Finally, the emission of benzaldehyde and 1,3-bis(1,1-dimethylethyl)-benzene at 3 dpi was 2.2- and 12.3-fold higher at 3 dpi than at 7 dpi.

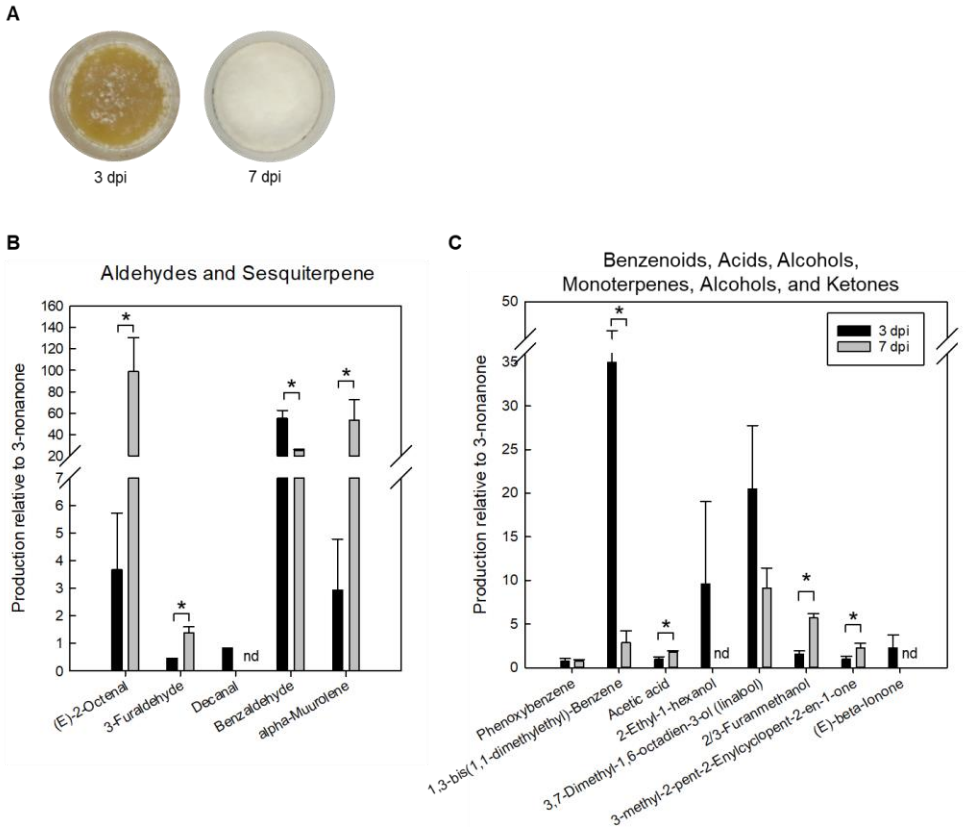

Figure 3. VOCs detected in* M. laxa *in vitro culture that were commonly detected in the nectarine-M. laxa study. *(**A**) Images of* M. laxa *mycelium on the top of peach-based medium corresponding to each sampling point (3 and 7 dpi). VOCs are grouped into (**B**) aldehydes and sesquiterpene and (**C**) benzenoids, acids, alcohols, monoterpenes, alcohols, and ketones. Data is presented relative to the internal standard (3-nonanone). Each value represents the mean (n = 3) ± Standard Error. Symbol (*) indicates significant differences (P ≤ 0.05) between 3- and 7-days post inoculation (dpi) for each VOC. Non-detected compound are indicated as "nd".*

Discussion

The fruit VOCs profile in response to *M. laxa* is also affected by developmental stage

By analyzing the VOCs profile of nectarine tissues with different susceptibility to *M. laxa,* results allowed to group VOCs according to their relationship with fruit susceptibility to the pathogen, while suggesting the most influential volatiles explaining the brown rot disease. Besides, VOCs can be emitted either by fruit in response to *M. laxa*, by the pathogen as development or virulence mechanisms or by both organisms. Herein, out of the total VOCs detected in the nectarine-*M. laxa* study,

20 compounds were emitted by all samples. Cluster 1 groups, in general, VOCs that were more abundant in mature than in immature tissues, but also, some VOCs that were more emitted in control than in *M. laxa*-inoculated tissues. For instance, acid compounds such nonanoic and octanoic, produced by all the analyzed samples, were also detected in slices of mature nectarines (Giné-Bordonaba et al., 2014) as well as the ketone 6-pentyl-2H-pyran-2-one, detected in skin and pulp of mature peaches (Aubert and Milhet, 2007). Regarding the production of VOCs by *M. laxa* grown in an *in vitro* peach-based medium, these results are the closest approach to the nectarine tissue for discerning among which VOCs can be also emitted by the pathogen, but in any case, we cannot assume that the rest of VOCs are exclusively emitted by the host. Hence, out of VOCs in C1, five of them were not detected in *M. laxa in vitro* culture, suggesting that they were produced by the host at the mature stage, and probably, involved in susceptibility factors. In this sense, El-Sayed et al. (2014), who also detected 6-pentyl-2H-pyran-2-one in ripe but not in unripe peach (with attached leaves), found that this compound is attractive to the New Zealand Flower Thrips (causing pest in mature stone fruit), and hence, contributing to the onset of the pest. Altogether points out that compounds such 6-pentyl-2H-pyran-2-one could act as susceptibility factors promoting brown rot disease.

Among the VOCs emitted by all samples and, in general, more abundant in immature fruit (especially immature control 'Venus' nectarine, grouped in C4 cluster), almost all were emitted by the host (i.e., not detected in *M. laxa in vitro* culture) except for aldehyde benzaldehyde. Among the compounds exclusively emitted by the host, 2,5-furandicarboxaldehyde (aldehyde) and (E)-2-hexen-1-ol (alcohol) were more emitted in immature than in mature samples, and the presence of *M. laxa* only impaired the VOCs profile in immature but not in mature tissues. Other authors also reported the production of 2,5-furandicarboxaldehyde in immature peaches (Bacvonkralj et al., 2014) and (E)-2-hexen-1-ol in unripe but also in commercially ripe nectarines (Aubert et al., 2003). Besides, (E)-2-hexen-1-ol was also detected in *B. cinerea*-inoculated peaches, in which the production was significantly lower than that in healthy fruit after 48 h of storage (Liu et al., 2018), which is a similar pattern to inoculated and control immature 'Venus' nectarines of our study. Hence, all suggest that these VOCs are overall typical of immature tissues and that can be altered by fungi during infection processes as a strategy to infect fruit. Furthermore, in this study, the production of (E,E)-2,4-heptadienal (aldehyde) by the resistant 'Venus' immature tissue was slightly lower than that from its control, and higher in *M. laxa*-inoculated susceptible tissues than their controls. In line with these results, other authors also detected (E,E)-2,4-

heptadienal in peaches, which also showed a high positive correlation with flesh firmness, suggesting that it accumulates in immature peach fruit (Sánchez et al., 2012). Besides, this compound is produced from linolenic acid via lipoxygenase (LOX) pathway in leek (Nielsen et al., 2004), and Balsells-Llauradó et al. (2020) found that *PpLOX3* gene is upregulated in response to *M. laxa* in resistant immature nectarines if compared to control fruit. Taken all together reveals that these compounds (e.g., (E)-2-hexen-1-ol and (E,E)-2,4-heptadienal) are produced by the host at the immature stage and seem to be involved in resistance to *M. laxa*.

Brown rot development may be favored by positively correlated fruit VOCs, although some are emitted by *M. laxa*

Among the VOCs emitted by visual symptomatic *M. laxa* tissues (rotted fruit), and positively correlated with brown rot disease (overall located in the C2 cluster), 4 VOCs were also found in *M. laxa in vitro* culture, indicating that they could be produced by *M. laxa* itself or by the fruit in response to the pathogen. Since the terpene alpha-muurolene and the ketone 3-methyl-2-pent-2-enylcyclopent-2-en-1-one were exclusively produced by rotted tissue, this suggests that they were probably emitted by the pathogen rather than by the host. A BLAST search in either *Rosaceae* or *Prunus* organisms revealed no matches with the codifying gene for the alpha-muurolene synthase (*COP3*) of the fungus *Marasmius oreades* (Hiltunen et al., 2021). Although it cannot be discarded of being a fruit VOC, all evidence points towards that alpha-muurolene is emitted by the pathogen. In this sense, Thelen et al. (2005) detected alpha-muurolene in tomato leaves infected by *B. cinerea* and Mang et al. (2015) found that this VOC is emitted by *M. fructicola* and *M. fructigena* in *in vitro* cultures. The function of this compound is poorly understood, but some authors relate the emissions of this compound with the toxigenicity of the phytopathogenic fungus *Aspergillus flavus* (Josselin et al., 2021) or emitted by the saprotrophic fungi *Hypholoma fasciculare* against ectomycorrhizal fungus *Pisolithus tinctorius* (Baptista et al., 2021). Hence, in our study, alpha-muurolene could be emitted by *M. laxa* as a virulence factor.

In addition to the VOCs emitted by rotted tissues and shared with the *M. laxa in vitro* culture, the alcohol 2-ethyl-1-hexanol and the terpene (E)-beta-ionone were emitted by all samples of the nectarine-*M. laxa* study. The detection of 2-ethyl-1-hexanol, as previously described in other peach and nectarine cultivars (Giné-Bordonaba et al., 2014; Xin et al., 2018), was similarly emitted in all samples of our study, suggesting that it is a fruit VOC, or seems not to be involved in *M. laxa* response. On the other

hand, (E)-beta-Ionone was significantly highly produced in very rotted fruit of both cultivars if compared to control and immature samples. Hence, although (E)-beta-ionone is produced by several peach cultivars (Montero-Prado et al., 2013; Xin et al., 2018), our results suggest that in the presence of *M. laxa*, the emission of this compound is enhanced, either by the action of the fungus or just because the pathogen itself is able to produce it, leading to an increased susceptibility of the tissue. Some of these VOCs, such as (E)-beta-ionone, are derived from the terpenoid metabolism, and specifically from (9Z)-beta-carotene, which are also highly induced in *M. laxa* infected nectarines (Balsells-Llauradó, unpublished). Recently, Brambilla et al. (2021) found that infected plants of barley emit (E)-beta-ionone, which in turn, induces resistance in neighbor plants. Hence, susceptible tissue of our study could be using such compounds, which can be emitted by the pathogen or by the host, as a fruit-fruit signaling to induce resistance on the neighbor fruit.

Out of the VOCs that were emitted by rotted fruit and positively correlated with brown rot disease, 6 were not detected in the *M. laxa in vitro* culture, indicating that they may be exclusively produced by fruit. Some of them, like the ketone 2-hydroxy-gamma-butyrolactone, have already been described in peach leaf extract (Ozpinar et al., 2017). A closely related compound to the aldehyde (E,E)-2,6-nonadienal (i.e., (E,Z)-2,6-nonadienal) is also emitted by peaches (Wang et al., 2009; Xi et al., 2017), and besides, applied as a fumigant, it reduced *Botrytis* growth on strawberries (Archbold et al., 1997). However, on the contrary to that described in *Botrytis,* this compound seemed to favor, or at least, it did not prevent brown rot since the disease developed in the tissues in which it was detected. On the other side, since (3E,6E)-nonadien-1-yl-acetate, (Z)-3-nonen-1-ol and benzyl nitrile (although not considered VIP compound) were exclusively produced by rotted tissues, results presented herein point out that these compounds are produced by the host as a response to the *M. laxa* disease. To our knowledge, (3E,6E)-nonadien-1-yl-acetate and (Z)-3-nonen-1-ol have been detected in several melon cultivars (Shi et al., 2020), but no studies have revealed neither their implication in fruit diseases nor their emission by fungi. Further studies should be conducted to explore whether these VOCs are produced by the fruit and could somehow favor the disease susceptibility.

Negatively correlated fruit VOCs may be potential antifungal compounds against *M. laxa*

Finally, VOCs that were negatively correlated with brown rot (overall located in C3), were, in general, lower emitted in *M. laxa*-inoculated fruit than in their respective control tissues. However, among them, three compounds (3-furaldehyde, 2/3-furanmethanol and decanal), were also emitted by *M. laxa in vitro* culture. Although our methodology was not able to discern between the alcohol 2- or 3-furanmethanol, Liu et al. (2018) reported that 2-furanmethanol is emitted by *B. cinerea*-inoculated peaches but neither in *M. fructicola*-inoculated nor control fruit. These results are in line with our study in which 2/3-furanmethanol was not detected in *M. laxa*-inoculated fruit. Besides, 2-furanmethanol is one of the main bioactive compounds produced by a *Bacillus* strain (DM6120) that suppresses the mycelial growth of *Colletotrichum nymphaeae* (Alijani et al., 2021). Regarding the aldehyde decanal, which is commonly emitted by peaches and nectarines (Montero-Prado et al., 2013; Giné-Bordonaba et al., 2014), it is also emitted by active molds on aged model materials (e.g., *Alternaria alternata* on silk and *Cladosporium herbarum* on paper) (Sawoszczuk et al., 2015). Besides, its exogenous application significantly inhibits the germination and development of *Penicillium expansum in vitro*, by decreasing the oxidative phosphorylation as one of the main inhibitory actions (Zhou et al., 2020). Hence, based on our results, two main hypotheses can arise: (1) the fact that they were overall lower or even not detected in rotted tissues, suggests that these VOCs were probably generated by the host rather than emitted by the pathogen itself. In this sense, the host could be reducing its emission since the fruit tissue was already invaded by the pathogen, and thus could drive the energy towards other metabolisms. Alternatively, another hypothesis could be that (2) although *M. laxa* can produce these VOCs for its own development (i.e., observed during *in vitro* culture on the peach juice based-medium), the results presented herein suggest that the pathogen was not producing them, since they were low or almost not detected in rotted samples. In turn, *M. laxa* could be repressing the emission of these VOCs in attempt to inhibit the negative effect that these compounds may have on the pathogen. Therefore, further studies are necessary to confirm these hypotheses and validate the negative effects of these compounds on *M. laxa*.

Furthermore, among the VOCs that were lower in *M. laxa*-inoculated fruit than in their respective control tissue, and negatively correlated with *M. laxa* disease, 5 VOCs were not detected in *M. laxa in vitro* culture, indicating its implication exclusively as a fruit

response to *M. laxa*. Herein, the compounds 2-ethyl furan and the aldehyde (E)-2-decenal were detected in all samples at different levels of emission (mostly lower in *M. laxa*-inoculated than in their controls), whereas 4-cyclopentene-1,3-dione, butyrolactone and 5-hydroxymethylfurfural, were almost not or lower emitted by rotted tissues compared to the other tissues. For instance, (E)-2-decenal is also detected in bean infected with *Colletotrichum lindemuthianum* and besides, it completely inhibits the mycelia growth of *C. lindemuthianum* and *B. cinerea* when the compound is exposed to the atmosphere of each pathogen (Quintana-Rodriguez et al., 2018). Furthermore, derivates of the ketone butyrolactone, also detected in peach cultivars (Xin et al., 2018), showed antifungal effect towards *B. cinerea* (Cazar et al., 2005). In this line, the aldehyde 5-hydroxymethylfurfural, a product of the degradation of furfural, which is also emitted by peaches inoculated with *R. stolonifer* (Liu et al., 2018) and by immature peaches (Bacvonkralj et al., 2014), also showed inhibition of the cell growth of some yeast strains (Liu et al., 2004). Hence, since these compounds were lower in susceptible tissues than in their respective control tissue and were not produced by the fungus itself in the tested conditions, altogether indicates that these compounds may have an antifungal activity, and *M. laxa* repressed or inhibited their production as a strategy to infect the fruit. In a study conducted with inoculated pears with either *P. expansum* or *R. stolonifer*, the most effective compounds (i.e., negatively correlated with incidence) reduced or even completely controlled mycelial growth of these pathogens in *in vitro* conditions (Torregrosa et al., 2020). Therefore, future studies could be conducted towards studying the antifungal effect of these compounds and their role as sustainable products for brown rot control.

Conclusions

The results from this study demonstrate that the degree of visual brown rot symptoms was associated with the VOCs profile of control and *M. laxa*-inoculated samples. Besides, the *M. laxa in vitro* culture allowed to discern which of the detected VOCs could also be produced by the pathogen. The different VOC profile in response to *M. laxa*, also sheds light on the different susceptibility to *M. laxa* of the different samples studied herein. Hence, the group of positively correlated VOCs with brown rot disease (e.g., (E,E)-2,6-nonadienal), some of them shared with the VOCs emitted by *M. laxa in vitro* culture (e.g., alpha-muurolene), was crucial for determining which of them may favor the susceptibility of nectarines to *M. laxa* infection. In turn, negatively correlated VOCs with brown rot, could be selected as potential antifungal compounds (e.g., (E)-2-decenal and butyrolactone). Overall, the results presented herein improve the

knowledge of *M. laxa* infection on nectarines and highlights target volatiles that may serve as potential brown rot control compounds.

Author contributions

Marta Balsells-Llauradó: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Data curation. Rosario Torres: Conceptualization, Supervision, Project administration, Writing- Reviewing and Editing. Gemma Echeverría: Formal analysis, Writing- Reviewing and Editing. Núria Vall-llaura: Conceptualization, Methodology, Investigation, Writing- Reviewing and Editing. Neus Teixidó: Investigation, Resources, Writing- Reviewing and Editing. Josep Usall: Supervision, Funding acquisition, Writing- Reviewing and Editing.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Abbas, F., Ke, Y., Yu, R., Yue, Y., Amanullah, S., Jahangir, M. M., et al. (2017). Volatile terpenoids: multiple functions, biosynthesis, modulation and manipulation by genetic engineering. *Planta* 246, 803–816. doi:10.1007/s00425-017-2749-x.

Alijani, Z., Amini, J., Ashengroph, M., Bahman, B., and Mozafari, A. A. (2021). Biocontrol of strawberry anthracnose disease caused by *Colletotrichum nymphaeae* using *Bacillus atrophaeus* strain DM6120 with multiple mechanisms. *Trop. Plant Pathol. 2021* 1, 1–15. doi:10.1007/S40858-021-00477-7.

Alkan, N., and Fortes, A. M. (2015). Insights into molecular and metabolic events associated with fruit response to post-harvest fungal pathogens. *Front. Plant Sci.* 6, 889. doi:10.3389/fpls.2015.00889.

Archbold, D. D., Hamilton-Kemp, T. R., Barth, M. M., and Langlois, B. E. (1997). Identifying Natural Volatile Compounds That Control Gray Mold (*Botrytis cinerea*) during Postharvest Storage of Strawberry, Blackberry, and Grape. *J. Agric. Food Chem.* 45, 4032–4037. doi:10.1021/jf970332w.

Aubert, C., Günata, Z., Ambid, C., and Baumes, R. (2003). Changes in Physicochemical Characteristics and Volatile Constituents of Yellow- and White-Fleshed Nectarines during. *J. Agric. Food Chem.* 51, 3083–3091.

Aubert, C., and Milhet, C. (2007). Distribution of the volatile compounds in the different parts of a white-fleshed peach (*Prunus persica* L. Batsch). *Food Chem.* 102, 375–384. doi:10.1016/j.foodchem.2006.05.030.

Bacvonkralj, M., Jug, T., Komel, E., Fajt, N., Jarni, K., Živković, J., et al. (2014). Effects of ripening degree and sample preparation on peach aroma profile characterization by headspace solid-phase microextraction. *Turkish J. Agric. For.* 38, 676–687. doi:10.3906/tar-1307-129.

Baldwin, I. T., Halitschke, R., Paschold, A., Von Dahl, C. C., and Preston, C. A. (2006). Volatile signaling in plant-plant interactions: "Talking trees" in the genomics era. *Science (80-.).* 311, 812–815. doi:10.1126/science.1118446.

Balsells-Llauradó, M., Silva, C. J., Usall, J., Vall-llaura, N., Serrano-Prieto, S., Teixidó, N., et al. (2020). Depicting the battle between nectarine and *Monilinia laxa*: the fruit developmental stage dictates the effectiveness of the host defenses and the pathogen's infection strategies. *Hortic. Res.* 7, 1–15. doi:10.1038/s41438-020-00387-w.

Baptista, P., de Pinho, P. G., Moreira, N., Malheiro, R., Reis, F., Padrão, J., et al. (2021). *In vitro* interactions between the ectomycorrhizal *Pisolithus tinctorius* and the saprotroph *Hypholoma fasciculare* fungi: morphological aspects and volatile production. *Mycology* 12, 216–229. doi:10.1080/21501203.2021.1876778 In.

Baró-Montel, N., Torres, R., Casals, C., Teixidó, N., Segarra, J., and Usall, J. (2019a). Developing a methodology for identifying brown rot resistance in stone fruit. *Eur. J. Plant Pathol.* 154, 287–303. doi:10.1007/s10658-018-01655-1.

Baró-Montel, N., Vall-llaura, N., Usall, J., Teixidó, N., Naranjo-Ortíz, M. A., Gabaldón, T., et al. (2019b). Pectin methyl esterases and rhamnogalacturonan hydrolases: weapons for successful *Monilinia laxa* infection in stone fruit? *Plant Pathol.* 68, 1381–93. doi:10.1111/ppa.13039.

