



Unraveling Interactions of the Necrotrophic Fungal Species *Botrytis cinerea* With 1-Methylcyclopropene or Ozone-Treated Apple Fruit Using Proteomic Analysis

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Gray mold caused by the necrotrophic fungus *Botrytis cinerea* is one of the major postharvest diseases of apple fruit. The exogenous application of 1-methylcyclopropene (1-MCP) and gaseous ozone (O₃) is commonly used to ensure postharvest fruit quality. However, the effect of these treatments on the susceptibility of apple fruit to postharvest pathogens remains largely unknown. Herein, the effect of O₃ and 1-MCP treatments on the development of gray mold on apple fruit (cv. "Granny Smith") was investigated. Artificially inoculated apple fruits, treated or not with 1-MCP, were subjected for 2 months to cold storage [0°C, relative humidity (RH) 95%] either in an O₃-enriched atmosphere or in a conventional cold chamber. Minor differences between 1-MCP-treated and control fruits were found in terms of disease expression; however, exposure to ozone resulted in a decrease of disease severity by more than 50% compared with 1-MCP-treated and untreated fruits. Proteomic analysis was conducted to determine proteome changes in the mesocarp tissue of control and 1-MCP- or O₃-treated fruits in the absence or in the presence of inoculation with *B. cinerea*. In the non-inoculated fruits, 26 proteins were affected by 1-MCP, while 51 proteins were altered by ozone. Dynamic changes in fruit proteome were also observed in response to *B. cinerea*. In O₃-treated fruits, a significant number of disease/defense-related proteins were increased in comparison with control fruit. Among these proteins, higher accumulation levels were observed for allergen, major allergen, ACC oxidase, putative NBS-LRR disease resistance protein, major latex protein (MLP)-like protein, or 2-Cys peroxiredoxin. In contrast, most of these proteins were down-accumulated in 1-MCP-treated fruits that were challenged with *B. cinerea*. These results suggest that ozone exposure may contribute to the reduction

of gray mold in apple fruits, while 1-MCP was not effective in affecting this disease. This is the first study deciphering differential regulations of apple fruit proteome upon *B. cinerea* infection and postharvest storage treatments, underlying aspects of host response related to the gray mold disease.

Keywords: apple proteome, gaseous O₃, Granny Smith, gray mold, postharvest treatments, 1-methylcyclopropene

INTRODUCTION

Apple (*Malus domestica* Borkh) is one of the most popular fruit crops cultivated throughout the world, with more than 80,000,000 tonnes of annual production (FAO, 2018). Apple fruits are characterized by their long storage capacity that can be extended by exogenous application of compounds interfering with ethylene biosynthesis or ethylene activity that delay fruit ripening (Karagiannis et al., 2018). However, during the long period of storage, apples become susceptible to various biotic (fungi and bacteria deterioration) and abiotic factors (physiological disorders), which lead to great economic losses. Among the biotic disorders, fungal pathogens play a predominant role in fruit postharvest decay. It is estimated that more than 90 fungal species may be agents of postharvest diseases on apple fruits, and some of them can cause devastating losses that can reach up to 25% (Jijakli and Lepoivre, 2004).

Gray mold caused by the necrotrophic pathogen *Botrytis cinerea* is a major postharvest disease of apple fruits throughout the world (Konstantinou et al., 2011; Nybom et al., 2020). Infections by the pathogen occur mostly through wounds caused during harvest or during postharvest processes in the packing house, while *B. cinerea* can also infect apple fruits during bloom or just after fruit setting through the open calyx of the fruits. However, the disease symptoms appear on the infected fruits during storage. Symptoms consist in the appearance of light tan to dark brown lesions that are irregular in shape without well-defined margins between healthy and decayed tissues (Konstantinou et al., 2011; Nybom et al., 2020).

As is typical, necrotrophic pathogen *B. cinerea* during the infection process modulates the host environment by lowering the pH of the host tissues, a condition conducive for fungal development and increase in virulence (Alkan et al., 2013; Rascle et al., 2018). Host tissue acidification is mediated through the production of gluconic, fumaric, and citric acids and is regulated by *PacC*, a conserved transcription factor that is involved in pH regulation in several phytopathogenic fungi including *B. cinerea* (Rascle et al., 2018). The lowering of pH within host tissues enhances the activity of several cell wall-degrading enzymes, such as polygalacturonases, employed by necrotrophic pathogens to destroy pectin and the primary cell walls of the host, to cause cell death or to alter the integrity of the host cells (Blanco-Ulate et al., 2014).

Despite that immunity of apple cultivars to gray mold does not exist, there is a variability in the level of their susceptibility to the disease, as has been shown by several previous studies assessing cultivars' susceptibility level with either natural infections or artificial inoculations (Spotts et al., 1999;

Konstantinou et al., 2011). These differences in the level of cultivar susceptibility are associated with fruit ripening-related characteristics, such as the rate of softening as well as with the chemical constituents of the peel and the flesh tissues, including polyphenol biosynthesis and pathogenesis-related (PR) protein activity (Ma et al., 2018). Particularly, the activation of ethylene biosynthesis in the harvested fruit has been found to play a critical role in the induction of resistance responses that are associated with PR proteins, polyphenolic compounds, or lignin biosynthesis (Akagi et al., 2011).

Apple, as a climacteric fruit, is characterized by a burst of respiration and ethylene production during its long storage life, which subsequently lead to a rapid fruit ripening accompanied by an undesirable fruit softening (Karagiannis et al., 2020). The 1-methylcyclopropene (1-MCP), an inhibitor of ethylene action, has been widely used in horticultural crops, particularly in apple fruits, to delay ripening, especially following long-term storage (Li et al., 2016; Minas et al., 2018). Also, 1-MCP applications have proven to be beneficial for the control of many physiological disorders, such as apple superficial scald (Karagiannis et al., 2018). Moreover, the effects of 1-MCP treatments on decay development are controversial and differ among the several fruit commodities tested (Li et al., 2016). In some cases, 1-MCP applications increased the susceptibility of the treated fruits, while there are some examples in which 1-MCP reduced the growth of postharvest pathogens (Mir et al., 2001; Díaz et al., 2002; Janisiewicz et al., 2003; Marcos et al., 2005). Meanwhile, ozone treatments can also delay climacteric fruit ripening by directly oxidizing ethylene, while, in addition, it can reduce the residues of various mycotoxins and fungicides (Karaca and Velioglu, 2007; Minas et al., 2018). However, apple fruit exposure to a high concentration of ozone may stimulate oxidative damage and physiological disorders such as superficial scald (Yaseen et al., 2015; Lv et al., 2019). Ozone has been tested against several pathogens on several horticultural fruits, with contradictory results (Palou et al., 2002; Karaca and Velioglu, 2007).

Proteomic analysis has been used to investigate complex biological processes such as the responses of fruit tissues to pathogens (Papavasileiou et al., 2020). With the use of proteomics, the response of *B. cinerea* to plant-based elicitors and hormones (Liñeiro et al., 2016) and the *in vitro* secretome of *B. cinerea* related to pathogenesis (González-Fernández et al., 2015) have been investigated. In apple fruits, proteomic studies have focused mostly on the investigation of protein changes during the ripening process (Zheng et al., 2013; Shi et al., 2014), while the available information on the effect of postharvest treatments and pathogen's presence on the modulation of apple proteome is limited (Burton-Moles et al., 2015).

In this work, we characterize the effect of 1-MCP and gaseous O₃ on the development of gray mold of apple (cv. Granny Smith) fruits. To improve our fundamental understanding of *B. cinerea* development and its response to 1-MCP and gaseous O₃ treatments, we conducted proteomic analysis to quantitatively investigate protein profiles of apple mesocarp of control and 1-MCP- or O₃-treated fruits following inoculation with *B. cinerea* to identify proteins potentially involved in fruit responses to pathogen attack.

MATERIALS AND METHODS

Fruit Material

Apple fruits (cv. Granny Smith) used in the experiments were collected from an orchard located in the region of Imathia (northern Greece) at the commercial harvest stage. The fruits selected for the experiments originated from trees that did not receive any fungicide application during the last 2 months before harvest. The selected fruits were healthy, without any visual defect, and they had an average weight of 150 g. The fruits immediately after harvest were transferred to the fruit storage facilities of AUTH and stored at a conventional chamber at 1°C and 95% relative humidity (RH).

The harvested fruits were divided into three groups, one for each treatment (untreated control, 1-MCP-treated, and O₃-treated fruits; see below), and placed on single-layer wooden trays with plastic vents. For each treatment, three replicates of 10 fruits were used for artificial inoculations, as well as for the ripening measurements after 60 days of storage.

Treatments With 1-Methylcyclopropene and O₃

The application of 1-MCP (SmartFresh™, AgroFresh Inc., Rohm and Haas, Spring House, PA, United States) was conducted 24 h after harvest. 1-MCP was applied at the commercially recommended dose of 500 µl L⁻¹ for 24 h at 1°C. The application of O₃ was initiated after the artificial inoculation of the fruits and was continuous during the entire cold storage period. For the production and monitoring of ozone concentration, an oxygen generator (model SEP-100, Anseros Klaus Non-nenmacher GmbH, Tübingen, Germany), a corona discharge ozone generator (model COM-AD-04, Anseros Klaus Non-nenmacher GmbH, Tübingen, Germany), and an ozone analyzer (model MP-6060, Anseros Klaus Non-nenmacher GmbH, Tübingen, Germany) were used.

Ripening Parameters of Apple Fruits

Ripening parameters, including firmness, titratable acidity (TA), and total soluble solids, of apple fruits were measured on the harvest day and after 60 days storage period, as previously outlined (Karagiannis et al., 2020). On the harvest day, the ripening status of apple fruits was performed using 30 replicate fruits (10 × 3 fruits in three independent replications). Similarly, after the end of the storage period, ripening parameters were conducted on 30 replicate fruits per treatment (control, 1-MCP-treated, and O₃-treated fruits; 10 × 3 fruits in three independent

replications). Fruit firmness was measured in two opposite sites of each fruit after peeling of skin using a texture analyzer (model 53205, T.R. Turoni Srl, Forlì, Italy) with a 12 mm probe. Soluble solids content (SSC) measurement was performed in juice using a refractometer (Atago PR-1, Atago Co., Ltd., Tokyo, Japan) and TA by acid–base titration of maleic acid (Karagiannis et al., 2020).