Brambilla, A., Sommer, A., Ghirardo, A., Wenig, M., Knappe, C., Weber, B., et al. (2021). Immunity-associated volatile emissions of β-ionone and nonanal propagate defence responses in neighbouring barley (*Hordeum vulgare*) plants. *J. Exp. Bot.* doi:10.1093/JXB/ERAB520.

Cazar, M. E., Astudillo, L., and Naturales, L. D. P. (2005). Antimicrobial butyrolactone I derivatives from the Ecuadorian soil fungus *Aspergillus terreus* Thorn. var *terreus*. *World J. Microbiol. Biotechnol.* 21, 1067–1075. doi:10.1007/s11274-004-8150-5.

Cindi, M. D., Soundy, P., Romanazzi, G., and Sivakumar, D. (2016). Different defense responses and brown rot control in two *Prunus persica* cultivars to essential oil vapours after storage. *Postharvest Biol. Technol.* 119, 9–17. doi:10.1016/j.postharvbio.2016.04.007.

Dini, M. (2019). Resistance to brown rot in peach. Available at: http://guaiaca.ufpel.edu.br:8080/handle/prefix/4957.

El-Sayed, A. M., Mitchell, V. J., and Suckling, D. M. (2014). 6-Pentyl-2H-pyran-2-one: A Potent Peach-Derived Kairomone for New Zealand Flower Thrips, *Thrips obscuratus*. *J. Chem. Ecol.* 40, 50–55. doi:10.1007/s10886-014-0379-3.

Giné-Bordonaba, J., Cantin, C. M., Larrigaudière, C., López, L., López, R., and Echeverria, G. (2014). Suitability of nectarine cultivars for minimal processing: The role of genotype, harvest season and maturity at harvest on quality and sensory attributes. *Postharvest Biol. Technol.* 93, 49–60. doi:10.1016/j.postharvbio.2014.02.007.

Gotor-Vila, A., Teixidó, N., Di Francesco, A., Usall, J., Ugolini, L., Torres, R., et al. (2017). Antifungal effect of volatile organic compounds produced by *Bacillus amyloliquefaciens* CPA-8 against fruit pathogen decays of cherry. *Food Microbiol.* 64, 219–225. doi:10.1016/J.FM.2017.01.006.

Hiltunen, M., Ament-Velásquez, S. L., and Johannesson, H. (2021). The Assembled and Annotated Genome of the Fairy-Ring Fungus *Marasmius oreades*. *Genome Biol. Evol.* 13. doi:10.1093/GBE/EVAB126.

Josselin, L., De Clerck, C., De Boevre, M., Moretti, A., Haïssam Jijakli, M., Soyeurt, H., et al. (2021). Volatile Organic Compounds Emitted by *Aspergillus flavus* Strains Producing or Not Aflatoxin B1. *Toxins 2021, Vol. 13, Page 705* 13, 1–19. doi:10.3390/TOXINS13100705.

Liu, Q., Zhao, N., Zhou, D., Sun, Y., Sun, K., Pan, L., et al. (2018). Discrimination and growth tracking of fungi contamination in peaches using electronic nose. *Food Chem.* 262, 226–234. doi:10.1016/j.foodchem.2018.04.100.

Liu, Z. L., Slininger, P. J., Dien, B. S., Berhow, M. A., Kurtzman, C. P., and Gorsich, S. W. (2004). Adaptive response of yeasts to furfural and 5-hydroxymethylfurfural and new chemical evidence for HMF conversion to 2,5-bis-hydroxymethylfuran. *J. Ind. Microbiol. Biotechnol.* 31, 345–352. doi:10.1007/S10295-004-0148-3.

López-Gresa, M. P., Payá, C., Ozáez, M., Rodrigo, I., Conejero, V., Klee, H., et al. (2018). A new role for green leaf volatile esters in tomato stomatal defense against *Pseudomonas syringe* pv. *tomato*. *Front. Plant Sci.* 871, 1–12. doi:10.3389/fpls.2018.01855.

Mang, S. M., Racioppi, R., Camele, I., Rana, G. L., and D'Auria, M. (2015). Use of volatile metabolite profiles to distinguish three *Monilinia* species. *J. Plant Pathol.* 97, 55–59. doi:10.4454/JPP.V97I1.005.

Mari, M., Bautista-Baños, S., and Sivakumar, D. (2016). Decay control in the postharvest system: Role of microbial and plant volatile organic compounds. *Postharvest Biol. Technol.* 122, 70–81. doi:10.1016/j.postharvbio.2016.04.014.

Montero-Prado, P., Bentayeb, K., and Nerín, C. (2013). Pattern recognition of peach cultivars (*Prunus persica* L.) from their volatile components. *Food Chem.* 138, 724–731. doi:10.1016/j.foodchem.2012.10.145.

Mustafa, M. H., Bassi, D., Corre, M.-N., Lino, L. O., Signoret, V., Quilot-Turion, B., et al. (2021). Phenotyping brown rot susceptibility in stone fruit: A literature review with emphasis on peach. *Horticulturae* 7, 115. doi:10.3390/horticulturae7050115.

Nielsen, G. S., Larsen, L. M., and Poll, L. (2004). Formation of Volatile Compounds in Model Experiments with Crude Leek (*Allium ampeloprasum* Var. *Lancelot*) Enzyme Extract and Linoleic Acid or Linolenic Acid. *J. Agric. Food Chem.* 52, 2315–2321. doi:10.1021/jf030600s.

Ozpinar, H., Dag, S., and Yigit, E. (2017). Alleophatic effects of benzoic acid, salicylic acid and leaf extract of *Persica vulgaris* Mill. (Rosaceae). *South African J. Bot.* 108, 102–109. doi:10.1016/J.SAJB.2016.10.009.

Quintana-Rodriguez, E., Rivera-Macias, L. E., Adame-Alvarez, R. M., Torres, J. M., and Heil, M. (2018). Shared weapons in fungus-fungus and fungus-plant interactions? Volatile organic compounds of plant or fungal origin exert direct antifungal activity *in vitro*. *Fungal Ecol.* 33, 115–121. doi:10.1016/j.funeco.2018.02.005.

Romanazzi, G., Sanzani, S. M., Bi, Y., Tian, S., Gutiérrez Martínez, P., and Alkan, N. (2016). Induced resistance to control postharvest decay of fruit and vegetables. *Postharvest Biol. Technol.* 122, 82–94. doi:10.1016/j.postharvbio.2016.08.003.

Sánchez, G., Besada, C., Badenes, M. L., Monforte, A. J., and Granell, A. (2012). A Non-Targeted Approach Unravels the Volatile Network in Peach Fruit. *PLoS One* 7, 1–11. doi:10.1371/journal.pone.0038992.

Sawoszczuk, T., Syguła-Cholewińska, J., and del Hoyo-Meléndez, J. M. (2015). Optimization of headspace solid phase microextraction for the analysis of microbial volatile organic compounds emitted by fungi: Application to historical objects. *J. Chromatogr. A* 1409, 30–45. doi:10.1016/j.chroma.2015.07.059.

Shi, J., Wu, H., Xiong, M., Chen, Y., Chen, J., Zhou, B., et al. (2020). Comparative analysis of volatile compounds in thirty nine melon cultivars by headspace solid-phase microextraction and gas chromatography-mass spectrometry. *Food Chem.* 316, 1–8. doi:10.1016/J.FOODCHEM.2020.126342.

Thelen, J., Harbinson, J., Jansen, R., Van Straten, G., Posthumus, M. A., Woltering, E. J., et al. (2005). The sesquiterpene α-copaene is induced in tomato leaves infected by *Botrytis cinerea*. *J. Plant Interact.* 1, 163–170. doi:10.1080/17429140600968177.

Torregrosa, L., Echeverria, G., Illa, J., Torres, R., and Giné-Bordonaba, J. (2020). Spatial distribution of flavor components and antioxidants in the flesh of 'Conference' pears and its relationship with postharvest pathogens susceptibility. *Postharvest Biol. Technol.* 159, 111004. doi:10.1016/j.postharvbio.2019.111004.

Vickers, C. E., Gershenzon, J., Lerdau, M. T., and Loreto, F. (2009). A unified mechanism of action for volatile isoprenoids in plant abiotic stress. *Nat. Chem. Biol.* 5, 283–291. doi:10.1038/nchembio.158.

Wang, Y. J., Yang, C. X., Li, S. H., Yang, L., Wang, Y. N., Zhao, J. B., et al. (2009). Volatile characteristics of 50 peaches and nectarines evaluated by HP-SPME with GC-MS. *Food Chem.* 116, 356–364. doi:10.1016/j.foodchem.2009.02.004.

Xi, W., Zheng, Q., Lu, J., and Quan, J. (2017). Comparative analysis of three types of peaches: Identification of the key individual characteristic flavor compounds by integrating consumers' acceptability with flavor quality. *Hortic. Plant J.* 3, 1–12. doi:10.1016/j.hpj.2017.01.012.

Xin, R., Liu, X., Wei, C., Yang, C., Liu, H., Cao, X., et al. (2018). E-nose and gc-ms reveal a difference in the volatile profiles of white- and red-fleshed peach fruit. *Sensors (Switzerland)* 18. doi:10.3390/s18030765.

Zhou, T., Ye, B., Yan, Z., Wang, X., and Lai, T. (2020). Uncovering proteomics changes of *Penicillium expansum* spores in response to decanal treatment by iTRAQ. *J. Plant Pathol.* 102, 721–730. doi:10.1007/S42161-020-00486-6/FIGURES/7.

Supplementary Material

Supplementary Table S1. Relative quantification of VOCs detected in the nectarine-*M. laxa* study. Data is presented relative to the internal standard (3-nonanone). Each value represents the mean (n = 3). Labels indicate the cultivar ('Venus', Ven; 'Albared', Alb), the developmental stage (mature, M; immature, IM) and tissue (control, CK; *M. laxa*-inoculated, ML). Non-detected compound is indicated as "nd".

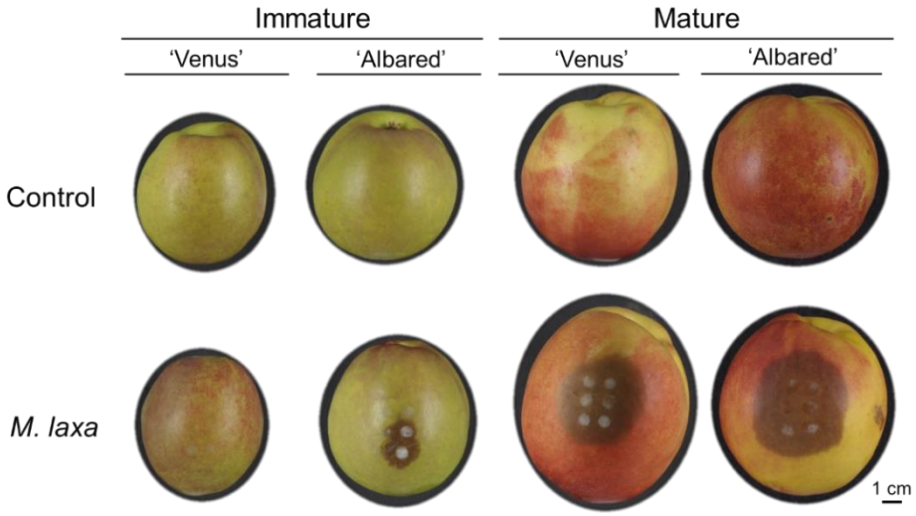
Label	3,7-Dimethyl-	2-Ethyl-1-hexanol	2-Ethyl-furan	(E,E)-2,4-Heptadienal	2,5-Furandicarb	(E,E)-2,6-Nonadienal	3-methyl-2-pent-2-	4-acetyl-2,3,4,5,5-	(E)-2-Decenal
Ven-IM-CK	1.48	0.89	2.33	0.79	4.54	0.22	nd	0.80	1.26
Ven-IM-ML	6.08	0.46	1.01	0.59	0.71	0.20	nd	0.46	1.18
Alb-IM-CK	0.66	0.49	1.16	0.60	1.69	0.11	nd	0.41	0.37
Alb-IM-ML	4.21	0.57	1.03	0.70	0.78	0.48	1.23	0.36	0.43
Ven-M-CK	80.58	0.91	1.83	0.33	0.89	0.08	nd	0.39	1.86
Ven-M-ML	40.48	0.83	0.40	0.55	0.72	0.41	1.29	0.60	0.99
Alb-M-CK	1.18	0.72	1.76	0.49	0.59	0.11	nd	0.43	1.45
Alb-M-ML	0.95	0.94	0.32	0.63	0.64	0.26	1.82	0.73	0.48

Label	(E)-2-Hexen-1-ol	2H-Pyran-2,6(3H)-dione	6-Pentyl-2H-pyran-2-one	2-Hydroxy-gamma-butyrolactone	(E)-2-Octenal	3-6-Nonadien-1-yl-acetate	3-Furaldehyde	3-Furancarboxylic acid	2/3-Furanmethanol
Ven-IM-CK	1.38	nd	0.47	nd	1.66	nd	3.80	nd	1.27
Ven-IM-ML	0.57	nd	0.39	nd	1.68	nd	0.87	nd	0.33
Alb-IM-CK	0.72	0.70	0.22	1.48	0.54	nd	3.23	1.12	1.09
Alb-IM-ML	1.15	0.64	nd	1.13	0.80	0.32	1.97	nd	0.35
Ven-M-CK	0.39	1.06	20.86	1.19	1.68	nd	5.10	nd	3.10
Ven-M-ML	0.36	nd	9.98	1.73	2.43	3.63	0.70	nd	nd
Alb-M-CK	0.15	nd	27.82	0.90	3.14	nd	1.19	nd	0.45
Alb-M-ML	0.34	nd	11.00	1.22	1.94	1.29	0.97	nd	nd

Label	(Z)-3-Nonen-1-ol	4-Cyclopentene-1,3-dione	5-Hydroxymethylfurfural	Acetic acid	alpha-Muurolene	Benzaldehyde	2-Hydroxy-benzaldehyde	1,3-bis(1,1-dimethylethyl)-Benzene	Benzyl nitrile
Ven-IM-CK	nd	0.90	20.69	9.95	nd	36.34	25.46	0.34	nd
Ven-IM-ML	nd	0.31	1.11	3.50	nd	32.27	5.73	0.39	nd
Alb-IM-CK	nd	0.58	11.41	10.33	nd	18.11	0.15	0.54	nd
Alb-IM-ML	0.74	0.41	3.67	3.67	0.74	18.19	0.17	0.44	8.34
Ven-M-CK	nd	1.18	7.71	10.13	nd	nd	3.63	0.72	nd
Ven-M-ML	8.05	nd	0.83	6.62	21.83	nd	1.66	0.66	nd
Alb-M-CK	nd	0.34	1.37	3.78	nd	15.91	0.23	0.59	nd
Alb-M-ML	5.09	nd	0.85	7.68	13.64	24.11	nd	0.34	nd

Label	Trans-beta-Ionone	Butyrolactone	Decanal	Phenoxybenzene	n-Decanoic acid	Nonanoic acid	Octanoic acid
Ven-IM-CK	0.93	0.52	1.09	3.04	0.66	2.78	1.81
Ven-IM-ML	1.20	0.18	1.55	1.96	0.43	2.48	1.81
Alb-IM-CK	0.84	0.15	0.43	1.51	0.41	1.94	1.44
Alb-IM-ML	0.91	nd	0.44	1.40	0.66	2.64	1.67
Ven-M-CK	1.35	0.92	1.26	4.54	0.95	3.51	2.46
Ven-M-ML	4.67	nd	0.80	2.92	0.72	2.82	2.16
Alb-M-CK	0.87	0.18	1.04	2.95	0.80	3.05	2.26
Alb-M-ML	3.46	nd	0.64	2.72	0.40	3.06	2.47

Supplementary Figure S1. Control and *M. laxa*-inoculated immature and mature nectarines of 'Venus' and 'Albared' cultivars at 3 dpi.



5. GENERAL DISCUSSION

This section aims to depict the most relevant findings of this thesis along with the current literature, from our research conducted in preharvest, postharvest, and to host-pathogen interaction studies. A special emphasis on some secondary metabolisms of nectarines related to responses to brown rot is also encompassed. Most of the results and their discussion could open new doors of study, which are also mentioned herein.

Brown rot disease caused by *Monilinia* spp. in stone fruit is greatly controlled in the field since it can infect fruit during its development and at postharvest, where the main fruit losses occur. Hence, the environmental factors present along the chain, from field to consumer's house, play a significant role in the onset of brown rot prevention and control. Among environmental conditions, light is an interesting factor to be studied both in the field and postharvest. In the field, some practices such as fruit bagging, aiming to protect the fruit from fungal diseases, alter the light that fruit receives. Hence, it can influence the development of natural fungal disease incidence (fruit decay) before harvest and the fruit susceptibility to *Monilinia* spp. Artificial lighting can also be determinant along the postharvest period, influencing many aspects of the global fruit quality (e.g., fruit decay or fruit susceptibility to *Monilinia* spp.). Furthermore, to delve into the understanding of brown rot in stone fruit, global host-pathogen interaction studies have an exceptional value since they may provide an overall perspective on defense mechanisms (fruit) and virulence factors (pathogen) ongoing during their interaction, which can be further validated with more detailed studies focusing on specific metabolites/compounds.

5.1. Effect of lighting treatments and fruit bagging

5.1.1. Preharvest: does fruit bagging affect fruit quality and prevent fruit decay?

Fruit bagging during preharvest is a control strategy used in the field to control pests and diseases and to improve the physical appearance of fruit in both conventional and organic orchards (Sharma et al., 2014; Campbell et al., 2021). In addition, fruit bagging modulates the light that fruit perceives (Xu et al., 2010) and thus, alters many physiochemical properties, including those metabolisms described as necessary for facing pathological diseases (Ilić and Fallik, 2017; Ali et al., 2021). For this reason, one of the objectives of this thesis was **to evaluate the effect of fruit bagging on fruit quality and fruit decay** in commercial organic nectarines (chapter 2).

Fruit quality depends on the changes that fruit undergoes throughout its development but also depends on other external factors such as **cultivar type**, tree management, and canopy position, among others, as mentioned in the Introduction section (1.1.3). The flesh of nectarines, either white or yellow, is very related to differences in some quality parameters such as the pH and titratable acidity, i.e., yellow-flesh cultivars have higher titratable acidity and lower pH than white-flesh cultivars (Gil et al., 2002). To avoid this factor, all cultivars tested in this thesis have yellow flesh. Fruit position within

the canopy tree influences the sunlight radiation received by the fruit in the field, affecting many aspects of stone fruit (e.g., size, fruit firmness, ripening process, and color) (Minas et al., 2018). Accordingly, to minimize such effects within each batch of fruit used in these studies, fruits were harvested in the same sunny side of the canopy and height, as well as it was confirmed that all four cultivars tested in all chapters of the thesis had similar open vase training system (**Figure 1**).

Figure 1. *Nectarine trees of the four nectarine cultivars tested in this thesis ('Fantasia', 'Venus', 'Nectatinto' and 'Albared'), that display some bagged fruits.*

Current research conducted on fruit bagging of peaches and nectarines reveals differences in some **fruit quality parameters** between bagged and unbagged fruit. For instance, bagged peaches with white paper bags had a higher CIRG (color index of red grapes) value and anthocyanin content than unbagged peaches (Liu et al.,

2015). However, other studies also show inconsistent effects on quality parameters between fruit bagging conditions. Whereas Campbell et al. (2021) found that parameters such as yield, weight, and diameter were not affected by bagging compared to unbagged organic peaches, Zhang et al. (2015) observed that bagged peaches were bigger (e.g., width) and had a higher weight than unbagged ones. Many factors could explain such inconsistent results since the effect of fruit bagging may rely on several factors, ranging from the type of bag to the intrinsic properties of the cultivar. In this thesis, fruit bagging slightly altered fruit quality on harvest day compared to unbagged fruit in a cultivar-dependent manner (chapter 2). For instance, whereas the 'Venus' cultivar's unbagged fruit was bigger than the bagged one, 'Albared' nectarines showed an opposite tendency. The effect of the type of cultivar has been studied on many aspects of fruit such as quality parameters (e.g., sugars, organic acids, firmness, color, and sensory evaluation) (Colaric et al., 2005; Iglesias and Echeverría, 2009; Xi et al., 2017) and metabolite production like fruit volatile organic compounds (VOCs) (Wang et al., 2009; Xi et al., 2017). Hence, the intrinsic properties due to the cultivar itself could finally explain the differences in the bagging conditions between cultivars. So, the cultivar type should be considered to better assess the effect of fruit bagging on fruit quality.

Changes in quality parameters between bagging conditions can also relay, for instance, on the altered **solar radiation** that fruit perceives when they are bagged. In our study, bagged fruit received 24% less solar radiation than unbagged fruit. Such alteration affects photosynthesis and, in turn, the plant's energy balance (Folta and Carvalho, 2015). In our study, the DA index, which assesses the absorbance difference and it is correlated with the flesh chlorophyll content, was also altered between bagging conditions (chapter 2). However, such effect differed among cultivars, in line with other studies. For instance, whereas bagged nectarines had lower chlorophyll content than unbagged ones (Zhang et al., 2015), bagged organic peaches had a higher DA index than unbagged ones (Campbell et al., 2021). Situations with high solar radiation (e.g., high altitudes) can induce oxidative stress in fruit that can cause a degradation of chlorophylls, and thus, a decrease in their content (Fernández-Cancelo, unpublished). Nevertheless, in our study, all parameters on harvest day (and after either storage condition) were within international recommendations (OECD, 2010; European Commission, 2019) and literature (Crisosto et al., 1999; Crisosto and Crisosto, 2005; Reig et al., 2012; Bonany et al., 2014). Hence, fruit bagging slightly affects the fruit quality of nectarines, but its effect is minimal for their acceptance in the market.