Fungal Strain and Artificial Inoculations

A *Botrytis cinerea* isolate (Bc Z08) that had been obtained from infected apple fruits and identified for the requirements of monitoring studies conducted by our group was used in the study. Inoculum was produced by cultivating the isolate on Potato Dextrose Agar (PDA) for 7 days at 25°C under continuous light. Conidia were suspended from the petri dishes using a scalpel, and inoculum suspensions were prepared in sterile distilled water supplemented with 0.1% (v/v) Tween 80 (PanReac Applichem, Germany). Inoculum suspensions density was adjusted at 4×10^5 conidia ml⁻¹, using a hemocytometer counting chamber (Neubauer, Heinz Herenz Hamburg, Germany).

On all the fruit sets, artificial inoculations were conducted 48 h after harvest (24 h after 1-MCP application on 1-MCP-treated fruit). Before inoculation, the fruits had been surface-sterilized by immersion in a 70% (v/v) ethanol solution for 2 min. Then, fruits were wounded with a sterile needle (5 mm) and inoculated with 45 µl of conidial suspension. Control fruits were similarly wounded and inoculated with 45 µl of distilled sterile water.

Storage Conditions and Disease Incidence/Severity Measurements

Following inoculation, the fruits were incubated for 3 h at 20°C to allow fungal spores to germinate and then transferred for cold storage. Control fruits and fruits that had been treated with 1-MCP were stored in a conventional cold chamber (1°C and RH 95%), while the rest of fruits were stored in an ozone-enriched atmosphere chamber (O₃ treatment) (0.3 µl L⁻¹, 1°C, RH 95%). Ozone generation was performed as described above.

After 60 days of cold storage, disease incidence (%) was measured by counting the number of the fruits with decay symptoms around the inoculation point, and the disease severity was determined by measuring the lesion diameter (cm) around the inoculation point.

Proteomic Analysis

After 60 days of cold storage, 20–30 g of skinless flesh of the mesocarp were carefully removed from each fruit antipodal to the inoculation point, to avoid any rotten tissue and the potential presence of fungal mycelia. Samples were cut into small pieces, placed in polyethylene bags (10 fruits per bag), frozen instantly in liquid nitrogen, and stored at -80°C until further analysis. In total, 30 fruits (3 × 10 fruits in each replicate) were utilized for the analysis. Apple protein extraction was performed with 5 g of mesocarp tissue based on a phenol extraction protocol (Karagiannis et al., 2020). Two-dimensional electrophoretic separation followed Minas et al. (2016) described procedure using a BIO-RAD system. For

imaging statistical analysis, silver nitrate staining was followed by two-dimensional gel electrophoresis (2-DE) scanning with a Bio-Rad GS-800 Calibrated Densitometer equipped with PDQuest Advanced 2-DE Gel Analysis Software, whereas for mass spectrum analysis, a compatible modified silver nitrate staining protocol using Silver Stain Plus kit (Bio-Rad) was performed according to Tanou et al. (2015). Statistics were done by one-way analysis of variance for significance level ($P \leq 0.05$). Student's *t*-test (significance level 95%) was applied to compare means. The statistically significant differences were further combined by a quantitative 2.0-fold change of spot volume. At least three biological replicates were performed for each fruit treatment.

Selected of interest spots underwent tryptic in-gel digestion and peptide fragments analyzed by matrix-assisted laser desorption ionization MS (MALDI-MS) in a time-of-flight MS (TOF-MS) (Ultraflex II, Bruker Daltonics, Bremen, Germany) as detailed by Ainalidou et al. (2016). Peptide mixtures were injected in a MALDI-TOF mass spectrometer (Autoflex-Speed, Bruker Daltonics). Raw files were searched against the Uniprot *Malus domestica* protein database using the (MASCOT Server v2.0). The mass error tolerance on the Mascot server was set to 25 ppm, methionine oxidation was considered as a variable modification, and cysteine carbamidomethylation was considered as a fixed modification. All information regarding peptide sequences, accession numbers, Mascot scores, and sequence coverage are provided in **Supplementary Table S1B**. For non-identified spots, peptide fragments were loaded on an LC-MS/MS using a LTQ Orbitrap XL Mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) coupled online with a nanoLC Ultimate 3000 chromatography system (Dionex, Sunnyvale, CA) (Karagiannis et al., 2016). Raw files were searched against the ncbi *M. domestica* protein database using PD 1.4 and the SEQUEST HT search engine. Protein identification required minimal XCorr values of 2.0 and 2.5 for charge states of doubly and triply charged precursor ions, respectively (**Supplementary Table S1A**). Manual protein BLAST against current databases was performed where protein annotations were missing. Identifications are based on at least two peptides per protein. This work is Minimum Information About a Proteomics Experiment (MIAPE) compliant.

Data Analysis

The Pearson chi-square test was used to compare disease incidence values on fruits of the three different treatments. Disease incidence percentage values were arcsine transformed for statistical analysis. Data on disease severity in terms of lesion diameter were subjected to one-way analysis of variance and the least significant differences (Duncan) at $P = 0.05$ were used for means comparison. Statistical analysis transacted using SPSS v21.0 (SPSS, Chicago, IL, United States). All the graphical presentation was created with GraphPad Prism (GraphPad Prism version 8.0.0 for Windows, GraphPad Software, San Diego, California United States)¹.