Out of the wide variety of light's effects on fruit, another typical result caused by the incidence of light on fruit is the induction of pigmentation. For that reason, fruit bagging is also used to improve fruit appearance, such as increasing the lightness of the peel in organic peaches (Campbell et al., 2021). So, all of these can affect the final acceptance of the consumer. In unpublished preliminary results from our group (Postharvest pathology), color attributes were measured in bagged and unbagged fruit of four nectarine and peach cultivars. Results showed that the color value *a* (high value means high red color) in bagged fruit (e.g., 'Pollero' peaches and 'Big top' nectarines) was higher than the one in unbagged fruit, whereas the color value *b* (high value means high yellow color) in bagged fruit was lower than the one in unbagged fruit. Hence, future studies can be conducted towards analyzing the pigment content and color attributes in bagged and unbagged organic nectarines to further quantify the visual differences observed between bagging conditions and cultivars (**Figure 2**).

Figure 2. *Bagged (B) and unbagged (UB) nectarines of 'Fantasia' and 'Nectatinto' cultivars on harvest day.*

Nevertheless, preharvest fruit bagging in mid-early and late-season nectarines mainly reduces **fruit decay** in preharvest but specifically during the postharvest handling chain. In our experiments, after storing the previously bagged fruit or not in the field under two postharvest conditions (darkness and treatment T1), results highlighted that fruit bagging clearly reduced fruit decay in most cultivars (chapter 2). Even though the incidence of fruit decay of unbagged fruit was low (below 35%) in early-mid cultivars, there was a 100% reduction in almost all bagged fruit of these cultivars. However, both late cultivars had a higher incidence of fruit decay in unbagged fruit (up to 65 and 85% of incidence for 'Nectatinto' and 'Albared', respectively), indicating

that these orchards had an increased inoculum pressure. Nevertheless, whereas fruit decay incidence in 'Nectatinto' was not significantly controlled by fruit bagging, this practice highly reduced (100% of reduction) the fruit decay in 'Albared' stored under darkness. Hence, fruit bagging reduces fruit decay, especially when stored under darkness during postharvest.

Even though the bagging technique was first utilized in Japan in the 20th century for pears and grapes (Sharma et al., 2014), scarce information exists related to its **economic viability**. Blasi et al. (2017) conducted an economic evaluation of the use of fruit bagging to protect peach against the Mediterranean fruit fly in the South of Italy with silver paper bag parchments. The authors concluded that the final income of the bagged orchard was almost 260 € ha^{-1} lower than in the case of conventional farming. Nevertheless, they did not specify whether they considered the losses occurring during the postharvest storage or not. Allran (2017) and Ali et al. (2021) already highlighted the importance of economic analysis for the benefits of bagging for each orchard and/or considering whether the management is conducted by either homeowners or commercial growers. Accordingly, we conducted, for the first time, an economic evaluation attending to both the orchard characteristics (yield, production) and prices (bags, fruit, workers) that are currently in the Lleida area and the postharvest losses due to fungal fruit decay after postharvest storage (chapter 2). Considering all of that, for instance, in one hectare of an organic orchard of 22.5 T of production, fruit bagging is rentable both in early-mid cultivars (+ 1,125 €) and late cultivars (+ 10,575 €) compared to unbagged fruit. Hence, if fruit bagging is applied in similar orchards to the Lleida area, bagging would be cost-effective, especially in late-season cultivars.

5.1.2. Postharvest: How do lighting treatments affect the fruit quality of bagged and unbagged fruit? Do they influence fruit decay and fruit susceptibility to brown rot?

After harvest, fruit is submitted to different environments along the postharvest chain, including artificial lighting, as detailed in the Introduction (section 1.1.4.1). Besides, for instance, once at consumer's houses, 35% of surveyed people usually hold nectarines at home up to an average of 3 days (Porat et al., 2018), which could also influence the onset of pathological diseases. Hence, another objective of this thesis was **to evaluate the effect of postharvest treatments (darkness and a lighting treatment) on fruit quality and the prevention of fruit decay** (chapter 2). In our study, one of the main observed consequences was the fruit weight loss in both

unbagged and bagged fruit after postharvest under either lighting treatment relative to initial quality. Weight loss, a common consequence due to dehydration of fruit during shelf life, is a complex process that is affected by preharvest factors (cultivar differences, fruit size, orchard practices, etc.), harvest factors (maturity stage, weather conditions, etc.), and postharvest factors (pre-treatments and storage conditions). However, research integrating all these factors is still lacking (Lufu et al., 2020). More than 8% of water losses, based on weight at harvest, is enough to cause visual fruit shriveling, according to Crisosto et al. (2020). Although in some cultivars like 'Fantasia' and 'Nectatinto', such reduction was considerably higher than 8% in either bagging conditions or under darkness, shriveling was only merely appreciable, i.e., without abundant shriveling symptoms (**Figure 3**). Regarding the lighting treatment effect, results showed that the reduction on size parameters (weight and CD) relative to initial data was higher after storage under darkness than under treatment T1 in 'Fantasia' (of either bagging condition) and bagged 'Albared' and unbagged 'Nectatinto' nectarines (chapter 2). Despite all the mentioned factors affecting water loss, the darkness storage favored the loss of water content herein. To our knowledge, no studies are conducted on the role of artificial visible lighting on the weight loss of peaches or nectarines, and current studies conducted with other types of lights are controversial. Nassarawa et al. (2021) reported that treatments with LED light inhibited the weight loss of vegetables (e.g., brussels sprouts and broccoli). Still, others such as Abdipour et al. (2019) found that a combination of UV-B and UV-C treatments greatly reduced weight loss in peaches compared to untreated samples. However, from the results of this thesis, it can be concluded that lighting treatment T1 during postharvest maintains better the fruit quality of most cultivars than darkness.

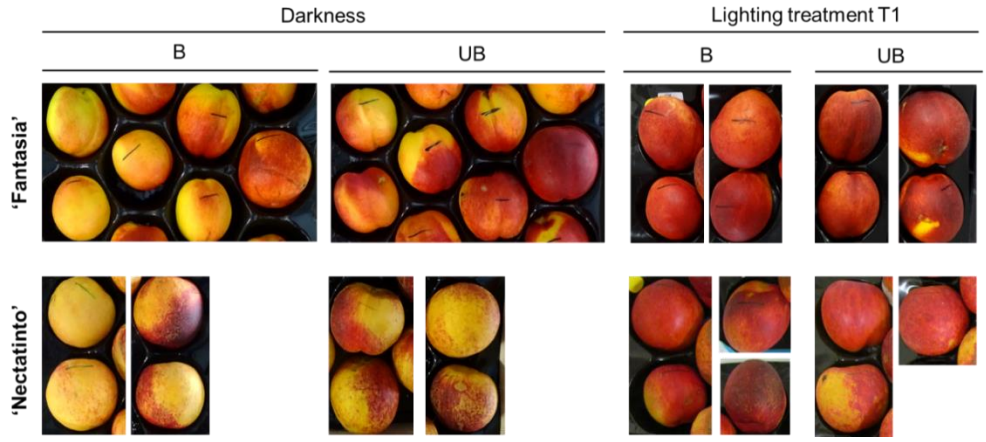


Figure 3. *Bagged (B) and unbagged (UB) nectarines of 'Fantasia' and 'Nectatinto' cultivars after postharvest storage under darkness or lighting treatment T1.*

In addition to fruit weight loss, another quality parameter that was impaired about fruit quality on harvest day was SSC, TA, and the **SSC/TA ratio**. Results showed that postharvest storage increased SSC/TA relative to initial quality. However, the SSC/TA increased under lighting treatment T1 depending on the cultivar and bagging condition (e.g., in bagged 'Albared' nectarines) (chapter 2). Other results of this study showed that the incidence of **fruit decay** of bagged 'Albared' nectarines under lighting treatment T1 was significantly higher (45%) than that under darkness (0%). Natural fungi infections mainly originate in the field but cause fruit decay during postharvest (quiescent or latent infections). However, fruit can also be infected during postharvest, even during cold storage, for the case of *Monilinia* spp. (Bernat et al., 2019). Recent studies describe the importance of good postharvest handling to reduce the activation and/or development of latent infections caused by *Monilinia* spp. (Garcia-Benitez et al., 2020). Hence, factors such as artificial lighting throughout fruit shelf life should also be considered. In an attempt to relate fruit quality's influence to fruit decay, results suggested that the increase of SSC/TA caused by T1 in 'Albared' bagged fruit could have favored fruit decay's appearance, compared to bagged fruit under darkness (chapter 2). Concrete sections of the visible light spectrum can also induce changes in fruit quality of peaches (Zhang et al., 2018). For instance, blue light treatment increases total sugar content in strawberries (Xu et al., 2014) and peaches (Gong et al., 2015) during storage, indicating that light may be mobilizing the sugars through carbon metabolism (Falchi et al., 2020). Consequently, all these soluble solid compounds can be catabolized for stone fruit pathogens, including *M. laxa* and *M. fructicola* (Kou et al., 2018; Rodríguez-Pires et al., 2020b). Actually, *M. laxa* considerably

expresses genes involved in carbohydrate metabolic processes during disease spread in mature nectarines (chapter 3). Since carbon availability is a key factor triggering the production and secretion of small fungal virulence factors required for pathogenicity (Prusky et al., 2020), the increase of SSC/TA seen in this thesis could have promoted an optimal environment for fungi, leading to the activation of latent infections.

As mentioned, *Monilinia* spp. conidia can be transferred from infected to uninfected fruit during storage or by direct contact with other fruit (Bernat et al., 2017, 2019). For this reason, we conducted studies **to test the fruit susceptibility to artificial inoculations performed after harvest with *Monilinia* spp. in unbagged and bagged** fruit of four nectarine cultivars. The first overview of results highlighted that some cultivars (e.g., 'Nectatinto' and 'Albared') were more susceptible than others ('Venus') (chapter 1-suppl. Table S1). Stone fruit cultivars have intrinsic characteristics that lead to a different brown rot susceptibility (Gununu et al., 2019). So that could explain the differences in brown rot susceptibility among cultivars. Regarding the lighting treatment effect on inoculated bagged fruit, results of 'Fantasia' cultivar showed that when storing *M. laxa*-inoculated nectarines, only the severity under T2 was significantly lower than darkness and T1, from 3 to 4 dpi, so lighting treatment T2 reduced the disease in terms of severity (lesion diameter length) compared to the other treatments. Canessa et al. (2013) reported that constant light and a photoperiod of light/dark considerably reduces the lesion areas caused by *B. cinerea* in *A. thaliana* plants compared to continuous darkness. Either the pathogen or the fruit has photoreceptors that activate specific internal responses (Folta and Carvalho, 2015). Thus, the tested lighting treatments could alter the virulence of *M. laxa* or the susceptibility of nectarines during the interaction. In turn, *M. laxa* is developed on fruit, although it can be retarded by high-intensity treatments such as T2.

Regarding *M. fructicola,* it increased incidence, spread faster, and displayed more disease symptoms (e.g., conidiation on fruit surface) than *M. laxa* irrespective of the bagging and lighting condition (**Figure 4**), indicating that lighting treatments differentially impair brown rot development depending on whether they were inoculated with *M. laxa* or *M. fructicola.* In the case of bagged fruit, inoculated fruit with *M. fructicola* presented a distinct development under the different lighting treatments, in which T1 favored the incidence of *M. fructicola* (95% of incidence since 3 dpi) compared to the other conditions (65% of incidence already at 2 dpi) (chapter 1). Visible light tends to decrease disease symptoms (e.g., conidiation, mycelial growth, and conidial germination) in several species such *B. cinerea* (Schumacher, 2017), and

as already mentioned for *M. laxa*-inoculated nectarines. However, results indicate that *M. fructicola* is differentially affected by lighting treatments (compared to literature and *M. laxa*), and the underlying mechanisms are unknown.

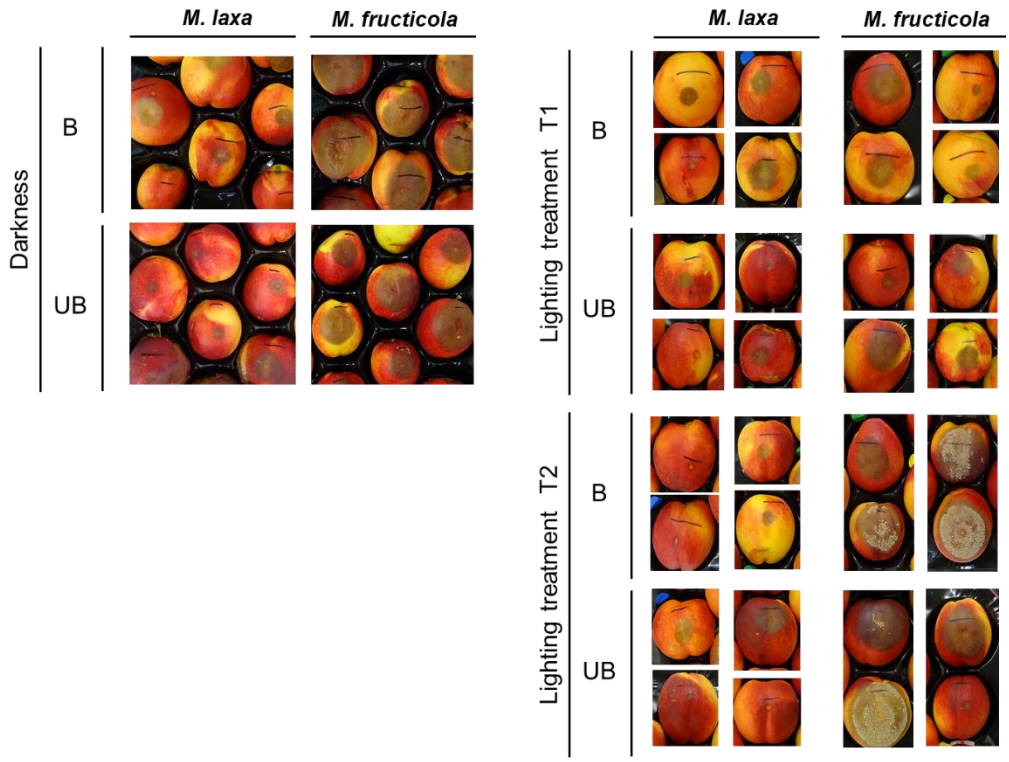


Figure 4. *Images of bagged (B) and unbagged (UB) 'Fantasia' nectarines inoculated with* M. laxa *and* M. fructicola *after 4 days of postharvest storage under lighting treatments (darkness, T1 and T2).*

Light is a source of energy and information for plants, but it can also be a stressor. A photoperiod regime results in stress characterized by ROS production, jasmonic acid (JA) accumulation, and, eventually, programmed cell death (Roeber et al., 2020). Thus said, plant immune responses to pathogen attacks can be affected by the length and intensity of the lighting period (Roeber et al., 2020). From all of that, two questions can be raised: 1) Which metabolisms that are important for brown rot response can be affected by lighting treatments? 2) How do these lighting treatments influence fungal performance and capacity to infect nectarines?

5.1.3. Which metabolisms can be affected by light (either at preharvest or postharvest) that, in turn, impair the fruit response to brown rot?

The production of ethylene, involved in defense against necrotrophic pathogens, among other processes such as development and ripening, can be altered by exposition to light. Hence, another aim of this thesis was **to evaluate the effect of lighting postharvest treatments on ethylene production of mock fruit and artificially inoculated nectarines with *Monilinia* spp.** The ethylene production is nectarine cultivar dependent, but in general, the ethylene climacteric peak of nectarines usually starts approximately the third day of storage at 20 ºC (Giné-Bordonaba et al., 2014), while the ethylene emission can keep increasing up to 10 days after storage (Fernández-Trujillo et al., 1998). Contrary to previous studies, in this thesis, ethylene levels of mock fruit of all conditions (bagged, unbagged, and under the 3 lighting treatments) started to slowly increase at 2-3 dpi. However, the climacteric burst was not clearly detected within the analyzed period. Remarkably, ethylene production in 'Fantasia' nectarines (mock fruit) was affected by neither the lighting treatment nor the fruit bagging condition (chapter 1), although fruit bagging impaired the ethylene production on harvest day in 'Fantasia' nectarines (chapter 2). Information regarding the downstream molecular mechanisms involved in the light-mediated control of ethylene production is limited and mainly relies on the role of red and far-red light in plant species like *Arabidopsis* and tobacco plants (Zdarska et al., 2015). In contrast, studies conducted on fruit, and specifically on peaches or nectarines, are scarce. In this sense, Gong et al. (2015) demonstrate how blue light can induce changes in ethylene to accelerate postharvest ripening in peaches. Although other types of lights, such as concrete sections of the light spectrum, alter the ethylene emission, our results reveal that neither artificial lighting nor bagging conditions change the ethylene pattern of mock nectarines, indicating no susceptibility to such external factors.

Concerning inoculated nectarines with *Monilinia* spp., the **ethylene production** by the pathosystem increased since early time points in almost all conditions tested compared to mock fruit. However, the magnitude of production depended on *Monilinia* spp., fruit bagging conditions, and lighting treatments (chapter 1). Interestingly, some fungi can produce ethylene (Chanclud and Morel, 2016), but its function in brown rot development is still unclear (Vall-llaura et al., under review). Recent studies from our group found that *Monilinia* spp. can produce ethylene under certain *in vitro* conditions (Vall-llaura et al., personal communication). Thus, the

recorded ethylene production in this thesis could also be influenced by the pathogen's production. Herein, the ethylene production by the *M. laxa*-unbagged nectarines under T1 and T2 was lower than darkness, and only the ethylene emission by the *M. fructicola* interaction under T1 at 3 dpi was lower than darkness and T2. However, bagged fruit revealed almost an opposite pattern. In bagged fruit, the ethylene production by the *M. laxa* pathosystem under both T1 and T2 was higher than darkness. In contrast, the production by the *M. fructicola* interaction under T1 at 3 dpi peaked compared to the other conditions. Ethylene is a response plant factor to numerous biotic and abiotic stresses (Glazebrook, 2005; Müller and Munné-Bosch, 2015), so here, ethylene could be involved in defense against *Monilinia* spp. attack. Alternatively, results also suggested that it could be acting as a susceptible factor, although this role is not clear enough, and the dual role of this phytohormone has already been described in other pathosystems (Van Loon et al., 2006). Hence, like described in peach fruit (Baró-Montel et al., 2019a) or peach petals (Vall-llaura et al., 2020), it can be hypothesized that the host could be conducting different responses depending on the *Monilinia* spp. (and also the virulence of the strain). Hence, the species was the main factor altering the ethylene production compared to mock fruit. In addition to the dual modulation of ethylene caused by *Monilinia* spp., the ethylene production of inoculated fruit also depended on the fruit bagging condition and, to a lesser extent, on lighting treatments.

To assess the effect of light in preharvest, i.e., **fruit bagging**, first evaluations of brown rot incidence were conducted on bagged and unbagged nectarines, that were artificially inoculated with *Monilinia* spp. No differences between bagging conditions were observed in either cultivar under darkness, indicating that the susceptibility to *Monilinia* spp. was not affected by the bagging condition under that storage (Suppl. Table S1 of chapter 1). Nevertheless, brown rot development changed between fruit bagging conditions when inoculated fruit was stored under lighting treatments T1 and T2. So, results elucidated the importance of the light in preharvest and its effect under lighting postharvest treatments. As already highlighted in chapter 1, studies should be conducted to analyze intrinsic fruit properties such as quality parameters that could be ultimately correlated with brown rot development under certain postharvest conditions. The quality parameters differing between fruit bagging conditions (chapter 2) might explain some susceptible phenotypes to artificially inoculated nectarines (chapter 1). For instance, the development of brown rot is positively associated with sucrose (Baró-Montel et al., 2020) and SSC (Gradziel, 1994). However, any clear relation can be extracted from the analyzed fruit quality parameters of

'Fantasia' nectarines and their relation to fruit susceptibility. Therefore, other studies of intrinsic fruit properties such as antioxidant parameters could be conducted in all four cultivars since brown rot (e.g., caused by *M. laxa*) can be correlated with antioxidant content (Obi et al., 2020).

Light, such as UV-B radiation, does not penetrate the flesh; there may be a possible signaling pathway between skin and flesh (Santin et al., 2021), which in turn, can impair the fruit response to brown rot. Herein, the development of *Monilinia* spp. on inoculated unbagged fruit under lighting treatments was considerably different from the one observed in *Monilinia* spp.-inoculated bagged nectarines. In *M. laxa*-inoculated unbagged nectarines incubated under lighting treatments, the incidence under T1 and T2 was significantly lower than darkness. In contrast, almost no differences were observed among conditions in *M. fructicola*-inoculated unbagged nectarines (chapter 1). In other studies with *B. cinerea,* a closely related species to *Monilinia* spp., the infection with this pathogen progresses better when the hyphae are protected from direct light, i.e., under darkness or once hyphae have penetrated the host (Schumacher, 2017). All suggest that the altered solar light received by the fruit due to bagging, together with the effect of lighting postharvest treatments, clearly affected the fruit surface and the downstream defense mechanisms. The exposure to light may cause stress responses in those cases in which light has exceeded the energetic demand of the plant or their capacity to dissipate the excessive light (Huang et al., 2019). Secondary fruit **metabolites** play a crucial role in protective functions (Khare et al., 2020). For instance, in *Arabidopsis* plants continuously exposed to high light intensity (1,200 mmol m^{-2} s^{-1}, 200-800 nm), the expression of genes related to abscisic acid (ABA) and phenylpropanoid pathway was highly expressed, suggesting that the response to high-intensity light stress was through dynamic regulation of hormonal network (Huang et al., 2019). Terpenoids are one of the secondary metabolisms that can be altered by light. In fact, light downregulates the mevalonate (MVA) pathway genes, but it stimulates the methylerythritol phosphate (MEP) pathway-related genes (Tholl, 2015). Among others, the UV-B irradiation has been shown to impair the peach fruit volatiles, like reducing the monoterpene linalool production and increasing the sesquiterpene accumulation (E,E)-α-farnesene (Liu et al., 2017). Preliminary studies of our group revealed that some terpenoid genes (*PpHMGS, PpPFT1, PpFOLK, PpSQS, PpSM2, PpLIS1*, and *PpLIS2*) were overall not induced by the effect of treatment T1 compared to total darkness in healthy nectarines (Annex 1). However, further studies could continue in that direction. Terpenoids are also implicated in biotic responses, as seen in the overall upregulation

of terpenoid genes in response to *M. laxa* in mature nectarines incubated under darkness (chapters 3 and 4). Very little data are available about the role of light in counteracting a fungal infection on peach fruit (Santin et al., 2018). Hence, studying postharvest fruit behavior toward combined biotic and abiotic factors would reflect the complex environment that crops face daily. Studies should be conducted towards the protective role that some metabolisms (e.g., terpenoids) can have under the combination of the light effect (either solar light or lighting treatments) and *Monilinia* spp. inoculations on stone fruit.

Furthermore, all results presented herein denote that *M. laxa* and *M. fructicola* infections on nectarines were differentially affected by lighting treatments. Still, such alterations depended on the bagging condition when fruit was stored under lighting postharvest treatments. In this line, *in vitro* studies of this thesis also confirmed the different photomorphogenesis of *M. laxa* and *M. fructicola*. However, the incidence of brown rot development on bagged fruit triggered by *M. fructicola* under T2 was like that under darkness (both different to T1). So, further studies deciphering the impaired effect of either T1 or T2 on *M. fructicola* are encouraged. Several fungal biological responses have been described for each monochromatic section of the spectrum (Schumacher, 2017; Veloso and van Kan, 2018). For instance, green light (around 540 nm) represses mycelial growth (Zhu et al., 2013), whereas blue (about 450 nm) and red (around 650 nm) light restrain conidiation (Tan, 1975). In this thesis, the light spectrum of both lighting treatments was different (**Figure 5**), in which, for instance, the orange/red wavelength of T1 is higher than the T2. Hence, the differences between T1 and T2, in terms of photoperiod, intensity, and color, could also explain the distinct brown development caused by *M. fructicola* on bagged fruit.

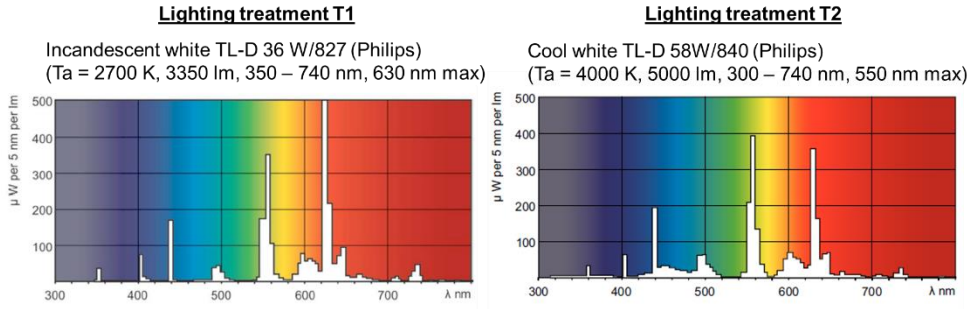


Figure 5. *The light spectrum of lighting treatments T1 and T2 used in chapters 1 and 2. Color, power, color code, the temperature of color, light flux, range, and maximum wavelength are indicated (Philips).*

5.1.4. How do postharvest lighting treatments influence *M. laxa* and *M. fructicola* behavior and their capacity to infect fruit?

Fungal morphogenesis is greatly regulated by light. In this sense, the last objective regarding light factor was **to decipher the effect of the postharvest lighting treatments on the behavior of *M. laxa* and *M. fructicola* in *in vitro* conditions and their capacity to infect fruit.** For that, in this thesis, (unbagged) fruit was inoculated with either *M. laxa* or *M. fructicola* that were previously incubated for 7 days under lighting treatments (T1, T2, and darkness). Then, the fruit was incubated in darkness to assess the disease development (chapter 1). The incidence of fruit inoculated with *M. laxa* that was previously incubated under darkness was significantly higher than when the pathogen was previously exposed to either T1 or T2 light treatments. Contrary to *M. laxa,* the incidence of fruit inoculated with *M. fructicola* previously incubated under T1 significantly peaked at the beginning of the infection, and its severity was significantly higher than the other conditions along the incubation time. Interestingly, these results were like those obtained when analyzing the effect of lighting treatments in *Monilinia* spp.-unbagged fruit interaction. Therefore, overall results suggested that disease development relied on the pathogen's light effect when infecting unbagged fruit.

Before conducting these experiments, there were no other studies available aiming to analyze the capacity of *M. laxa* and *M. fructicola* to infect stone fruit, such as nectarines, after being incubated to lighting treatments. Only some studies were conducted in other pathosystems such as *P. digitatum*-oranges (Lafuente et al., 2018). The darkness exposition of *P. digitatum* leads to an increased capability to infect oranges compared to cultures submitted to non-continuous light. Recently, studies of our group have also shown that *M. laxa* was more aggressive in infecting nectarines of the 'Extreme 563' cultivar after being incubated under darkness than under white light, whereas *M. fructicola* was more aggressive after being exposed to white light (Verde-Yáñez, under review). Hence, overall suggests that lighting treatments affected *Monilinia* spp. by altering their capacity to infect fruit, and thus, brown rot development in a species-specific manner.

To understand the different behavior of *M. laxa* and *M. fructicola* in infecting nectarines after being exposed to lighting treatments, an *in vitro* ecophysiology study was proposed as a valuable tool to understand such behavior and development on fruit. The results presented in this thesis clearly showed that light differentially altered the **phenotype** of *M. laxa* and *M. fructicola* in *in vitro* conditions, in which *M. laxa* had

a broader photomorphogenic response to light than *M. fructicola.* As detailed in chapter 1, the morphology of conidia of *M. laxa* was altered by lighting treatments, and the conidial viability under T2 was lower than darkness. Both alterations could explain the reduced aggressiveness of *M. laxa* after being exposed to either T1 or T2 before infecting fruit, as already suggested for *P. digitatum*-oranges by Lafuente et al. (2018). In the case of *M. fructicola*, although lighting treatments also altered the morphology of conidia, there were no differences in the conidial viability among lighting treatments. Hence, contrary to *M. laxa,* results can infer that the altered *M. fructicola* conidia under T1 or T2 affected neither conidial viability nor capacity to infect fruit. Light can have a different effect among fungal species (e.g., regulation of carotenoid production is different among *Phycomyces*) but also among phytopathogens (e.g., carbohydrate metabolism) (Tisch and Schmoll, 2010). In this line, *M. fructicola* seems to be less altered by the different lighting treatments than *M. laxa*. Hence, *M. fructicola* could better in facing the changes of the light spectrum (e.g., future increases in high UV-B radiation) more than *M. laxa.*

Fungal growth and conidiation under *in vitro* conditions are correlated to fungal aggressiveness on nectarines, as seen for an *M. fructicola* isolate on wounded nectarines (Janisiewicz et al., 2013). Herein, lighting treatments (T1 and T2) made *M. laxa* to grow and produce more conidia on PDA-T, compared to darkness, whereas it occurred the opposite for *M. fructicola* (chapter 1). However, when fruit was inoculated with *Monilinia* spp. that were previously incubated under each lighting treatment, both pathogen behaviors on fruit showed almost the opposite pattern than that observed *in vitro.* Besides, nectarines inoculated with *M. laxa* previously incubated under any lighting treatment barely showed conidiation on fruit surface (only 10% of fruits), whereas *M. fructicola* produced a lot of conidiation on fruit surface and early in time (low incubation and latency periods) under T1 and T2 compared to darkness (**Figure 6**). So, both *Monilinia* spp. on fruit behaved contrary to that in *in vitro* conditions. Hence, from the results obtained for the growth rate and conidiation in *in vitro* conditions, we cannot infer its capacity to infect fruit. Light-induced alterations observed under *in vitro* development were not maintained when the pathogen interacted with fruit. Thus, which other *in vitro* parameters, altered by lighting treatments, could shed light on the infection capabilities?

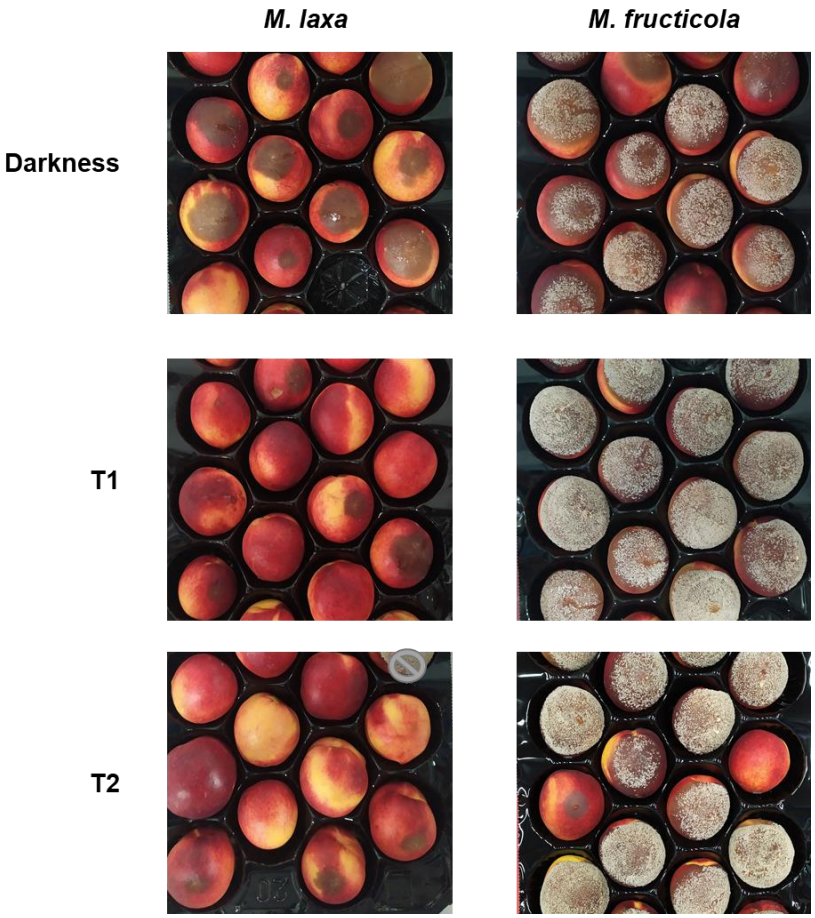


Figure 6. *Effect of the capacity of* Monilinia *spp. to infect nectarines of 'Fantasia' cultivar. Nectarines (unbagged) were inoculated with* M. laxa *or* M. fructicola *that were previously grown on PDA-T medium and incubated under each lighting treatment (darkness, T1, T2). Images were taken at 7 dpi. Similar results were obtained for the 'Venus' cultivar.*

Another phenotypical feature raised from the *in vitro* ecophysiology studies is the impaired pigmentation that fungal cultures had among lighting treatments. For instance, the *M. laxa* colony turned mainly hazel after being exposed to T1 and T2 on PDA-T, compared to the white phenotype under darkness conditions. As mentioned in chapter 1, long-term effects related to exposure to light can involve the accumulation of pigments such as carotenoids, implicated in protecting cells from ROS due to its antioxidant nature, and also melanin, involved in protection against environmental stresses (e.g., UV radiation) and also in full virulence to infect stone fruit (Fuller et al., 2015; Corrochano, 2019; Yu et al., 2020). De Cal and Melgarejo (1993) showed that an *M. laxa* occurring natural mutant for the melanin biosynthetic pathway

(albino) could not induce peach twig blight. Thus said, further studies should be conducted to decipher the role of fungal pigments in *Monilinia* spp. in *in vitro* conditions, especially during the infection process on stone fruit.

Overall results highlighted the different behavior of *M. laxa* and *M. fructicola* in both *in vitro* and *in vivo* development. The **dual behavior of these species** have been recorded for several authors at both phenotypical and transcriptional level (De Miccolis Angelini et al., 2018; Baró-Montel et al., 2020; Vall-llaura et al., 2020) but also at the genomic level (Marcet-Houben et al., 2021). For instance, results from De Miccolis Angelini et al. (2018) revealed that the expression of some *Monilinia* spp. genes (e.g., pectate lyase and glycoside hydrolase family 5 protein) in the fungal mycelium is impaired between darkness and light conditions (combination of daylight and near-UV lamps). Such differences also depend on the *Monilinia* species, specifically for some isolates of *M. fructicola* and *M. laxa*. However, the mechanisms underlying the impaired photoresponse due to lighting treatments and how they can influence the capacity of pathogens to infect fruit are still not studied. In addition, the results presented herein revealed some differences in the behavior between T1 and T2 lighting treatments, which can be derived, among others (e.g., photoperiod period), from their light spectrum (**Figure 4**). Each treatment has peaks at different wavelengths, indicating the differences in light quality (wavelength of electromagnetic spectrum, i.e., color) among treatments. Several biological responses of *B. cinerea* have been described for each monochromatic section of the spectrum (Schumacher, 2017), as previously mentioned in chapter 1. Recently, some photoreceptors and related regulatory proteins (e.g., velvet regulatory family) have been described and characterized in *M. laxa* (Rodríguez-Pires et al., 2021), indicating the capability of *M. laxa* to sense light. Currently, Verde-Yañez and coauthors are assessing the effect of monochromatic sections of the visible spectrum on the three main *Monilinia* spp. in *in vitro* conditions (both at phenotypic and transcriptomic level) but also on their capacity to infect nectarines. In addition, future studies towards studying the effect of UV-B radiation, which will increase as a consequence of global climate change (Hashimoto et al., 1990), should also be addressed.

Overall, considering the light both in the field (fruit bagging) and during postharvest (lighting treatments) is relevant for the improvement of global fruit quality (including the reduction of fruit decay), but also for their impact not only in fruit resistance/susceptibility to brown rot, but also for its effect on *Monilinia* spp. morphogenesis.

5.2. The relevance of host-pathogen interactions studies to understand brown rot development

Before this thesis, there was no detailed information on the simultaneous transcriptional responses of the interaction between *M. laxa* and peach or nectarine. In contrast to the other studies that have studied *Monilinia* spp. for one side and stone fruit on the other, a dual RNA-Sequencing (RNA-Seq) provides information on which metabolisms are expressed by both players during the interaction along the infection time course. Thus, an objective of this thesis was **to unravel the global strategies deployed simultaneously by nectarine and *M. laxa* during their interaction** through identifying the main host defense responses involved in resistance or susceptibility to brown rot and determining putative and relevant strategies employed by the pathogen to cause disease (chapter 3). This chapter showed that immature and mature 'Venus' nectarines were differentially susceptible to *M. laxa,* and this was the key point to find the differential strategies that both *M. laxa* and nectarine perform during i) an interaction in which the disease progressed and, ii) an interaction in which the disease did not develop. Hence, we performed a dual RNA-Seq analysis in two developmental stages of fruit that were either resistant (immature) or susceptible (mature) to *M. laxa.*

From literature, it is already known that although fruit can be infected by *Monilinia* spp. at any developmental stage, peaches become less susceptible to *M. laxa* at the stage in which growth (cell enlargement and elongation) starts, between 4 and 8 weeks before harvest (Guidarelli et al., 2014). For that reason, we carefully studied the commercial harvest day following the grower's recommendations. So fruit one month before harvest and commercial harvest day were harvested for the "immature" and "mature" stages, respectively. Sampling was conducted to obtain samples with a progressive evolution of *M. laxa* spread. De Cal (personal communication) found that within 6 h post-inoculation (hpi), *M. laxa* can establish on fruit surface and start germination if the external conditions are favorable. Hence, it was selected as the first time point to be analyzed. Following time points were sampled along the infection time course at 14, 24, 48, and 72 hpi. The study revealed a good homogeneity among replicates and good separations among samples by Principal Component Analysis (PCA) that we believe were crucial for the optimal development of the RNA-Seq and subsequent analysis.

Results showed that, although no disease symptoms were observed on immature fruit, analyses showed an increase of *M. laxa* biomass and mapped reads suggesting that

the pathogen was also active on immature fruit (e.g., the *MlACT* relative expression at 14 hpi was 1.90-fold higher than that at 6 hpi). On the other side, the disease greatly progressed in mature tissues, coinciding with the massive increase of *M. laxa* biomass and mapped reads (e.g., the *MlACT* relative expression at 72 hpi was 40.48-fold higher than that at 6 hpi). Besides, the expression profile of nectarine genes of each sample (PCA results) was grouped according to their developmental stage and infection status. The expression patterns of *M. laxa* suggested that the pathogen utilized different strategies depending on the host developmental stage. Similar behaviors were obtained for *B. cinerea*, *Fusarium acuminatum*, and *Rhizopus stolonifer* on mature green (resistant) and red ripe (susceptible) tomatoes, in which pathogens modified their infection strategies in response to the developmental stage of the host (Petrasch et al., 2019) and that tomato responses were according to fruit stage (Silva et al., 2021). In our work, *M. laxa*-inoculated samples for *M. laxa* genes were very close in the PCA at 6 hpi of both tissues; for that reason, the differential expression (DE) analysis was conducted through pairwise comparisons between 14, 24, and 48 hpi compared to 6 hpi, for each fruit developmental stage. In turn, DE analysis of nectarine genes was conducted comparing inoculated vs control fruit for each developmental stage and time point. Overall, results concluded that the nectarine susceptibility to *M. laxa* was developmentally controlled and that nectarine and *M. laxa* synchronized their transcriptional response during their interaction.

Therefore, in this section, the main strategies employed by *M. laxa* in mature and immature fruit will be addressed as well as the host defense responses involved in resistance or susceptibility to brown rot (**Figure 7**). Finally, as highlighted by KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichments of the RNA-Seq study, a special mention will be conducted on the role of some secondary metabolites (i.e., terpenoid metabolism and volatile organic compounds, VOCs) that could be participating in the resistance or susceptibility of nectarine to brown rot.

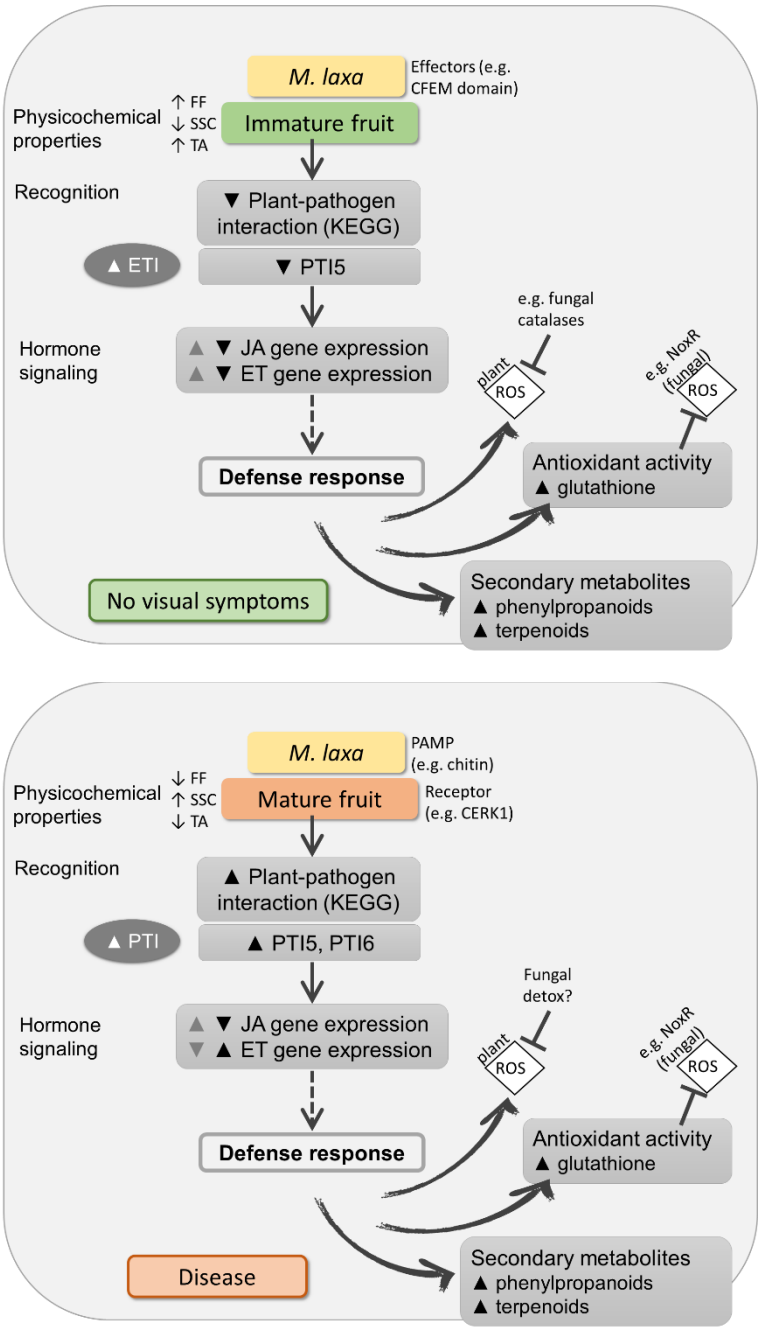


Figure 7. *Main metabolisms elucidated in the RNA-Seq analysis during the interaction of* M. laxa*-nectarine in immature (up) and mature (down) fruit. Abbreviations: Flesh firmness (FF), soluble solids content (SSC), titratable acidity (TA), pathogen-associated molecular patterns (PAMP), PAMP-triggered immunity (PTI), effector-triggered immunity (ETI), transcriptional activators (PTI5 and PTI6), jasmonic acid (JA), ethylene (ET).*

5.2.1. Which are the main strategies employed by *M. laxa* to develop on nectarine tissues with different levels of susceptibility to it?