¹ www.graphpad.com

RESULTS

The Effect of 1-Methylcyclopropene and O₃ Treatments on the Ripening Characteristic of Apple Fruit

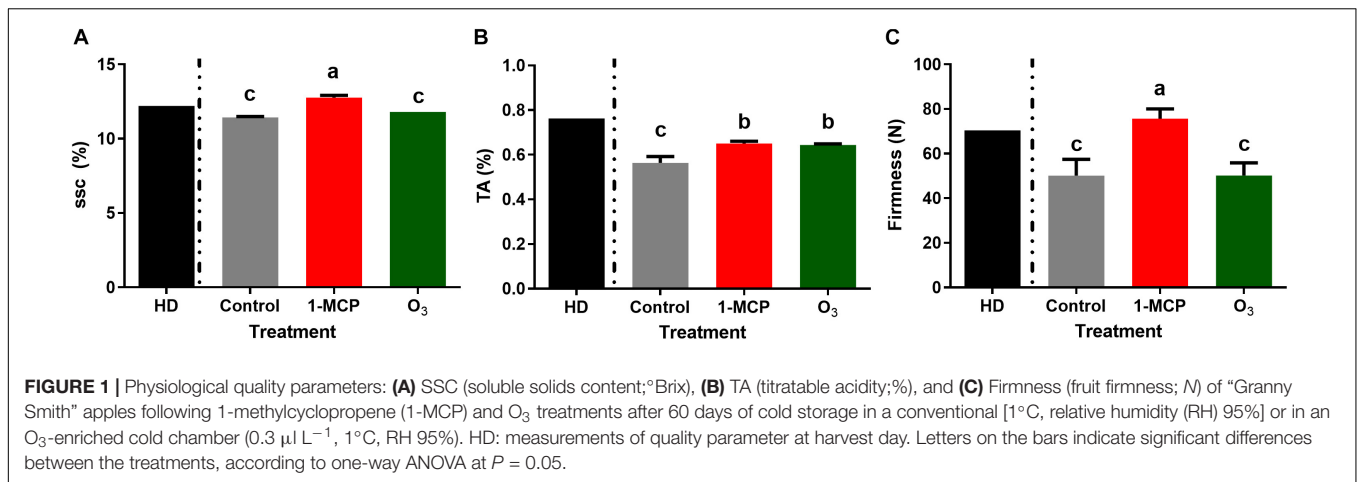
Measurements of fruit qualitative parameters on the harvest day revealed that mean SSC, TA, and fruit firmness levels were of 12.2°Brix, 0.76%, and 70.5 N, respectively (**Figure 1**). In 1-MCP-treated fruits after 60 days of storage, most of the studied quality parameters remained at harvest day's level. After 60 days of storage, SSC values were significantly higher ($P < 0.05$) in 1-MCP-treated fruits (12.8°Brix) compared with control (11.43°Brix) and O₃-treated (11.8°Brix) fruits (**Figure 1A**). Similarly, fruit firmness was substantially higher ($P < 0.05$) in 1-MCP-treated fruits (75.6 N) compared with that of control (50.1 N) or O₃-treated (50.2 N) fruits (**Figure 1C**). TA levels were substantially reduced in the three postharvest conditions compared with the initial ones determined on harvest day. Moreover, the reduction in TA was higher in untreated control fruits compared with that observed in 1-MCP- or O₃-treated fruits (**Figure 1B**).

The Effect of 1-Methylcyclopropene and O₃ Treatments on Gray Mold Incidence and Severity

Measurements of gray mold incidence on apple fruit treated with 1-MCP or O₃ showed that these treatments did not affect ($P > 0.05$) disease incidence compared with that on the control fruits. In detail, the rates of the disease incidence in response to control, 1-MCP, and O₃ treatment were 86.6, 76.6, and 93.3%, respectively (**Figure 2A**). However, distinct differences were observed among the different treatments regarding disease severity. The lower ($P < 0.05$) lesion diameter was observed in O₃-treated apple fruits with a mean value of 2.55 cm, while no difference ($P > 0.05$) was observed between 1-MCP-treated and control fruits, with mean lesion diameter values of 5.95 and 6.07 cm, respectively (**Figures 2B,C**). These results indicated that the O₃ application significantly suppressed *Botrytis cinerea*, while 1-MCP treatment had no significant impact on the pathogen.

Characterization of Apple Fruit Proteome Following Long-Term Storage

To better understand the mechanisms behind the observed resistance of apple fruits to *B. cinerea* of O₃-treated fruits, 2-DE analysis was performed to identify protein changes between the postharvest treatments tested (**Figures 3A,B**). Among all treatments (inoculated or not), more than 500 protein spots were detected. At the same time, 98 of them exhibited significant differences in their abundance of either inoculated or not 1-MCP- and O₃-treated apple fruits according to the Student *t*-test with 95% confidence interval, further validated by 2-fold change threshold (**Figures 3C,D** and **Supplementary Table S3**). According to the mass spectrum analysis, 138 proteins were identified, while 19 proteins were detected in more than one spot, and 10 proteins were not identified. In detail,