Plant pathogenic fungi such as *M. laxa* infect fruit when external conditions (e.g., environmental and physicochemical fruit properties) are favorable for the success of the pathogen (Luo et al., 2005; Gununu et al., 2019). If conditions are not good, fungi can remain quiescent (Prusky et al., 2013), cause latent infections (Gell et al., 2008), or search for other survival pathways. The RNA-Seq results obtained in this thesis revealed that the pathogen used different transcriptional machinery according to the fruit developmental stage (chapter 3). For instance, up to 118 unique upregulated differential expressed genes (DEGs) of *M. laxa* were found at 48 hpi on immature tissue, and up to 562 upregulated *M. laxa* DEGs were found at 48 hpi on mature tissue. Besides, the top 5 up-regulated *M. laxa* DEGs (see table 1, chapter 3) were also unique for each nectarine developmental stage. However, the magnitude of the transcriptional response was higher (e.g., 3-fold of DEGs with GO annotations at 48 hpi) in mature than immature tissue along with the infection course. Hence, although disease symptoms were not observed on immature fruit, these results suggested that the pathogen was active at later time points since it expressed genes related to survival mechanisms to remain on immature nectarines. In contrast, in mature fruit, where the disease progressed, the switch on the transcriptional machinery (between 6 and 14 hpi) was towards a necrotrophic infection mechanism.

Analyzing in detail the putative strategy of *M. laxa* on immature fruit, it was observed that *M. laxa* had DEGs belonging to the CAZymes functional category since early time points. **CAZymes** are a family of cell wall degrading enzymes (CWDE) that necrotrophic pathogens use to break down plant tissues to infect a particular host. Among them, a member of the glycosidase hydrolase family 31 (*Monilinia_056600*), which was highly expressed at 24 hpi, was also recently predicted in the *M. laxa* 8L proteome as a secreted enzyme (Rodríguez-Pires et al., 2020a). Besides, mutants of glycoside hydrolase of *Magnaporthe oryzae* (*MoGLS2*) show decreased virulence (reduced incidence and smaller lesions) when infecting rice and barley seedlings (Li et al., 2016). Hence, *M. laxa* at least partially activated the machinery to penetrate host cells. Alternatively, plant pathogenic fungi can also secrete **effector proteins** to alter the defense responses of host cells or to kill them by inducing plant programmed cell death (Mukhtar et al., 2016). In our study, results revealed that *M. laxa* DEGs with the CFEM domain (common in several fungal extracellular membrane proteins) and known to be involved in pathogenesis (**Table 1**) were only present at 14 hpi. As mentioned in chapter 3, among the DEGs containing CFEM domains, *Monilinia_077410* is a

homolog of *BcCFEM1* from *B. cinerea*, an effector shared by many *Botrytis* spp. (Valero-Jiménez et al., 2019). Recently, a protein (MFRU_002g05260) with this domain has been predicted as an effector protein in *M. fructicola* (Vilanova et al., 2021). Besides, its transient expression in *Nicotiana benthamiana* leaves was able to induce cell death in the infiltrated area. Together with the expression of DEGs with signal peptide annotations, herein suggested that *M. laxa* could be secreting effector proteins to suppress the host PTI response.

Table 1. *List of Pfam (protein family database) obtained in Pfam enrichments of* M. laxa *upregulated DEGs (category, description, and function/process) in immature nectarines.*

Immature		
Category	**Description**	**Function / Process**
PF05730	CFEM domain	Pathogenesis
PF07690	Major Facilitator Superfamily	Membrane transport
PF00083	Sugar (and other) transporter	Membrane transport
PF00106	Short chain dehydrogenase	Other
PF13561	Enoyl-(Acyl carrier protein) reductase	Other
PF08659	KR domain	Other
PF00232	Glycosyl hydrolase family 1	CAZy
PF14310	Fibronectin type III-like domain	Adhesion
PF01915	Glycosyl hydrolase family 3 C-terminal domain	CAZy
PF00933	Glycosyl hydrolase family 3 N terminal domain	CAZy
PF00107	Zinc-binding dehydrogenase	Redox
PF08240	Alcohol dehydrogenase GroES-like domain	Redox
PF01073	3-beta hydroxysteroid dehydrogenase/isomerase family	Other
PF06628	Catalase-related immune-responsive	Redox
PF00199	Catalase	Redox

Notwithstanding, *M. laxa* was not able to cause disease on immature nectarines. From 14 hpi onwards, the *M. laxa* biomass started to decrease, followed by a decline of the *M. laxa* mapped reads, a reduction of up-regulated *M. laxa* DEGs, and a switch of *M. laxa* transcriptional machinery. Remarkably, most of the upregulated *M. laxa* DEGs (>50 DEGs) at 24 hpi were related to fungal membrane transport (**Table 1** and chapter 3). All these results suggested that *M. laxa* could be starting a process of **autolysis** as it expressed genes of membrane transporter proteins that could be used to feed on its remains, which ultimately would help the pathogen to survive on the fruit surface. Fungal autolysis involves partial permeabilization of cells and leakage of intracellular

material, widely described in filamentous fungi for the self-digestion of aged hyphal (White et al., 2002). This process implies enzymic activities such as hydrolase. In our study, among the 5 top upregulated *M. laxa* DEG found at 48 hpi, *Monilinia__034450* has GO terms related to hydrolase activity and the CAZy annotation of Glycosyl hydrolase family 3, suggesting that such gene could be implicated in hydrolysis during the autolytic process.

Finally, at 48 hpi on immature fruit, some upregulated *M. laxa* DEGs were involved in response to **oxidative stress**. Actually, the highest induced DEG among all time points of immature fruit was an oxidoreductase gene. Besides, the pathogen was also expressing 10 DEGs with **catalase activity** at that time. Fungal catalases have been reported to detoxify H_2O_2 (accumulated in the infection zone) during the infection of tomato leaves with *B. cinerea* (Schouten et al., 2002). Hence, our results suggested that *M. laxa* was under oxidative stress conditions, maybe due to the ROS produced by host cells, and thus, tried to scavenge ROS generated. In parallel, **ROS production** by fungi is also described as a key factor in developmental processes in various phytopathogens, and the lack of fungal ROS-producing systems can affect their virulence (Segal and Wilson, 2018). Herein, some genes encoding the Nox regulator R (NoxR), which belongs to the NADPH oxidase (Nox) complex and is involved in fungal ROS production (Kim et al., 2011; Li et al., 2016a), were found to be upregulated at 24 hpi in immature tissue. Hence, our results also suggest that *M. laxa* could also produce ROS through either NADPH oxidase complex or the action of alcohol oxidase, as already mentioned in chapter 3. Overall, these results provide several potential strategies that the pathogen used to survive on immature nectarines. So, targeting these specific genes involved in response to oxidative stress, nutrient transport, and carbohydrate catabolism could reduce latent or quiescent infections.

In contrast to immature fruit, *M. laxa* expressed a very different transcriptional machinery on mature nectarines. Specifically, *M. laxa* DEGs only shared the Redox category of Pfam enrichments between immature and mature tissues (**Tables 1 and 2**). Between 6 and 14 hpi, *M. laxa* experienced a transcriptional reprogramming, coinciding with the onset of disease symptoms typical of a necrotrophic phase (penetration initiation and maceration), **growth, and spread** of the pathogen (e.g., upregulated DEGs with GO and Pfam annotations of transcription/translation and filamentous growth). In addition, *M. laxa* also expressed DEGs with GO involved in proteolysis predominantly in protein translation. Proteolytic activity (**Table 2**) was prevalent throughout disease progression. Still, Pfam enrichments related to such activity (e.g., PF09286) peaked at 14 hpi, probably towards the invasion of host cells, since the penetration of the pericarp tissues occurred between 14 and 24 hpi. In fact,

a single protease gene was the highest upregulated DEG (***Monilinia_077490***) at all time points on mature nectarines. This gene codifies for a protein detected in the exoproteome of three different isolates of *M. laxa* (including ML8L isolate, the same used in this thesis) in *in vitro* peach cultures (Rodríguez-Pires et al., 2020b). Besides, this *M. laxa* gene is orthologous to the acid protease Bcacp1 in *B. cinerea* and only expressed under acidic conditions during infection (Rolland et al., 2009). Acid proteases have a crucial role in colonization and infection, as seen during the pathogenesis of *Sclerotinia sclerotiorum* (Poussereau et al., 2001) or even can overcome host defense responses as seen in *Fusarium oxysporum* f. sp. *Lycopersici* (Jashni et al., 2015). Overall, results provide evidence of the plethora of mechanisms that *M. laxa* expressed during the early stages of the infection process to invade host cells.

Table 2. List of Pfam (protein family database) obtained in Pfam enrichments of M. laxa upregulated DEGs (category, description, and function/process) in mature nectarines.

Mature		
Category	**Description**	**Category**
PF09286	Pro-kumamolisin, activation domain	Proteolysis
PF01248	Ribosomal protein L7Ae/L30e/S12e/Gadd45 family	Transcription/Translation
PF00270	DEAD/DEAH box helicase	Transcription/Translation
PF00400	WD domain, G-beta repeat	Filamentous growth
PF00428	60s Acidic ribosomal protein	Transcription/Translation
PF04003	Dip2/Utp12 Family	Transcription/Translation
PF00271	Helicase conserved C-terminal domain	Transcription/Translation
PF00118	TCP-1/cpn60 chaperonin family	Transcription/Translation
PF00076	RNA recognition motif. (a.k.a. RRM, RBD, or RNP domain)	Transcription/Translation
PF01138	3' exoribonuclease family, domain 1	Transcription/Translation
PF03144	Elongation factor Tu domain 2	Transcription/Translation
PF00587	tRNA synthetase class II core domain (G, H, P, S, and T)	Transcription/Translation
PF00153	Mitochondrial carrier protein	Membrane transport
PF02668	Taurine catabolism dioxygenase TauD, TfdA family	Redox
PF09334	tRNA synthetases class I (M)	Transcription/Translation
PF07992	Pyridine nucleotide-disulphide oxidoreductase	Redox
PF00133	tRNA synthetases class I (I, L, M, and V)	Transcription/Translation
PF13450	NAD(P)-binding Rossmann-like domain	Redox

The abundant disease symptoms on mature fruit were also correlated with high transcriptional activity. Specifically, those transcripts related to translation, ribosomal proteins, and transcriptional factors (growth and cell cycle control) were prevalent throughout the infection. On mature fruit, *M. laxa* also expressed genes related to **ROS production**, like the Nox regulator R (upregulated at 24 hpi), a regulator subunit of the NADPH oxidase complex involved in fungal ROS production during host-pathogen interactions (Kim et al., 2011; Li et al., 2016). In addition, and according to its necrotrophic lifestyle, *Monilinia* spp. can produce a large arsenal of **CWDE** as virulence factors; for instance, the expression of up to 25 hydrolytic and carbohydrate-active genes have been described in three *Monilinia* spp. (*M. fructicola*, *M. laxa* and *M. fructigena*) (De Miccolis Angelini et al., 2018). Pectinolytic, proteolytic, cellulolytic, and xylanolytic activities were also found on nectarine juices inoculated with *M. fructicola, M. fructigena*, and *M. laxa* isolates (Garcia-Benitez et al., 2019). In this RNA-seq study, a protein codified by a glycoside hydrolase family 71 genes (***Monilinia_037020***), and a protein codified by a glycoside hydrolase family 28 gene (***Monilinia_000560***), both expressed at 14 hpi, were also detected in the exoproteome of three isolates of *M. laxa* (including ML8L) in *in vitro* peach cultures (Rodríguez-Pires et al., 2020a). Recently, Marcet-Houben et al. (2021) showed that these two *M. laxa* genes (*Monilinia_037020* and *Monilinia_000560*) were also present in five *Monilinia* spp. genomes, pointing out the importance of such genes as virulence factors on *Monilinia* spp. In this line, the putative protein function of *Monilinia_000560* was identified by Rodríguez-Pires et al. (2020a) as a polygalacturonase 2. It was upregulated on a minimal medium containing pectin compared with glucose, indicating its role in pectin hydrolysis. Furthermore, a rhamnogalacturonan hydrolase (glycoside hydrolase family 28, ***Monilinia_041700***), highly expressed at later time points (24 and 48 hpi), was also characterized in *M. laxa* infecting peaches (Baró-Montel et al., 2019b), indicating proteolytic activities in other stone fruit. Based on these data, targeting specific proteolytic genes and additional CAZymes may help inhibit or reduce the severity of disease in susceptible fruit.

5.2.2. Which are the main nectarine defenses involved in its resistance or susceptibility to *M. laxa*?

As mentioned before, the expression of nectarine genes was according to their developmental stage and infection status. In particular, *M. laxa*-inoculated samples of 6 hpi of each developmental stage were close to their respective control samples, indicating that nectarine genes switched their transcriptional machinery after 6 hpi in

each stage (chapter 3). That switch implied a larger transcriptional change in mature than immature fruit; for instance, mature nectarines expressed 3.5-fold more DEGs (inoculated vs control) than immature tissues. Likewise, the amount of DEGs increased over time, indicating that the host was conducting an extensive transcriptional reprogramming as the disease progressed, although the former failed in blocking the pathogen spread. Similar results were obtained in susceptible tomatoes (red ripe) that expressed a stronger immune response to *B. cinerea*, *F. acuminatum*, and *R. stolonifer* (1,538 upregulated genes) than resistant unripe fruit (475 upregulated genes) (Silva et al., 2021).

In our work, in the tissue where disease progressed, results pointed out that the host triggered the PAMP-triggered immunity (PTI) since the chitin elicitor receptor kinase 1 (codified by *CERK1*) (*Prupe.3G213100.1*) and the transcriptional activator *PTI6* (*Prupe.6G039700.1*) were upregulated in the mature tissue at 14 hpi. In fact, *PTI6* was also suggested to be a candidate gene in the disease resistance response of unwounded peaches inoculated with *M. fructicola* (Fu et al., 2021). In contrast, on immature fruit, since results elucidated that *M. laxa* putatively expressed effector proteins, results pointed out the activation of the effector-triggered immunity (ETI). In both cases, both PTI and ETI induced host hormone signaling.

Immature fruit inoculated with *M. laxa* was also very active transcriptionally, in which 63.60% of the total detected DEGs were upregulated compared to control fruit. The KEGG enrichment of these upregulated DEGs showed that enriched pathways peaked at or after 24 hpi, coinciding with the highest gene expression induction on immature fruit. Once the plant-pathogen interaction took place in terms of transcriptional activity (merely enriched at 24 hpi), the host hormone signaling transduction pathways' biosynthesis was induced. Pathways associated with **ethylene and jasmonic acid (JA)** (cysteine and methionine metabolism and α-linolenic acid pathways) were enriched at later time points. However, the gene expression of their biosynthetic genes of both pathways was induced, in general, since 14 hpi. These results suggested the involvement of these hormones in the *M. laxa*-nectarine interaction.

Specifically, the gene expression of the JA pathway in immature fruit revealed that multiple genes encoding the initial biosynthetic steps were induced in response to *M. laxa*. In contrast, this expression in the later steps was moderately activated. However, the JA pathway expression in resistant immature fruit was like susceptible tissue, so all points out that the response of the immature fruit, which should be towards resistance,

could be towards expressing other strategies. For instance, compounds of the JA pathway can be used as an elicitor of defense mechanisms (Tsao and Zhou, 2000). Recent studies have shown that methyl jasmonate increases the phenolic content, antioxidant capacity, and the activity of defense-related enzymes of fruits inoculated with *M. laxa* compared to control while decreasing the brown rot incidence and the lesion diameter on apricot fruit (Ezzat et al., 2021). Consequently, the JA activity is likely useful as a plant defense signaling molecule.

Regarding ethylene, the production in *M. laxa*-immature nectarine interaction was higher than the control only at 24 hpi, corresponding to the highest induction of the ethylene biosynthetic genes (e.g., *ACS2* and *ACO1*) in response to *M. laxa.* That production coincided with the peak of transcriptional responses related to fruit defense response against *M. laxa* (Van Der Ent and Pieterse, 2012). Alternatively, the pathogen could be inducing the host's ethylene production to accelerate ripening and make the tissue more conductive to infection (Hayama et al., 2006). However, in immature tissue, the pathogen did not develop the disease. The negative regulator *EBF1/2* gene was downregulated compared to control at two time points (6 and 24 hpi), which could explain why the inoculated levels fall to control levels.

Furthermore, immature fruit also activated other defense responses to avoid the pathogen invasion. In addition to the **ROS** produced by hosts to mediate defense genes activation through ethylene and ERFs (Müller and Munné-Bosch, 2015) or to cause oxidative stress to pathogens (Torres et al., 2006), the host responded to the putatively ROS generated by *M. laxa* by expressing a plethora of **antioxidant metabolisms**. These included genes related to redox-related amino acids (cysteine and methionine metabolism) and glutathione biosynthesis, widely used as antioxidants to overcome the pathogen oxidative burst. Recently, Papavasileiou et al. (2020) found that some genes of the glutathione metabolism (e.g., S-formylglutathione hydrolase and glutathione S-transferase) were more accumulated in peaches that presented mid-low incidence (below 67% and 18%) with either *M. fructicola* or *M. laxa* isolates, respectively, compared to control fruit. In our study, inoculated immature nectarines also induced the gene expression of the **phenylpropanoid pathway** in response to the pathogen, probably as an antioxidants mechanism or as substrates to reinforce the cell wall through lignin accumulation (Veloso and van Kan, 2018). For instance, pathways leading to lignin biosynthesis are activated as a typical response of grape berries to noble rot caused by *B. cinerea* (Blanco-Ulate et al., 2015).

In contrast, in mature nectarine, multiple KEGG pathways related to plant defense were enriched in response to *M. laxa* since early time points. Besides, this fruit displayed a larger number of induced genes than immature from 6 hpi and increased while the disease progressed, yet they were overall of the same pathways. The pathways associated with ethylene and JA were enriched almost through the whole infection process on mature fruit. Although the expression of the **JA pathway** at early steps was like that in immature fruit (i.e., DEGs were upregulated), some receptor genes were downregulated on inoculated mature fruit compared to control. Based on these data, it could be pointed out that *M. laxa* would be somehow blocking the JA signaling pathway since some fungi can hijack the JA signaling pathway to cause disease. For instance, *B. cinerea* can produce an exopolysaccharide that actives the salicylic acid pathway, which in turn antagonizes the JA signaling pathway, leading to fungal growth in tomato leaves (El-Oirdi et al., 2011).

Regarding **ethylene**, the *M. laxa*-inoculated mature nectarines produced lower quantities than control fruit only at 6 hpi, coinciding with an impaired upregulation of biosynthetic genes (e.g., *ACS1, ACS2, ACO1, ACO2,* and *ACO3*). Hence, these results suggested that the host could be mitigating the ethylene production in an attempt to maintain the firmness of the tissue and not making it more conductive to infection, a consequence that could derive from the *M. laxa* inoculation (Hayama et al., 2006; Van Der Ent and Pieterse, 2012). However, from 24 hpi, although the ethylene biosynthesis genes were highly induced in response to *M. laxa,* the ethylene production of *M. laxa*-inoculated fruit increased along time similarly to control nectarines, and pathogen succeeded and developed the disease. Lastly, like immature fruit, mature nectarines also highly induced the expression of **phenylpropanoids and glutathione metabolism**. Phenylpropanoids and related compounds were also upregulated in apricot in response to *M. laxa* (Ezzat et al., 2021). Since they have a crucial role in the biosynthesis of phenols, phytoalexins, and lignin (Lara et al., 2020), their putative role in mature nectarines against *M. laxa* infections could range from antioxidants activities to the reinforcement of cell walls.