these proteins were 2-methylene-furan-3-one-reductase (spot nos. 5,223 and 6,218), 5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase (spot nos. 7,720 and 7,728), ACC oxidase (ACO; spot nos. 1,132, 1,212, 1,220, 2,213, and 3,220), actin (spot nos. 23, 3,220, and 3,327), allergen (spot nos. 1,037 and 2,029), ATP-citrate synthase beta chain protein (spot nos. 8,606 and 9,603), beta galactosidase (spot nos. 3,719 and 9,708), CNP-60 (spot nos. 2,631 and 2,634), fructose-bisphosphate aldolase (spot nos. 6,222, 6,225, 7,222, and 9,227), glyceraldehyde-3-phosphate dehydrogenase (spot nos. 3,211, 9,313, and 9,314), L-3-cyanoalanine synthase (spot nos. 2,320, 3,322, and 3,324), major allergen (spot nos. 1,037, 3,040, and 4,016), NADP-dependent-D-sorbitol-6-phosphate dehydrogenase (spot nos. 5,627, 7,222, 7,226, 8,232, and 9,221), phosphoenolpyruvate carboxylase (spot nos. 6,834, 6,905, and 6,908), porin (spot no. 8,732, 9,117, 9,118), proteasome subunit alpha (spot nos. 4,105 and 8,123), serine/threonine protein phosphatase (spot nos. 109 and 1624), stem-specific protein (spot nos. 210 and 2,113), and V-ATPase (spot nos. 1,510, 1,511, and 2,727). Further information regarding the identified proteins and their functional categorization based on their gene ontology and literature is provided in **Supplementary Tables S1A,B**.

Functional Classification of Proteins Affected by 1-Methylcyclopropene and O₃ Treatments in Non-inoculated Fruit

To determine the apple proteins whose abundance changed (increased or decreased) by the 1-MCP and O₃ applications compared with the untreated control fruits, proteins were grouped into two sets: (1) a set of 26 proteins that were changed in non-inoculated fruits by the application of 1-MCP (control vs. 1-MCP) (**Figure 4B**), and (2) a set of 51 proteins that were altered in non-inoculated fruits by the O₃ application (control vs. O₃) (**Figure 4C**).

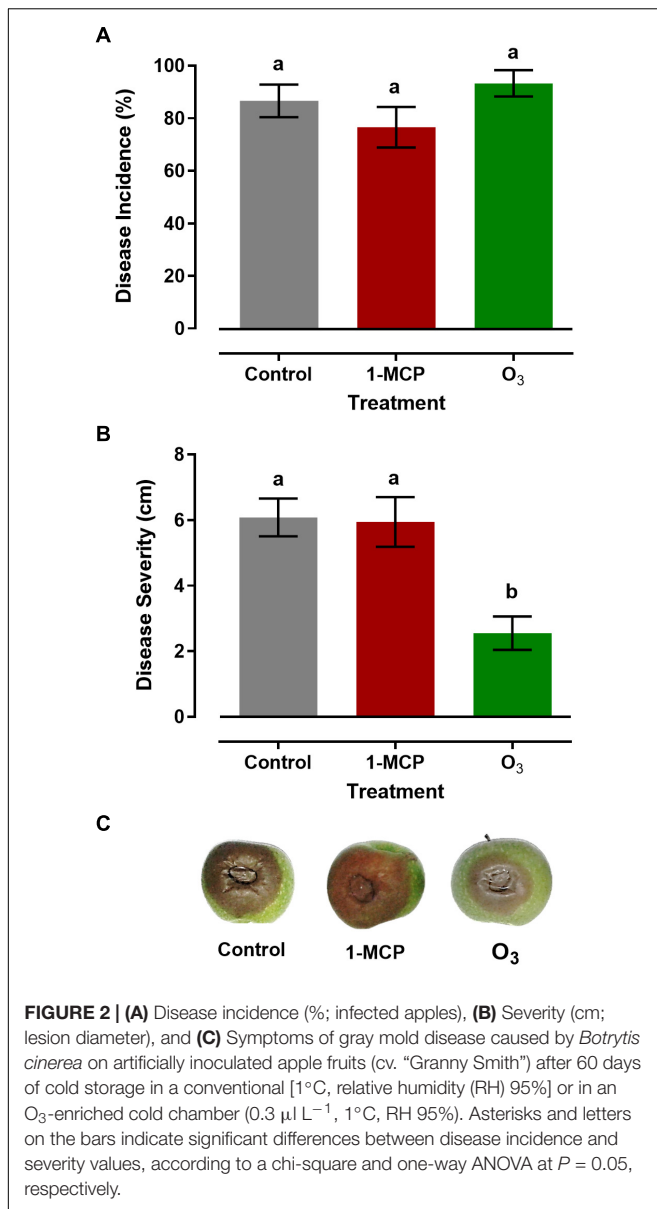
Functional analysis showed that 1-MCP application affected several proteins (*n* = 26) that were mainly involved in energy (26.92%), disease/defense (23.08%), and protein destination and storage (15.38%) (**Figure 4B**). Moreover, most of these proteins were down-accumulated (*n* = 18), while many of them were

involved in energy (27.78%), disease/defense (27.78%), and protein destination and storage (16.67%) (**Figure 5**). A similar functional classification was observed in up-accumulated (*n* = 8) proteins, which were mainly participated in energy (25%), disease/defense (12.5%), protein destination and storage (12.5%), and metabolism (12.5%) (**Figures 4B, 5**). The application of O₃ on apple fruits affected a major part of proteins (*n* = 51) that were mostly involved in disease/defense (25.4%), energy (15.6%), and protein destination and storage (15.6%). Most of these proteins were up-accumulated (*n* = 43) and functionally belong to disease/defense (37.5%), energy (25%), and protein destination and storage (25%). A similar function classification also had the down-accumulated proteins (*n* = 8) with rates of 23.2, 13.95, and 13.95% (**Figures 4C, 5**).

An overlap of 15 proteins modulated by both 1-MCP and O₃ treatments was detected, whereas 11 and 36 proteins were affected exclusively by 1-MCP and O₃ treatments, respectively (**Figures 4A,D**). These 15 proteins were mainly involved in energy (33.3%), protein destination and storage (26.6%), and disease/defense (13.3%) (**Figures 4A,D**), whereas the 11 and 36 solely 1-MCP- or O₃-triggered proteins were associated with disease/defense or their classification was unclear (**Figures 4A,D**).

Functional Classification of Proteins Modulated by 1-Methylcyclopropene and O₃ Treatments in Fruit Artificially Inoculated With *Botrytis cinerea*

Proteome comparisons were also performed to identify proteins modulated by 1-MCP and O₃ treatments in the presence of *B. cinerea* compared with the untreated and non-inoculated fruits (control). Subsequently, three sets of apple proteins were defined: (1) a set of 50 proteins that were differentiated by the presence of the pathogen in untreated fruits (control vs. control-Bc) (**Figure 6B** and **Supplementary Table S2**), (2) a set of 49 proteins that were influenced by the pathogen and 1-MCP treatment (control vs. 1-MCP-Bc) (**Figure 6C** and **Supplementary Table S2**), and (3) a set of 40 proteins that were



modulated by *B. cinerea* and O₃ treatment (control vs. O₃-Bc) (Figure 6D and Supplementary Table S2).

Functional classification revealed that the presence of *B. cinerea* significantly affected apple proteins, mainly those involved in disease/defense, metabolism, energy, and protein destination and storage. Inoculation with the pathogen triggered a great number of proteins (*n* = 50), which operated in disease/defense (30%), metabolism (30%), and energy (10%). In detail, most of these proteins were up-accumulated (*n* = 29) and involved in metabolism (38%) and disease/defense (31%), while down-accumulated proteins (*n* = 21) were associated with disease/defense (29%) and metabolism (19%) (Figures 5, 6B and Supplementary Table S2). The combination of artificial inoculations and 1-MCP treatment affected several proteins (*n* = 49) that are involved in metabolism (22%), protein

destination and storage (20%), disease/defense (16%), and energy (16%). A great number of these proteins were downregulated (*n* = 26) and functionally associated with disease/defense (23%), protein destination and storage (23%), metabolism (15%), and energy (15%). A similar functional classification was observed in the up-accumulated proteins, which were associated with metabolism (30%), protein destination and storage (17%), and energy (17%) (Figures 5, 6C and Supplementary Table S2). The combination of artificial inoculations and O₃ treatment affected 40 proteins that were mostly involved in metabolism (30%), disease/defense (25%), and protein destination and storage (13%). Among these 40 proteins, 23 were upregulated and mainly involved in disease/defense (39%) and metabolism (35%), while 17 proteins were downregulated proteins, and they were associated with metabolism (24%) and protein destination and storage (24%) (Figures 5, 6D and Supplementary Table S2).

Details of shared and distinct affected proteins among the untreated control and the 1-MCP- and O₃-treated fruits that had been inoculated with *B. cinerea* are shown in Figures 6A,D. The common proteins shared among the three conditions (*n* = 13) were involved in metabolism (54%), protein destination and storage (15.4%), and energy (15.4%) (Figure 6E). Proteins related to disease/defense (31.6%) and metabolism (21%) were predominant in the set of proteins (*n* = 19) exclusively affected by the presence of the pathogen on the untreated fruits (Figure 6E). Common proteins between control and 1-MCP-treated and artificially inoculated fruits (*n* = 4) were associated with disease/defense (75%) and energy (25%), while control's shared proteins with O₃-treated and inoculated fruits (*n* = 14) were mainly involved in disease/defense (43%) and metabolism (28%) (Figure 6E). In 1-MCP-treated apple fruits inoculated with *B. cinerea*, a set of unique proteins (*n* = 27) were identified that were mainly related to protein destination and storage (22.2%), disease/defense (18.5%), and energy (18.5%). Proteins triggered uniquely (*n* = 8) by O₃ treatment and the pathogen were associated with disease/defense (30%), while proteins commonly shared between O₃- and 1-MCP-treated fruits that had been inoculated with the pathogen (*n* = 5) were mostly associated with protein destination and storage (40%) (Figure 6E).

The changes in apple proteins among untreated control, 1-MCP-, and O₃-exposed fruits inoculated or not with *B. cinerea* compared with the non-inoculated and untreated fruits (control) are presented in Figure 5. Interestingly, the variations in accumulation levels of *B. cinerea* (Bc)-responsive proteins were prevented by O₃ treatment compared with control (Figure 5). It is noted that a set of 50 proteins were differentiated by the presence of the pathogen (control vs. control-Bc), whereas the O₃ treatment prevented the accumulation of almost half of them (*n* = 23), and their abundance was not different compared with that in the control. Such proteins included actin (spot nos. 23, 3,220, and 3,327), sorbitol dehydrogenase (spot no. 50), plastoglobulin (spot no. 317), deddyrin 8 (spot no. 1,507), stem-specific protein (spot no. 2,110), plasma membrane-associated cation-binding protein (spot no. 2,115), L-3-cyanoalanine synthase (spot no. 2,320), glyceraldehyde-3-phosphate dehydrogenase (spot no. 3,211, 9,313), ACCO (spot