5.2.3. Which can be the role of selected secondary metabolites in the resistance or susceptibility of nectarines to *M. laxa*?

Secondary metabolites actively participate in protective functions under stresses (Khare et al., 2020), either as preformed or inducible compounds after a pathogen attack (Alkan and Fortes, 2015). Based on data from RNA-Seq of 'Venus' nectarines inoculated with *M. laxa* (chapter 3), genes involved in the biosynthesis of secondary

metabolites (e.g., terpenoid biosynthetic genes) were more enriched in resistant immature than in susceptible mature nectarines. Besides, in immature tissues, the terpenoid metabolism (i.e., backbone, monoterpenoid, sesquiterpenoid, triterpenoid, and brassinosteroid biosynthetic genes) was enriched slightly later (from 14 hpi) than mature fruit, which was already enriched from 6 hpi. Hence, the last objectives of this thesis were **to assess the expression of nectarine terpenoid biosynthetic genes in response to *M. laxa* infection and to reveal the most significant VOCs released during the interaction of *M. laxa*-nectarine**.

Gene expression analyses were conducted using 'Albared' nectarines inoculated with *M. laxa* (chapter 4), following the same methodology previously described in 'Venus' nectarines. Both developmental stages (immature and mature) developed brown rot disease in this cultivar. The gene expression analysis in 'Venus' cultivar was assessed using the normalized read counts data from the RNA-Seq study (chapter 3). The study of VOCs was conducted using the same experimental design previously described in chapters 3 and 4 and with both cultivars 'Venus' and 'Albared' (chapter 5). This study showed a similar susceptibility of both cultivars to *M. laxa* as that previously observed in other chapters of this thesis (**Figure 8**).

To date, only one study is available in the fruit terpenoid response to *Monilinia* spp. Dini (2019) studied that immature nectarines emitted a different VOCs profile (mainly phenolics and terpenoids) to wounded immature nectarines, being this tissue slowly infected by *M. laxa* compared to ripe fruit. However, they did not find a promising relation. Hence, no studies aiming to analyze the terpenoid biosynthetic profile are conducted on unwounded nectarines inoculated with *Monilinia* spp. The RNA-Seq study demonstrated that the susceptibility to *M. laxa* depended on the fruit developmental stage and the intrinsic and induced physicochemical fruit properties. Previous studies on strawberries at two developmental stages reported that terpenoid biosynthetic genes (e.g., *HMGS2* and *DXS*) are differentially regulated between developmental stages in response to *B. cinerea* infection (Haile et al., 2019). Hence, the pattern of gene expression together with the emission of fruit volatiles by the resistant (i.e., immature 'Venus' nectarines) and susceptible nectarine tissues (i.e., immature 'Albared' and mature nectarines of 'Venus' and 'Albared') could help us to elucidate the putative role of terpenoid metabolism on determining fruit resistance/susceptibility to *M. laxa*. Results from chapters 4 and 5 revealed that in both cultivars, *M. laxa*-inoculated tissues induced a clear response against the pathogen if compared to the control tissue: the gene expression for most of the terpenoid genes

were up or downregulated in response to *M. laxa,* and some VOCs were also highly released or suppressed in response to the pathogen inoculation.

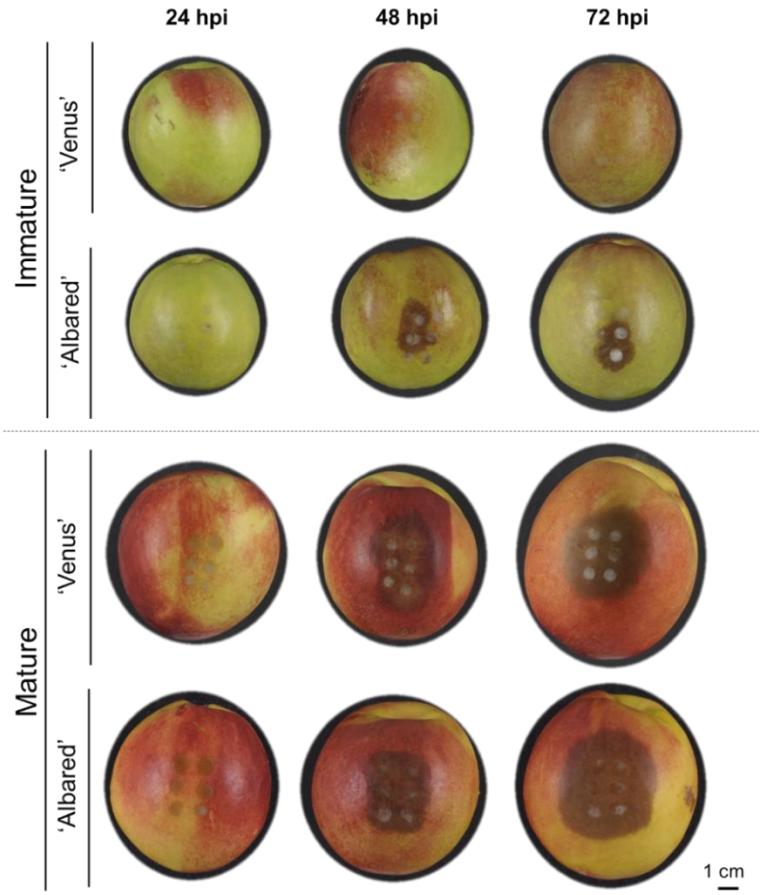


Figure 8. Monilinia laxa *spread on immature and mature tissues of 'Venus' and 'Albared' nectarines at different time points after inoculation.*

Terpenoids can be biosynthesized from either the cytosolic mevalonic acid (MVA) pathway or the plastidial methylerythritol phosphate (MEP/DOXP) pathway. The pathway used ultimately depends on the stimuli at which plants are submitted to adequate the end-compounds to properly face the stress (Tholl, 2015). In this thesis, both developmental stages of 'Venus' nectarines basically employed the **MVA pathway**, and not the MEP pathway, to respond against *M. laxa*. In this line, genes related to the terpenoid backbone biosynthesis (*PpIDI* and *PpFPS*) in 'Venus' and 'Albared' cultivars were overall upregulated, reinforcing that the end-compounds of

the terpenoid metabolism were related to pathogen response. Furthermore, results pointed out that ***PpHMGR1*** was the key-responsive gene in susceptible tissues (both 'Albared' tissues and mature 'Venus' nectarines), in line with previous studies demonstrating its key regulatory role of the MVA pathway (Hemmerlin et al., 2012).

Steroids (e.g., β-sitosterol and phytosterol stigmasterol) are involved in plant-pathogen interactions, as reported in *A. thaliana–Pseudomonas syringae* and berries-*B. cinerea* (Griebel and Zeier, 2010; Agudelo-Romero et al., 2015). Our results showed that the expression of **steroid biosynthetic genes** was induced by the pathogen in all inoculated tissues (both stages in both cultivars) along the infection process. Under biotic stress, *HMGR* paralogs direct the flux towards the production of stress-induced compounds (Hemmerlin et al., 2012). Some steroids (e.g., steroidal glycoalkaloids of the *Solanaceae* genus) have been shown to reduce the *in vitro* growth of one strain of *Monilinia* spp. (Cristea et al., 2017). In our results, since the expression of steroid biosynthetic genes was impaired in all fruit tissues in response to *M. laxa* inoculation, steroids were not likely to be crucial in protective functions. Hence, the steroids' expression is induced in response to *M. laxa*-inoculation, although its role remains unclear.

The role of farnesal or farnesal-related compounds in fruit-pathogen interactions is still unknown, yet its involvement against human pathogens is well-studied (Nagaki et al., 2011; Biva et al., 2019). Recently, Cantó-Tejero et al. (2021) showed that the application of farnesol, a structurally related compound to farnesal, to pepper leaf discs reduced the population of aphids. In our work, **farnesal-related genes** (e.g., *PpPFT* and *PpFOLK*) were barely induced in resistant tissues, whereas these genes were overall downregulated in susceptible tissues. These results suggested that the expression of farnesal-related genes could be repressed by the pathogen, pointing out a fruit resistance role. Further studies to unravel the potential of these terpenoid compounds on controlling brown rot development are encouraged.

Both the RNA-Seq study in the 'Venus' cultivar and the gene expression analysis in 'Albared' revealed the **biosynthesis of linalool** as a potential participant in the nectarine resistance to *M. laxa.* Although our results showed that the MVA pathway was more prevalent than the MEP one in response to the pathogen, it is known that the MVA and MEP pathways can exchange metabolites under different conditions (Hemmerlin et al., 2012). In other pathosystems, it has been described that the linalool biosynthesis and its accumulation is involved in plant defense (citrus leaves and fruits) against *Penicillium italicum* (Shimada et al., 2014). Herein, the overall upregulation of

PpLIS family members in the resistant tissue and the downregulation in susceptible ones suggested that linalool synthase expression could be implicated in protective functions against *M. laxa* infection. Furthermore, previous studies demonstrated that the application of linalool slightly reduced the *in vitro* growth of a strain of *M. laxa*, *M. fructicola,* and *M. fructigena* (Elshafie et al., 2015). Based on all this information, the results presented herein pointed out the role of linalool in nectarine defense against *M. laxa.*

Volatile organic compounds (VOCs) are secondary metabolites that can be emitted from plant cells in response to biotic and abiotic stresses due to their implication in protective functions (Baldwin 2010). Volatiles are classified in several chemical categories, such as alcohols, aldehydes, C6 compounds, C9 compounds, esters, ketones, and terpenoids, being the latter the largest class of plant VOCs (Wang et al., 2009; Montero-Prado et al., 2013; Abbas et al., 2017; Xi et al., 2017). As mentioned, studies of peach or nectarine (unwounded) volatiles emitted in response to *Monilinia* spp. are unavailable. Still, current studies are focused on reporting the different volatile profiles that fungal infections emit in postharvest chambers to monitor fungal contamination. In this sense, Liu et al. (2018) found that up to eight volatile compounds were significantly different between healthy peaches and those inoculated with *B. cinerea, M. fructicola,* or *R. stolonifer*. For that reason, the production of VOCs was also analyzed in fruit tissues with different susceptibility to brown rot (chapter 5). Besides, since fungi can also emit VOCs, we also aimed to investigate the VOCs profile emitted by *M. laxa* during *in vitro* conditions. We used a media based on peach juice to provide the closest approach to the nectarine tissue for discerning among VOCs that can also be emitted by the pathogen. However, it cannot be assumed that the host exclusively emitted the rest of the VOCs.

In this study, and based on the VOC profile, samples were grouped according to their developmental stage, infection status, and even the cultivar type. Out of the total VOCs obtained in all samples, 34 VOCs were selected to be further analyzed. Subsequent analyses allowed the get two groups of fruit volatiles (VIP ≥ 0.8) that were relevant for i) being emitted in response to *M. laxa* infection and positively correlated with it, and ii) being negatively correlated with brown rot disease. Among the VOCs exclusively produced by tissues that showed *M. laxa* disease symptoms (rotted tissues) and positively correlated with brown rot disease, some of them, like **alpha-muurolene** was also emitted by *M. laxa in vitro* culture. Similar results were obtained by Mang et al. (2015), in which they found that this compound was detected in the 10-day-old

colony of *M. fructicola* and *M. fructigena* grown *in vitro*, but not in *M. laxa.* Besides, a BLAST search was conducted in either *Rosaceae* or *Prunus* organisms, and it revealed no matches with the codifying gene for the alpha-muurolene synthase (*COP3*) of the fungus *Marasmius oreades* (Hiltunen et al., 2021). Alpha-muurolene can be a virulence factor like seen in other phytopathogenic fungi like *Aspergillus flavus* or other fungi-fungi interactions (Baptista et al., 2021; Josselin et al., 2021). Hence, all point out that alpha-muurolene is produced by *M. laxa* for its development as well as when infecting fruit, probably as a virulence factor.

The role of other VOCs with a significant positive correlation with brown rot disease, also produced by *M. laxa in vitro* culture, was slightly more controversial. Whereas some VOCs such as **2-ethyl-1-hexanol** were emitted by all samples at similar relative quantities, others such as **(E)-beta-ionone** were more produced in rotted tissues than the others *M. laxa*-inoculated with less or no symptoms and controls. Since this compound is related to induction resistance (Brambilla et al., 2021) and it is derived from the terpenoid metabolism, which is overall highly induced in nectarine in response to *M. laxa* (seen in previous chapters of this thesis), all suggest that (E)-beta-ionone, in addition of being emitted by the pathogen, was produced by the host in an attempt for signaling to induce host resistance.

Interestingly, results grouped VOCs emitted during rotted tissues, positively correlated with brown rot disease, and not detected in the *M. laxa in vitro* culture. These VOCs, such as **2-hydroxy-gamma-butyrolactone, (3E,6E)-nonadien-1-yl-acetate, and (Z)-3-nonen-1-ol,** do not have an implication in fruit diseases as found in the current literature. Hence, these compounds should be further explored to unravel more mechanisms underlying the nectarine responses to *M. laxa.*

On the other hand, this study presented a group of VOCs that, in general, were emitted lower or even not emitted in inoculated tissues than control tissues and were negatively correlated with brown rot disease. However, some were also detected in *M. laxa* in *in vitro* culture, specifically **3-furaldehyde, 2/3-furanmethanol, and decanal**. The raised question was which organism was the main responsible for the emission of these compounds: Why does the pathogen produce a compound that is also emitted by the fruit and may have antifungal properties if emitted by the host? The methodology used in this thesis could not discern who emitted those compounds, but based on the results, some hypotheses can arise, as mentioned in chapter 5. Among them, the hypothesis by which *M. laxa* could repress the emission of these compounds in an attempt to inhibit their antifungal function also has some evidence. For instance,

regarding decanal, which is also emitted by active molds on aged model materials (e.g., *Alternaria alternata* on silk and *Cladosporium herbarum* on paper) (Sawoszczuk et al., 2015), its exogenous application significantly inhibits the germination and development of *Penicillium expansum* in *in vitro* conditions, by decreasing the oxidative phosphorylation as one of the main inhibitory actions (Zhou et al., 2020). Hence, this compound emitted by nectarines may have a negative effect on *M. laxa*, and therefore, the pathogen would try to repress it. However, future studies such as gene expressing analyses and targeting biosynthetic genes of this compound of either the host or the pathogen would help elucidate its role in the interaction nectarine-*M. laxa*.

Finally, the VOCs study of this thesis revealed which VOCs were negatively correlated with brown rot incidence and not detected in *M. laxa* in *in vitro* culture, suggesting that these were putatively produced by the fruit and could serve as brown rot control strategies: 2-ethyl furan, (E)-2-decenal, 4-cyclopentene-1,3-dione, butyrolactone and 5-hydroxymethylfurfural. For instance, **(E)-2-decenal and butyrolactone** have shown an antifungal effect on the growth of *B. cinerea* in *in vitro* conditions (Cazar et al., 2005; Quintana-Rodriguez et al., 2018). Several research has been conducted on reporting the effect of characteristic VOCs produced by stone fruit on the development of *Monilinia* spp. or related fungi under *in vitro* conditions. Some aldehydes, such as benzaldehyde, hexanal, and (E)-2-hexenal, and esters, such as benzyl-acetate, have exhibited a different degree of fungicidal and fungistatic effect on *M. fructicola*, *M. laxa,* and *B. cinerea* (Wilson, 1987; Caccioni et al., 1995; Neri et al., 2007). However, other authors report and highlight the importance of not considering each compound separately, as suggested in a study of citrus volatiles' effect on the germination and growth of *Penicillium* spp. (Droby et al., 2008). Therefore, further validation of the negative effects of these compounds on the development of *M. laxa* should be performed to confirm their potential role in controlling the brown rot disease.

Overall, this thesis has shown that some biosynthetic genes of the terpenoid metabolism of nectarine and VOCs emitted by the nectarine-*M. laxa* interaction have been shown to respond to *M. laxa* inoculation and could be potential candidates for future studies as key fruit response against brown rot development.

5.3. References

Abbas, F., Ke, Y., Yu, R., Yue, Y., Amanullah, S., Jahangir, M. M., et al. (2017). Volatile terpenoids: multiple functions, biosynthesis, modulation and manipulation by genetic engineering. *Planta* 246, 803–816. doi:10.1007/s00425-017-2749-x.

Abdipour, M., Hosseinifarahi, M., and Naseri, N. (2019). Combination method of UV-B and UV-C prevents post-harvest decay and improves organoleptic quality of peach fruit. *Sci. Hortic. (Amsterdam).* 256, 108564. doi:10.1016/j.scienta.2019.108564.

Agudelo-Romero, P., Erban, A., Rego, C., Carbonell-Bejerano, P., Nascimento, T., Sousa, L., et al. (2015). Transcriptome and metabolome reprogramming in *Vitis vinifera* cv. Trincadeira berries upon infection with *Botrytis cinerea*. *J. Exp. Bot.* 66, 1769–1785. doi:10.1093/jxb/eru517.

Ali, M. M., Anwar, R., Yousef, A. F., Li, B., Luvisi, A., De Bellis, L., et al. (2021). Influence of bagging on the development and quality of fruits. *Plants* 10, 1–17. doi:10.3390/plants10020358.

Alkan, N., and Fortes, A. M. (2015). Insights into molecular and metabolic events associated with fruit response to post-harvest fungal pathogens. *Front. Plant Sci.* 6, 889. doi:10.3389/fpls.2015.00889.

Allran, J. (2017). Investigation of peach fruit bagging to produce high quality fruit and to manage pests and diseases. Available at: https://tigerprints.clemson.edu/all_theses/2695/.

De Miccols Angelini, R. M., Abate, D., Rotolo, C., Gerin, D., Pollastro, S., and Faretra, F. (2018). D*e novo* assembly and comparative transcriptome analysis of *Monilinia fructicola*, *Monilinia laxa* and *Monilinia fructigena*, the causal agents of brown rot on stone fruits. 19, 1–21. doi:10.1186/s12864-018-4817-4.

Baptista, P., Guedes de Pinho, P., Moreira, N., Malheiro, R., Reis, F., Padrão, J., et al. (2021). *In vitro* interactions between the ectomycorrhizal *Pisolithus tinctorius* and the saprotroph *Hypholoma fasciculare* fungi: morphological aspects and volatile production. *Mycology* 12, 216–229. doi.org/10.1080/21501203.2021.1876778 In.

Baró-Montel, N., Giné-Bordonaba, J., Torres, R., Vall-llaura, N., Teixidó, N., and Usall, J. (2020). Scrutinising the relationship between major physiological and compositional changes during 'Merrill O'Henry' peach growth with brown rot susceptibility. *Food Sci. Technol. Int.* 27, 366–379. doi:10.1177/1082013220959988.

Baró-Montel, N., Vall-llaura, N., Giné-Bordonaba, J., Usall, J., Serrano-Prieto, S., Teixidó, N., et al. (2019a). Double-sided battle: The role of ethylene during *Monilinia* spp. infection in peach at different phenological stages. *Plant Physiol. Biochem.* 144, 324–333. doi:10.1016/j.plaphy.2019.09.048.

Baró-Montel, N., Vall-llaura, N., Usall, J., Teixidó, N., Naranjo-Ortíz, M. A., Gabaldón, T., et al. (2019b). Pectin methyl esterases and rhamnogalacturonan hydrolases: weapons for successful *Monilinia laxa* infection in stone fruit? *Plant Pathol.* 68, 1381–93. doi:10.1111/ppa.13039.

Bernat, M., Casals, C., Torres, R., Teixidó, N., and Usall, J. (2019). Infection risk of *Monilinia fructicola* on stone fruit during cold storage and immersion in the dump tank. *Sci. Hortic. (Amsterdam).* 256, 108589. doi:10.1016/j.scienta.2019.108589.

Bernat, M., Segarra, J., Casals, C., Teixidó, N., Torres, R., and Usall, J. (2017). Relevance of the

main postharvest handling operations on the development of brown rot disease on stone fruits. *J. Sci. Food Agric.* 97, 5319–5326. doi:10.1002/jsfa.8419.

Biva, I. J., Ndi, C. P., Semple, S. J., and Griesser, H. J. (2019). Antibacterial performance of terpenoids from the australian plant *Eremophila lucida*. *Antibiotics* 8, 6–11. doi:10.3390/antibiotics8020063.

Blanco-Ulate, B., Amrine, K. C., Collins, T. S., Rivero, R. M., Vicente, A. R., Morales-Cruz, A., et al. (2015). Developmental and metabolic plasticity of white-skinned grape berries in response to *Botrytis cinerea* during noble rot. *Plant Physiol.* 169, pp.00852.2015. doi:10.1104/pp.15.00852.

Blasi, E., Pancino, B., Passeri, N., and Franco, S. (2017). Environmental and economic benefits of the preharvest fruit bagging technique: Trade-off evaluation in a Mediterranean area. *Acta Hortic.* 1160, 313–318. doi:10.17660/ActaHortic.2017.1160.45.