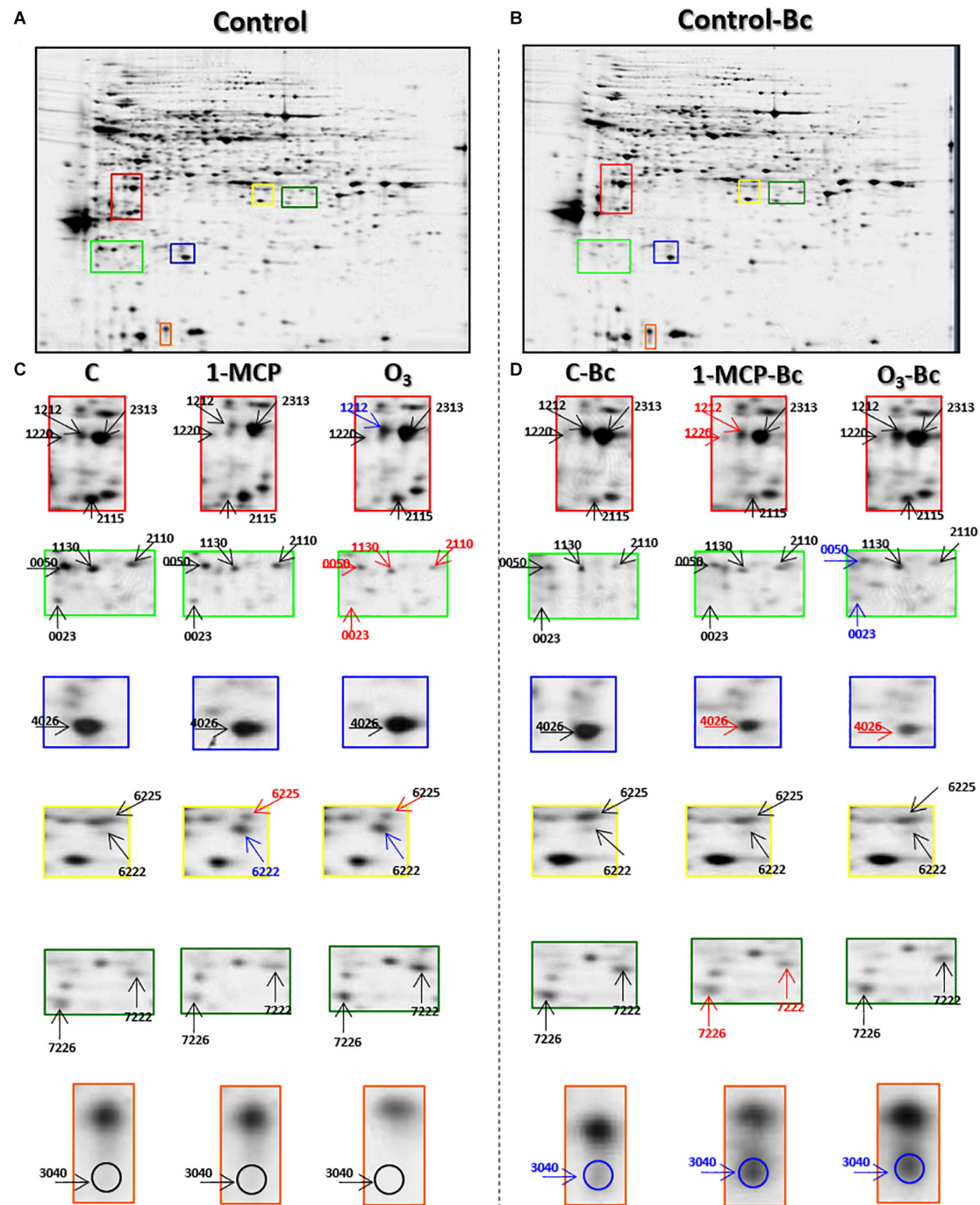
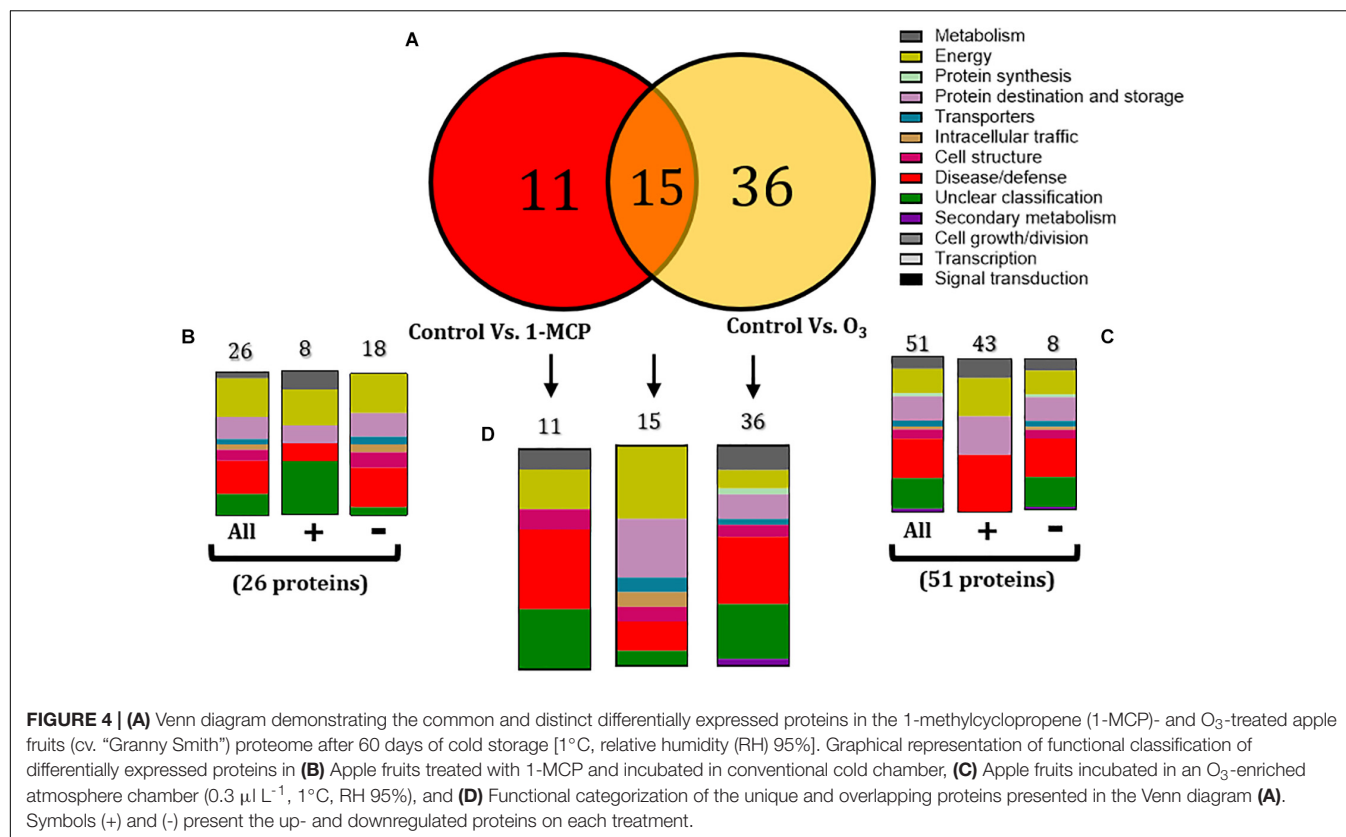