Bonany, J., Carbó, J., Echeverria, G., Hilaire, C., Cottet, V., Iglesias, I., et al. (2014). Eating quality and European consumer acceptance of different peach (*Prunus persica* (L.) Batsch) varieties. *J. Food, Agric. Environ.* 12, 67–72.

Brambilla, A., Sommer, A., Ghirardo, A., Wenig, M., Knappe, C., Weber, B., et al. (2021). Immunity-associated volatile emissions of β-ionone and nonanal propagate defence responses in neighbouring barley (*Hordeum vulgare*) plants. *J. Exp. Bot.* doi:10.1093/JXB/ERAB520.

Caccioni, D. R. L., Tonini, G., and Guizzardi, M. (1995). Antifungal activity of stone fruit aroma compounds against *Monilinia laxa* (Aderh. et Ruhl.) Honey and *Rhizopus stolonifer* (Ehrenb.): *In vivo* trials. *J. Plant Dis. Prot.* 102, 518–525.

Campbell, D., Sarkhosh, A., Brecht, J. K., Gillett-Kaufman, J. L., Liburd, O., Melgar, J. C., et al. (2021). Bagging organic peaches reduces physical injuries and storage decay with minimal effects on fruit quality. *HortScience* 56, 52–58. doi:10.21273/HORTSCI15391-20.

Canessa, P., Schumacher, J., Hevia, M. A., Tudzynski, P., and Larrondo, L. F. (2013). Assessing the effects of light on differentiation and virulence of the plant pathogen *Botrytis cinerea*: Characterization of the white collar complex. *PLoS One* 8. doi:10.1371/journal.pone.0084223.

Cantó-Tejero, M., Casas, J. L., Marcos-García, M. Á., Pascual-Villalobos, M. J., Florencio-Ortiz, V., and Guirao, P. (2021). Essential oils-based repellents for the management of *Myzus persica*e and *Macrosiphum euphorbiae*. *J. Pest Sci.* 1, 1–15. doi:10.1007/s10340-021-01380-5.

Cazar, M. E., Astudillo, L., and Naturales, L. D. P. (2005). Antimicrobial butyrolactone I derivatives from the Ecuadorian soil fungus *Aspergillus terreus* Thorn. var *terreus*. *World J. Microbiol. Biotechnol.* 21, 1067–1075. doi:10.1007/s11274-004-8150-5.

Chanclud, E., and Morel, J. B. (2016). Plant hormones: a fungal point of view. *Mol. Plant Pathol.* 17, 1289–1297. doi:10.1111/MPP.12393.

Colaric, M., Veberic, R., Stampar, F., and Hudina, M. (2005). Evaluation of peach and nectarine fruit quality and correlations between sensory and chemical attributes. *J. Sci. Food Agric.* 85, 2611–2616. doi:10.1002/jsfa.2316.

Corrochano, L. M. (2019). Light in the fungal world: From photoreception to gene transcription and beyond. *Annu. Rev. Genet.* 53, 149–170. doi:10.1146/annurev-genet-120417-031415.

Crisosto, C. H., and Crisosto, G. M. (2005). Relationship between ripe soluble solids concentration (RSSC) and consumer acceptance of high and low acid melting flesh peach and nectarine (*Prunus persica* (L.) Batsch) cultivars. *Postharvest Biol. Technol.* 38, 239–246. doi:10.1016/j.postharvbio.2005.07.007.

Crisosto, C. H., Echeverría, G., and Manganaris, G. A. (2020). "Peach and Nectarine," in *Manual on postharvest handling of Mediterranean tree fruits and nuts*, eds. C. H. Crisosto and G. M. Crisosto, 53–87.

Crisosto, C. H., Mitchell, F. G., and Ju, Z. (1999). Susceptibility to chilling injury of peach, nectarine, and plum cultivars grown in California. *HortScience* 34, 1116–1118. doi:10.21273/hortsci.34.6.1116.

Cristea, S., Manole, M. S., Zala, C., Jurcoane, Ștefana, Dănăilă -Guidea, S., Matei, F., et al. (2017). *In vitro* antifungal activity of some steroidal glycoalkaloids on *Monilinia* spp.

De Cal, A., and Melgarejo, P. (1993). Effects of pyroquilon on the infection process of *Monilinia laxa* causing peach twig blight. *Pestic. Sci.* 39, 267–269. doi:10.1002/ps.2780390403.

Dini, M. (2019). Resistance to brown rot in peach. Available at: http://guaiaca.ufpel.edu.br:8080/handle/prefix/4957.

Droby, S., Eick, A., Macarisin, D., Cohen, L., Rafael, G., Stange, R., et al. (2008). Role of citrus volatiles in host recognition, germination and growth of *Penicillium digitatum* and *Penicillium italicum*. *Postharvest Biol. Technol.* 49, 386–396. doi:10.1016/j.postharvbio.2008.01.016.

El-Oirdi, M., El-Rahman, T. A., Rigano, L., El-Hadrami, A., Rodriguez, M. C., Daayf, F., et al. (2011). *Botrytis cinerea* manipulates the antagonistic effects between immune pathways to promote disease development in tomato. *Plant Cell* 23, 2405–2421. doi:10.1105/tpc.111.083394.

Elshafie, H. S., Mancini, E., Sakr, S., De Martino, L., Mattia, C. A., De Feo, V., et al. (2015). Antifungal activity of some constituents of *Origanum vulgare* L. essential oil against postharvest disease of peach fruit. *J. Med. Food* 18, 929–934. doi:10.1089/jmf.2014.0167.

European Commission (2019). COMMISSION DELEGATED REGULATION (EU) 2019/428.

Ezzat, A., Szabó, S., Szabó, Z., Hegedűs, A., Berényi, D., and Holb, I. J. (2021). Temporal patterns and inter-correlations among physical and antioxidant attributes and enzyme activities of apricot fruit inoculated with *Monilinia laxa* under salicylic acid and methyl jasmonate treatments under shelf-life conditions. *J. Fungi* 7. doi:10.3390/jof7050341.

Falchi, R., Bonghi, C., Drincovich, M. F., Famiani, F., Lara, M. V., Walker, R. P., et al. (2020). Sugar metabolism in stone fruit: Source-sink relationships and environmental and agronomical effects. *Front. Plant Sci.* 11, 1–14. doi:10.3389/FPLS.2020.573982.

Fernández-Trujillo, J. P., Cano, A., and Artés, F. (1998). Physiological changes in peaches related to chilling injury and ripening. *Postharvest Biol. Technol.* 13, 109–119. doi:10.1016/S0925-5214(98)00006-4.

Folta, K. M., and Carvalho, S. D. (2015). Photoreceptors and control of horticultural plant traits. *HortScience* 50, 1274–1280. doi:10.21273/hortsci.50.9.1274.

Fu, W., da Silva Linge, C., and Gasic, K. (2021). Genome-Wide Association study of brown rot

(*Monilinia* spp.) tolerance in peach. *Front. Plant Sci.* 12, 1–14. doi:10.3389/fpls.2021.635914.

Fuller, K. K., Loros, J. J., and Dunlap, J. C. (2015). Fungal photobiology: visible light as a signal for stress, space and time. *Curr. Genet.* 61, 275–288. doi:10.1007/s00294-014-0451-0.

Garcia-Benitez, C., Casals, C., Usall, J., Sánchez-Ramos, I., Melgarejo, P., and De Cal, A. (2020). Impact of postharvest handling on preharvest latent infections caused by *Monilinia* spp. in nectarines. *J. Fungi* 6, 1–14. doi:10.3390/jof6040266.

Garcia-Benitez, C., Melgarejo, P., Sandin-España, P., Sevilla-Morán, B., and De Cal, A. (2019). Degrading enzymes and phytotoxins in *Monilinia* spp. *Eur. J. Plant Pathol.* 154, 305–318. doi:10.1007/s10658-018-01657-z.

Gell, I., De Cal, A., Torres, R., Usall, J., and Melgarejo, P. (2008). Relationship between the incidence of latent infections caused by *Monilinia* spp. and the incidence of brown rot of peach fruit: factors affecting latent infection. *Eur. J. Plant Pathol.* 121, 487–498. doi:10.1007/s10658-008-9268-3.

Gil, M. I., Tomas-Barberan, F. A., Hess-Pierce, B., and Kader, A. A. (2002). Antioxidant capacities, phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars from California. *J. Agric. Food Chem.* 50, 4976–4982.

Giné-Bordonaba, J., Cantin, C. M., Larrigaudière, C., López, L., López, R., and Echeverria, G. (2014). Suitability of nectarine cultivars for minimal processing: The role of genotype, harvest season and maturity at harvest on quality and sensory attributes. *Postharvest Biol. Technol.* 93, 49–60. doi:10.1016/j.postharvbio.2014.02.007.

Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annu. Rev. Phytopathol.* 43, 205–227. doi:10.1146/annurev.phyto.43.040204.135923.

Gong, D., Cao, S., Sheng, T., Shao, J., Song, C., Wo, F., et al. (2015). Effect of blue light on ethylene biosynthesis, signalling and fruit ripening in postharvest peaches. *Sci. Hortic. (Amsterdam).* 197, 657–664. doi:10.1016/j.scienta.2015.10.034.

Gradziel, T. M. (1994). Changes in susceptibility to brown rot with ripening in three clingstone peach genotypes. *J. Amer. Soc. Hort. Sci* 119, 101–105. doi:10.21273/JASHS.119.1.101.

Griebel, T., and Zeier, J. (2010). A role for β-sitosterol to stigmasterol conversion in plant-pathogen interactions. *Plant J.* 63, 254–268. doi:10.1111/j.1365-313X.2010.04235.x.

Guidarelli, M., Zubini, P., Nanni, V., Bonghi, C., Rasori, A., Bertolini, P., et al. (2014). Gene expression analysis of peach fruit at different growth stages and with different susceptibility to *Monilinia laxa*. *Eur. J. Plant Pathol.* 140, 503–513. doi:10.1007/s10658-014-0484-8.

Gununu, P. R., Munhuweyi, K., Obianom, P. C., and Sivakumar, D. (2019). Assessment of eleven South African peach cultivars for susceptibility to brown rot and blue mould. *Sci. Hortic. (Amsterdam).* 254, 1–6. doi:10.1016/j.scienta.2019.04.067.

Haile, Z. M., Nagpala-De Guzman, E. G., Moretto, M., Sonego, P., Engelen, K., Zoli, L., et al. (2019). Transcriptome profiles of strawberry (*Fragaria vesca*) fruit interacting with *Botrytis cinerea* at different ripening stages. *Front. Plant Sci.* 10. doi:10.3389/fpls.2019.01131.

Hashimoto, M., Styrikovich, M., Nishioka, S., Rouviere, C., Williams, T., Ball, R., et al. (1990). "Human settlement; the energy, transport and industrial sectors; human health; air quality; and changes in ultraviolet-B radiation," in *Climate Change. The IPCC Impacts Assessment*, eds. W. M. Tegart, G. W. Sheldon, and D. C. Griffiths Available at: https://www.ipcc.ch/report/ar1/wg2/.

Hayama, H., Shimada, T., Fujii, H., Ito, A., and Kashimura, Y. (2006). Ethylene-regulation of fruit softening and softening-related genes in peach. *J. Exp. Bot.* 57, 4071–4077. doi:10.1093/jxb/erl178.

Hemmerlin, A., Harwood, J. L., and Bach, T. J. (2012). A raison d'être for two distinct pathways in the early steps of plant isoprenoid biosynthesis? *Prog. Lipid Res.* 51, 95–148. doi:10.1016/j.plipres.2011.12.001.

Hiltunen, M., Ament-Velásquez, S. L., and Johannesson, H. (2021). The assembled and annotated genome of the fairy-ring fungus *Marasmius oreades*. *Genome Biol. Evol.* 13. doi:10.1093/GBE/EVAB126.

Huang, J., Zhao, X., and Chory, J. (2019). The *Arabidopsis* transcriptome responds specifically and dynamically to high light stress. *Cell Rep.* 29, 4186-4199.e3. doi:10.1016/j.celrep.2019.11.051.

Iglesias, I., and Echeverría, G. (2009). Differential effect of cultivar and harvest date on nectarine colour, quality and consumer acceptance. *Sci. Hortic.* 120, 41–50. doi:10.1016/j.scienta.2008.09.011.

Ilić, Z. S., and Fallik, E. (2017). Light quality manipulation improves vegetable quality at harvest and postharvest: A review. *Environ. Exp. Bot.* 139, 79–90. doi:10.1016/j.envexpbot.2017.04.006.

Janisiewicz, W. J., Biggs, A. R., Ii, W. M. J., Vico, I., and Conway, W. S. (2013). Biological characteristics of *Monilinia fructicola* isolates from stone fruits in eastern West Virginia. *Can. J. Plant Pathol* 35, 315–327. doi:10.1080/07060661.2013.823465.

Jashni, M. K., Dols, I. H. M., Iida, Y., Boeren, S., Beenen, H. G., Mehrabi, R., et al. (2015). Synergistic action of a metalloprotease and a serine protease from *Fusarium oxysporum* f. sp. *lycopersici* cleaves chitin-binding tomato chitinases, reduces their antifungal activity, and enhances fungal virulence. *Mol. Plant-Microbe Interact.* 28, 996–1008. doi:10.1094/MPMI-04-15-0074-R/ASSET/IMAGES/LARGE/MPMI-04-15-0074-R_F6.JPEG.

Josselin, L., De Clerck, C., De Boevre, M., Moretti, A., Haïssam Jijakli, M., Soyeurt, H., et al. (2021). Volatile organic compounds emitted by *Aspergillus flavus* strains producing or not aflatoxin B1. *Toxins 2021, Vol. 13, Page 705* 13, 1–19. doi:10.3390/TOXINS13100705.

Khare, S., Singh, N. B., Singh, A., Hussain, I., Niharika, K., Yadav, V., et al. (2020). Plant secondary metabolites synthesis and their regulations under biotic and abiotic constraints. *J. Plant Biol.* 63, 203–216. doi:10.1007/s12374-020-09245-7.

Kim, H. jin, Chen, C., Kabbage, M., and Dickman, M. B. (2011). Identification and characterization of *Sclerotinia sclerotiorum* NADPH oxidases. *Appl. Environ. Microbiol.* 77, 7721–7729. doi:10.1128/AEM.05472-11.

Kou, J., Wei, Y., He, X., Xu, J., Xu, F., and Shao, X. (2018). Infection of post-harvest peaches by *Monilinia fructicola* accelerates sucrose decomposition and stimulates the Embden–

Meyerhof–Parnas pathway. *Hortic. Res.* 5. doi:10.1038/s41438-018-0046-x.

Lafuente, M. T., Alférez, F., and González-Candelas, L. (2018). Light-emitting diode blue light alters the ability of *Penicillium digitatum* to infect citrus fruits. *Photochem. Photobiol.* 94, 1003–1009. doi:10.1111/php.12929.

Lara, M. V., Bonghi, C., Famiani, F., Vizzotto, G., Walker, R. P., and Drincovich, M. F. (2020). Stone fruit as biofactories of phytochemicals with potential roles in human nutrition and health. *Front. Plant Sci.* 11, 1–21. doi:10.3389/fpls.2020.562252.

Li, H., Zhang, Z., He, C., Qin, G., and Tian, S. (2016a). Comparative proteomics reveals the potential targets of BcNoxR, a putative regulatory subunit of NADPH oxidase of *Botrytis cinerea*. *Mol. Plant-Microbe Interact.* 29, 990–1003. doi:10.1094/MPMI-11-16-0227-R.

Li, M., Liu, X., Liu, Z., Sun, Y., Liu, M., Wang, X., et al. (2016b). Glycoside hydrolase MoGls2 controls asexual/sexual development, cell wall integrity and infectious growth in the rice blast fungus. *PLoS One* 11, e0162243. doi:10.1371/journal.pone.0162243.

Liu, H., Cao, X., Liu, X., Xin, R., Wang, J., Gao, J., et al. (2017). UV-B irradiation differentially regulates terpene synthases and terpene content of peach. *Plant Cell Environ.* 40, 2261–2275. doi:10.1111/pce.13029.

Liu, Q., Zhao, N., Zhou, D., Sun, Y., Sun, K., Pan, L., et al. (2018). Discrimination and growth tracking of fungi contamination in peaches using electronic nose. *Food Chem.* 262, 226–234. doi:10.1016/j.foodchem.2018.04.100.

Liu, T., Song, S., Yuan, Y., Wu, D., Chen, M., Sun, Q., et al. (2015). Improved peach peel color development by fruit bagging. Enhanced expression of anthocyanin biosynthetic and regulatory genes using white non-woven polypropylene as replacement for yellow paper. *Sci. Hortic. .* 184, 142–148. doi:10.1016/j.scienta.2015.01.003.

Lufu, R., Ambaw, A., and Opara, U. L. (2020). Water loss of fresh fruit: Influencing pre-harvest, harvest and postharvest factors. *Sci. Hortic. (Amsterdam).* 272. doi:10.1016/j.scienta.2020.109519.

Luo, Y., Michailides, T. J., Morgan, D. P., Krueger, W. H., and Buchner, R. P. (2005). Inoculum dynamics, fruit infection, and development of brown rot in prune orchards in California. *Phytopathology* 95, 1132–1136. doi:10.1094/PHYTO-95-1132.

Mang, S. M., Racioppi, R., Camele, I., Rana, G. L., and D'Auria, M. (2015). Use of volatile metabolite profiles to distinguish three *Monilinia* species. *J. Plant Pathol.* 97, 55–59. doi:10.4454/JPP.V97I1.005.

Marcet-Houben, M., Villarino, M., Vilanova, L., De Cal, A., van Kan, J. A. L., Usall, J., et al. (2021). Comparative genomics used to predict virulence factors and metabolic genes among *Monilinia* species. *J. Fungi* 7. doi:10.3390/jof7060464.

Minas, I. S., Tanou, G., and Molassiotis, A. (2018). Environmental and orchard bases of peach fruit quality. *Sci. Hortic.* 235, 307–322. doi:10.1016/j.scienta.2018.01.028.

Montero-Prado, P., Bentayeb, K., and Nerín, C. (2013). Pattern recognition of peach cultivars (*Prunus persica* L.) from their volatile components. *Food Chem.* 138, 724–731. doi:10.1016/j.foodchem.2012.10.145.

Mukhtar, M. S., McCormack, M. E., Argueso, C. T., and Pajerowska-Mukhtar, K. M. (2016). Pathogen tactics to manipulate plant cell death. *Curr. Biol.* 26, 608–619.

doi:10.1016/j.cub.2016.02.051.

Müller, M., and Munné-Bosch, S. (2015). Ethylene response factors: A key regulatory hub in hormone and stress signaling. *Plant Physiol.* 169, 32–41. doi:10.1104/pp.15.00677.

Nagaki, M., Narita, T., Ichikawa, H., Kawakami, J., and Nakane, A. (2011). Antibacterial and antifungal activities of isoprenoids. *Trans. Mater. Res. Soc. Japan* 36, 55–58.

Nassarawa, S. S., Abdelshafy, A. M., Xu, Y., Li, L., and Luo, Z. (2021). Effect of Light-Emitting Diodes (LEDs) on the quality of fruits and vegetables during postharvest period: a review. *Food Bioprocess Technol.* 14, 388–414. doi:10.1007/s11947-020-02534-6.

Neri, F., Mari, M., Brigati, S., and Bertolini, P. (2007). Fungicidal activity of plant volatile compounds for controlling *Monilinia laxa* in stone fruit. 91, 30–35. doi:10.1094/PD-91-0030.

Obi, V. I., Montenegro, J., Barriuso, J. J., Saidani, F., Aubert, C., and Gogorcena, Y. (2020). Is the tolerance of commercial peach cultivars to brown rot caused by *Monilinia laxa* modulated by its antioxidant content? *Plants* 9, 589. doi:10.3390/plants9050589.

OECD (2010). Peaches and nectarines, International Standards for Fruit and Vegetables. OECD Publishing, Paris doi:https://doi.org/10.1787/9789264084926-en-fr.

Papavasileiou, A., Tanou, G., Samaras, A., Samiotaki, M., Molassiotis, A., and Karaoglanidis, G. (2020). Proteomic analysis upon peach fruit infection with *Monilinia fructicola* and *M. laxa* identify responses contributing to brown rot resistance. *Sci. Rep.* 10, 7807. doi:10.1038/s41598-020-64864-x.

Petrasch, S., Silva, C. J., Mesquida-Pesci, S. D., Gallegos, K., van den Abeele, C., Papin, V., et al. (2019). Infection strategies deployed by *Botrytis cinerea*, *Fusarium acuminatum*, and *Rhizopus stolonifer* as a function of tomato fruit ripening stage. *Front. Plant Sci.* 10, 1–17. doi:10.3389/fpls.2019.00223.

Philips Available at: https://www.lighting.philips.es/welcome [Accessed November 16, 2020].

Porat, R., Lichter, A., Terry, L. A., Harker, R., and Buzby, J. (2018). Postharvest losses of fruit and vegetables during retail and in consumers' homes: Quantifications, causes, and means of prevention. *Postharvest Biol. Technol.* 139, 135–149. doi:10.1016/j.postharvbio.2017.11.019.

Poussereau, N., Creton, S., Billon-Grand, G., Rascle, C., and Fevre, M. (2001). Regulation of acp1, encoding a non-aspartyl acid protease expressed during pathogenesis of *Sclerotinia sclerotiorum*. *Microbiology* 147, 717–726. doi:10.1099/00221287-147-3-717.

Prusky, D., Alkan, N., Mengiste, T., and Fluhr, R. (2013). Quiescent and necrotrophic lifestyle choice during postharvest disease development. *Annu. Rev. Phytopathol.* 51, 155–176. doi:10.1146/annurev-phyto-082712-102349.

Prusky, D., de Assis, L. J., Baroncelli, R., Benito, E. P., del Castillo, V. C., Chaya, T., et al. (2020). Nutritional factors modulating plant and fruit susceptibility to pathogens: BARD workshop, Haifa, Israel, February 25–26, 2018. *Phytoparasitica* 48, 317–333. doi:10.1007/s12600-020-00803-w.

Quintana-Rodriguez, E., Rivera-Macias, L. E., Adame-Alvarez, R. M., Torres, J. M., and Heil, M. (2018). Shared weapons in fungus-fungus and fungus-plant interactions? Volatile organic compounds of plant or fungal origin exert direct antifungal activity *in vitro*. *Fungal Ecol.*

33, 115–121. doi:10.1016/j.funeco.2018.02.005.

Reig, G., Alegre, S., Iglesias, I., Echeverría, G., and Gatius, F. (2012). Fruit quality, colour development and index of absorbance difference (I_{AD}) of different nectarine cultivars at different harvest dates. *Acta Hortic.* 934, 1117–1126. doi:10.17660/ActaHortic.2012.934.150.

Rodríguez-Pires, S., De Cal, A., Espeso, E. A., Rasiukeviciut, N., and Melgarejo, P. (2021). Light-photoreceptors and proteins related to *Monilinia laxa*. *J. Fungi* 7. doi:10.3390/jof7010032.

Rodríguez-Pires, S., Melgarejo, P., De Cal, A., and Espeso, E. A. (2020a). Pectin as carbon source for *Monilinia laxa* exoproteome and expression profiles of related genes. *Mol. Plant-Microbe Interact.* 33, 1116–1128. doi:10.1094/MPMI-01-20-0019-R.

Rodríguez-Pires, S., Melgarejo, P., De Cal, A., and Espeso, E. A. (2020b). Proteomic studies to understand the mechanisms of peach tissue degradation by *Monilinia laxa*. *Front. Plant Sci.* 11, 1–12. doi:10.3389/fpls.2020.01286.

Roeber, V. M., Bajaj, I., Rohde, M., Schmülling, T., and Cortleven, A. (2020). Light acts as a stressor and influences abiotic and biotic stress responses in plants. *Plant Cell Environ.*, 1–20. doi:10.1111/pce.13948.

Rolland, S., Bruel, C., Rascle, C., Girard, V., Billon-Grand, G., and Poussereau, N. (2009). pH controls both transcription and posttranslational processing of the protease BcACP1 in the phytopathogenic fungus *Botrytis cinerea*. *Microbiology* 155, 2097–2105. doi:10.1099/MIC.0.025999-0/CITE/REFWORKS.

Santin, M., Neugart, S., Castagna, A., Barilari, M., Sarrocco, S., Vannacci, G., et al. (2018). UV-B Pre-treatment alters phenolics response to *Monilinia fructicola* infection in a structure-dependent way in peach skin. *Front. Plant Sci.* 9. doi:10.3389/fpls.2018.01747.

Santin, M., Ranieri, A., Hauser, M. T., Miras-Moreno, B., Rocchetti, G., Lucini, L., et al. (2021). The outer influences the inner: Postharvest UV-B irradiation modulates peach flesh metabolome although shielded by the skin. *Food Chem.* 338. doi:10.1016/j.foodchem.2020.127782.

Sawoszczuk, T., Syguła-Cholewińska, J., and del Hoyo-Meléndez, J. M. (2015). Optimization of headspace solid phase microextraction for the analysis of microbial volatile organic compounds emitted by fungi: Application to historical objects. *J. Chromatogr. A* 1409, 30–45. doi:10.1016/j.chroma.2015.07.059.

Schouten, A., Tenberge, K. B., Vermeer, J., Stewart, J., Wagemakers, L., Williamson, B., et al. (2002). Functional analysis of an extracellular catalase of *Botrytis cinerea*. *Mol. Plant Pathol.* 3, 227–238. doi:10.1046/j.1364-3703.2002.00114.x.

Schumacher, J. (2017). How light affects the life of *Botrytis*. *Fungal Genet. Biol.* 106, 26–41. doi:10.1016/j.fgb.2017.06.002.

Segal, L. M., and Wilson, R. A. (2018). Reactive oxygen species metabolism and plant-fungal interactions. *Fungal Genet. Biol.* 110, 1–9. doi:10.1016/j.fgb.2017.12.003.

Sharma, R. R., Reddy, S. V. R., and Jhalegar, M. J. (2014). Pre-harvest fruit bagging: A useful approach for plant protection and improved post-harvest fruit quality - A review. *J. Hortic. Sci. Biotechnol.* 89, 101–113. doi:10.1080/14620316.2014.11513055.

Shimada, T., Endo, T., Fujii, H., Rodríguez, A., Peña, L., and Omura, M. (2014). Characterization of three linalool synthase genes from *Citrus unshiu* Marc. and analysis of linalool-mediated resistance against *Xanthomonas citri* subsp. *citri* and *Penicilium italicum* in citrus leaves and fruits. *Plant Sci.* 229, 154–166. doi:10.1016/j.plantsci.2014.09.008.

Silva, C. J., van den Abeele, C., Ortega-Salazar, I., Papin, V., Adaskaveg, J. A., Wang, D., et al. (2021). Host susceptibility factors render ripe tomato fruit vulnerable to fungal disease despite active immune responses. *J. Exp. Bot.* 72, 2696–2709. doi:10.1093/jxb/eraa601.

Tan, K. K. (1975). Interaction of near-ultraviolet, blue, red, and far-red light in sporulation of *Botrytis cinerea*. *Trans. Br. Mycol. Soc.* 64, 215–222. doi:10.1016/s0007-1536(75)80105-7.

Tholl, D. (2015). Biosynthesis and biological functions of terpenoids in plants. *Adv. Biochem. Eng. Biotechnol.* 148, 63–106. doi:10.1007/10_2014_295.

Tisch, D., and Schmoll, M. (2010). Light regulation of metabolic pathways in fungi. *Appl. Microbiol. Biotechnol.* 85, 1259–1277. doi:10.1007/s00253-009-2320-1.

Torres, M. A., Jones, J. D. G., and Dangl, J. L. (2006). Reactive oxygen species signaling in response to pathogens. 141, 373–378. doi:10.1104/pp.106.079467.

Tsao, R., and Zhou, T. (2000). Interaction of monoterpenoids, methyl jasmonate, and Ca^{2+} in controlling postharvest brown rot of sweet cherry. *HortScience* 35, 1304–1307. doi:10.21273/hortsci.35.7.1304.

Valero-Jiménez, C. A., Veloso, J., Staats, M., and Van Kan, J. A. L. (2019). Comparative genomics of plant pathogenic *Botrytis* species with distinct host specificity. *BMC Genomics* 20. doi:10.1186/s12864-019-5580-x.

Vall-llaura, N., Giné-Bordonaba, J., Usall, J., Larrigaudière, C., Teixidó, N., and Torres, R. (2020). Ethylene biosynthesis and response factors are differentially modulated during the interaction of peach petals with *Monilinia laxa* or *Monilinia fructicola*. *Plant Sci.* 299, 110599. doi:10.1016/j.plantsci.2020.110599.

Van Der Ent, S., and Pieterse, C. M. J. (2012). "Ethylene: multi-tasker in plant-attacker interactions," in *Annual Plant Reviews*, ed. Michael T. McManus (Blackwell Publishing Ltd), 343–377. doi:10.1002/9781118223086.ch13.

Van Loon, L. C., Rep, M., and Pieterse, C. M. J. (2006). Significance of inducible defense-related proteins in infected plants. *Annu. Rev. Phytopathol.* 44, 135–162. doi:10.1146/annurev.phyto.44.070505.143425.

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

Vilanova, L., Valero-Jiménez, C. A., and van Kan, J. A. L. (2021). Deciphering the *Monilinia fructicola* genome to discover effector genes possibly involved in virulence. *Genes (Basel).* 12, 1–15. doi:10.3390/genes12040568.

Wang, Y. J., Yang, C. X., Li, S. H., Yang, L., Wang, Y. N., Zhao, J. B., et al. (2009). Volatile characteristics of 50 peaches and nectarines evaluated by HP-SPME with GC-MS. *Food Chem.* 116, 356–364. doi:10.1016/j.foodchem.2009.02.004.

White, S., McIntyre, M., Berry, D. R., and McNeil, B. (2002). The autolysis of industrial filamentous fungi. *Crit. Rev. Biotechnol.* 22, 1–14.

Wilson, C. L. (1987). Fruit volatiles inhibitory to *Monilinia fructicola* and *Botrytis cinerea*. *Plant Dis.* 71, 316. doi:10.1094/pd-71-0316.

Xi, W., Zheng, Q., Lu, J., and Quan, J. (2017). Comparative analysis of three types of peaches: Identification of the key individual characteristic flavor compounds by integrating consumers' acceptability with flavor quality. *Hortic. Plant J.* 3, 1–12. doi:10.1016/j.hpj.2017.01.012.

Xu, F., Shi, L., Chen, W., Cao, S., Su, X., and Yang, Z. (2014). Effect of blue light treatment on fruit quality, antioxidant enzymes and radical-scavenging activity in strawberry fruit. *Sci. Hortic. (Amsterdam).* 175, 181–186. doi:10.1016/j.scienta.2014.06.012.

Xu, H. X., Chen, J. W., and Xie, M. (2010). Effect of different light transmittance paper bags on fruit quality and antioxidant capacity in loquat. *J. Sci. Food Agric.* 90, 1783–1788. doi:10.1002/jsfa.4012.

Yu, F.-Y., Chiu, C.-M., Lee, Y.-Z., Lee, S.-J., Chou, C.-M., You, B.-J., et al. (2020). Polyketide synthase gene expression in relation to chloromonilicin and melanin production in *Monilinia fructicola*. *Phytopatholog*, 1–64. doi:10.1094/phyto-02-20-0059-r.

Zdarska, M., Dobisová, T., Gelová, Z., Pernisová, M., Dabravolski, S., and Hejátko, J. (2015). Illuminating light, cytokinin, and ethylene signalling crosstalk in plant development. *J. Exp. Bot.* 66, 4913–4931. doi:10.1093/jxb/erv261.

Zhang, B. B., Xu, J. L., Zhou, M., Yan, D. H., and Ma, R. J. (2018). Effect of light quality on leaf photosynthetic characteristics and fruit quality of peach (*Prunus persica* L. Batch). *Photosynthetica* 56, 1113–1122. doi:10.1007/s11099-018-0820-x.

Zhang, B. Bin, Ma, R. J., Cai, Z. X., Yan, J., and Guo, J. Y. (2015). Effects of bagging on fruit quality of "Ruiguang 47" nectarine. in *VIIIth Intl. Peach Symposium*, ed. C. Xiloyannis et al. (Acta Hort. 1084, ISHS), 613–624.

Zhou, T., Ye, B., Yan, Z., Wang, X., and Lai, T. (2020). Uncovering proteomics changes of *Penicillium expansum* spores in response to decanal treatment by iTRAQ. *J. Plant Pathol.* 102, 721–730. doi:10.1007/S42161-020-00486-6/FIGURES/7.

Zhu, P., Zhang, C., Xiao, H., Wang, Y., Toyoda, H., and Xu, L. (2013). Exploitable regulatory effects of light on growth and development of *Botrytis cinerea*. *J. Plant Pathol.* 95, 509–517. doi:10.4454/JPP.V95I3.038.

6. CONCLUSIONS/ CONCLUSIONS/ CONCLUSIONES

The main conclusions derived from this thesis are:

1. *Monilinia laxa* has a broader morphogenic response to light than *M. fructicola* under *in vitro* conditions, ultimately altering their capacity to infect nectarines in a species-dependent manner. The lighting treatments delay the capacity of *M. laxa* to infect fruit but accelerate the onset of disease symptoms and conidiation of *M. fructicola.*

2. Light received by the nectarines during preharvest modifies the intrinsic fruit properties. If the fruit is stored under lighting postharvest treatments, these alterations ultimately influence its response to face *Monilinia* spp.

3. The ethylene production in *Monilinia* spp.-fruit interaction of the 'Fantasia' cultivar depends on the interaction with the pathogen rather than the bagging effect and/or lighting treatment.

4. Fruit bagging slightly alters fruit quality and ethylene production on harvest day, but its effects on fruit quality are almost subsided after the lighting treatment storage. Specifically, all fruit quality parameters on harvest day and after postharvest storage are within the official recommendations irrespective of the bagging condition.

5. Fruit bagging reduces and even prevents fruit decay incidence and is economically rentable. In particular, storage under darkness prevents fruit decay.

6. 'Venus' nectarine cultivar and *M. laxa* synchronize their transcriptional responses during their interaction.

7. *Monilinia laxa* uses different strategies depending on the nectarine developmental stage, such as deploying survival mechanisms in the less susceptible tissue or expressing a large arsenal of necrotrophic tools to infect a susceptible tissue.

8. The expression of 'Venus' nectarine genes in response to *M. laxa* is dependent on the fruit susceptibility to *M. laxa*; the mature susceptible tissue displays a stronger transcriptional response than the immature fruit, although both tissues express genes of similar metabolic pathways.

9. Terpenoid biosynthetic gene expression in nectarines is impaired in response to brown rot. In particular, the cytosolic MVA pathway is induced rather than the plastidial MEP pathway in response to *M. laxa* in the 'Venus' cultivar.

10. The expressions of farnesal-related and linalool biosynthetic genes are highlighted for being upregulated only in the resistant tissue to brown rot.

11. The VOCs profile of control and *M. laxa*-inoculated fruit is associated with the fruit developmental stage, cultivar type, and most notably to the susceptibility to *M. laxa.*

12. Some VOCs putatively emitted by the host either favor the disease susceptibility (e.g., (E,E)-2,6-nonadienal) or negatively affect *M. laxa* development (e.g., (E)-2-decenal and butyrolactone), being the latter, putative target volatiles for further brown rot control strategies.

Les conclusions principals que deriven d'aquesta tesi són:

1. *Monilinia laxa* presenta una resposta morfogènica a la llum més àmplia que *M. fructicola* en condicions *in vitro*, alterant finalment la seva capacitat d'infectar nectarines, d'una forma dependent de l'espècie. Els tractaments d'il·luminació retarden la capacitat de *M. laxa* per infectar la fruita, però acceleren l'aparició dels símptomes de la malaltia i la conidiació de *M. fructicola*.

2. La llum rebuda per les nectarines durant la precollita modifica les propietats intrínseques de la fruita. Si la fruita s'emmagatzema sota tractaments d'il·luminació durant el període de postcollita, aquestes alteracions acaben influint en la seva resposta davant de *Monilinia* spp.

3. La producció d'etilè en el desenvolupament de *Monilinia* spp. en la varietat 'Fantasia' depèn més de la interacció amb el patogen que de l'efecte de l'embossat i/o tractaments d'il·luminació.

4. L'embossat de la fruita altera, lleugerament, la qualitat de la fruita i la producció d'etilè el dia de la collita, però el seu efecte sobre la qualitat de la fruita gairebé disminueix després de l'emmagatzematge sota condicions de fotoperíode. Concretament, tots els paràmetres de qualitat de la fruita del dia de la collita i els de postcollita s'engloben dins de les recomanacions oficials, independentment de l'estat previ d'embossat.

5. L'embossat de la fruita redueix, i fins i tot prevé, la incidència de podrits naturals en la fruita, i és rentable econòmicament. En particular, l'emmagatzematge a la foscor prevé el desenvolupament dels podrits.

6. A nivell transcripcional, nectarines de la varietat 'Venus' i *M. laxa* sincronitzen les seves respostes durant la interacció entre elles.

7. *Monilinia laxa* utilitza diferents estratègies en funció de l'estadi de desenvolupament de la nectarina, com ara desplegar mecanismes de supervivència en el teixit menys susceptible o expressar un gran arsenal de mecanismes necrotròfics per infectar el teixit susceptible.

8. L'expressió dels gens de la nectarina 'Venus' en resposta a *M. laxa* depèn de la susceptibilitat del fruit a *M. laxa*; el teixit susceptible madur mostra una resposta transcripcional més accentuada que el fruit immadur, tot i que ambdós teixits expressen gens de vies metabòliques similars.

9. L'expressió dels gens de biosíntesi de terpenoides en les nectarines es veu alterada en resposta a la podridura marró. En concret, en la varietat 'Venus', la via citosòlica MVA s'indueix en resposta a *M. laxa* en lloc de la via plastídica MEP.

10. Les expressions dels gens biosintètics relacionats amb el farnesal i el linalol destaquen per estar regulats només en el teixit de nectarina resistent a la podridura marró.

11. El perfil de compostos orgànics volàtils (COVs) de fruita control i inoculades amb *M. laxa* està associat a l'estadi de desenvolupament del fruit, al tipus de varietat i, sobretot, a la susceptibilitat a la infecció.

12. Alguns COVs suposadament emesos per l'hoste afavoreixen la susceptibilitat a la malaltia (per exemple, el (E,E-2,6-nonadienal)) o afecten negativament el desenvolupament de *M. laxa* (per exemple, el (E)-2-decenal i butirolactona), sent aquests últims, possibles volàtils diana per a futures estratègies de control de la podridura marró.

Las conclusiones principales que derivan de esta tesis son:

1. En condiciones *in vitro, Monilinia laxa* presenta una respuesta frente a luz más amplia que *M. fructicola*, alterando en última instancia su capacidad para infectar nectarinas de una manera especie dependiente. Los tratamientos de iluminación retrasan la capacidad de *M. laxa* para infectar la fruta, mientras que aceleran la aparición de los síntomas de la enfermedad y la conidiación en *M. fructicola.*

2. La luz que reciben las nectarinas durante la precosecha modifica las propiedades intrínsecas de la fruta, y si la fruta se almacena debajo de tratamientos de iluminación durante el periodo de postcosecha, estas modificaciones influyen en la respuesta frente a *Monilinia* spp.

3. La producción de etileno durante el desarrollo de *Monilinia* spp. en la variedad 'Fantasia' depende más del patógeno que del efecto del embolsado y/o tratamiento de iluminación.

4. El embolsado de la fruta altera ligeramente la calidad de la fruta y la producción de etileno el día de cosecha, pero su efecto sobre la calidad de la fruta se diluye después del almacenamiento en condiciones de fotoperíodo. En concreto, todos los parámetros de calidad de la fruta evaluados a cosecha y postcosecha se encuentran dentro de las recomendaciones oficiales, independientemente de la condición de embolsado.

5. El embolsado de la fruta reduce, e incluso previene, la incidencia de podredumbre en la fruta y es económicamente rentable. En particular, el almacenamiento en la oscuridad previene la podredumbre de la fruta.

6. A nivel transcriptómico, la nectarina de la variedad 'Venus' y el patógeno *M. laxa* sincronizan sus respuestas durante su interacción.

7. *Monilinia laxa* utiliza diferentes estrategias según el estado de desarrollo de la nectarina, como desplegar mecanismos de supervivencia en el tejido menos susceptible o expresar un gran arsenal de mecanismos necrotróficos para infectar al tejido susceptible.

8. La expresión de genes de la nectarina 'Venus' en respuesta a *M. laxa* depende de la susceptibilidad del fruto frente a *M. laxa*; el tejido susceptible maduro muestra una respuesta transcripcional más acentuada que el fruto inmaduro, aunque ambos tejidos expresan genes de rutas metabólicas similares.

9. La expresión de genes de nectarina ligados a la biosíntesis de terpenoides se ve alterada como respuesta a la podredumbre parda. Específicamente, en la variedad 'Venus' se induce la vía citosólica MVA en respuesta a *M. laxa,* en lugar de la vía plastídica MEP.

10. Las expresiones de genes de biosíntesis relacionados con el farnesal y el linalool resaltan por estar reguladas solo en el tejido resistente a la podredumbre parda.

11. El perfil de compuestos orgánicos volátiles (COVs) de fruta control e inoculada con *M. laxa* está asociado con el estadio de desarrollo del fruto, variedad y, sobre todo, de la susceptibilidad del tejido a *M. laxa*.

12. Algunos COVs probablemente emitidos por el huésped favorecen el desarrollo de la enfermedad (p. ej., (E,E)-2,6-nonadienal) o afectan negativamente (p. ej., (E)-2-decenal y butirolactona), lo que los hace posibles volátiles diana para futuras estrategias de control de la podredumbre parda.

7. ANNEX

Annex 1

Mean normalized expression (MNE) of seven terpenoid biosynthetic genes (*PpHMGS, PpPFT1, PpFOLK, PpSQS, PpSM2, PpLIS1* and *PpLIS2*) in healthy nectarines (cv 'Big Top') on harvest day (0 h) and after postharvest storage (48 h, 72 and 7 days) under T1 treatment and darkness. Asterisks denote significant differences between T1 and darkness at each sampling point according to T-Student's test (**P* ≤ 0.05; ***P* ≤ 0.01).