FIGURE 3 | Representative silver-stained two-dimensional gel electrophoresis (2-DE) map of total proteins from **(A)** Untreated (control) and **(B)** Untreated inoculated (control-Bc) apple fruits (cv. “Granny Smith”). **(C,D)** Enlarged view panels of selected areas of control in relation to 1-methylcyclopropene (1-MCP)- and O_3 -treated apple fruits inoculated or not with the necrotrophic pathogen *Botrytis cinerea*. Blue, red, and black arrows demonstrate protein spots abundance increased, decreased, or remained unchanged, respectively, compared with the control. Enlarged in views of selected areas of full-length gels are provided as **Supplementary Figure S1**.

no. 3,220), glutamine synthetase (spot no. 4,314), glutamate decarboxylase (spot no. 4,517), aconitate hydratase (spot no. 5,840), AP-2 complex subunit alpha (spot no. 5,845), alpha-glucosidase (spot no. 5,845), apolipoprotein (spot no. 6,016),

oxygen-evolving enhancer protein (spot no. 6,019), glutathione-S-transferase (spot no. 6,019), phosphoenolpyruvate carboxylase (spot no. 6,908), voltage-gated potassium channel subunit beta (spot no. 7,220), and porin (spot no. 9,117) (**Figure 5**).



DISCUSSION

Applications of ozone and especially 1-MCP have been widely distributed throughout the world as postharvest treatments to extend the storage period of apple. However, most of these studies focus on the anti-ripening and antimicrobial efficacy of 1-MCP and ozone treatment, respectively, and knowledge concerning the molecular effects of these chemical applications on major postharvest spoilage agents of apple fruits is quite limited. In this study, the effect of the postharvest treatments with 1-MCP and ozone on the development of gray mold caused by *Botrytis cinerea* on apple fruit (cv. “Granny Smith”) was investigated. We further employed a comparative study of the proteomic profiles among non-inoculated and inoculated fruits that were either exposed to 1-MCP and ozone or remain untreated. Such an experimental approach provides insights into the responses of apple fruits to *B. cinerea* infection as well as into the physiological function of 1-MCP and ozone in apple postharvest biology.

The ripening of fleshy fruit is associated with changes in susceptibility to pathogen infection since susceptibility significantly increases during ripening (Papavasileiou et al., 2020). Apple is a typical climacteric fruit, and its ripening process and postharvest behavior is controlled by ethylene (Karagiannis et al., 2018). In line, apple fruit ripening parameters such as SSC, TA, and firmness underwent reduction in untreated control fruits during storage compared with harvest stage. The application of 1-MCP contributed to a retention of fruit physiological characteristics at levels comparable with those during harvest,

confirming previous studies (Janisiewicz et al., 2003; Kolniak-Ostek et al., 2014). Moreover, firmness and SSC remained unaffected in O₃-treated fruits, supporting recent works in apples (Lv et al., 2019; Karagiannis et al., 2020).

The phenotypic characterization of apple fruit responses to *B. cinerea* showed that applications of 1-MCP did not contribute to the suppression of the pathogen. Disease incidence and severity caused by *B. cinerea* in 1-MCP-treated fruits was similar to that observed on untreated control fruits. Unpublished data of our group suggested that 1-MCP applications on another apple cultivar (cv. “Red Delicious”) increased fruit susceptibility to *B. cinerea* (Karaoglanidis, unpublished data). Previous studies on the effect of 1-MCP treatments on the control of *B. cinerea* on several horticultural commodities provided variable results. For example, applications of 1-MCP failed to protect tomato and pear fruits by *B. cinerea* or even led to an increased susceptibility (Díaz et al., 2002; Akagi and Stotz, 2007; Min et al., 2018), while on cut carnation flowers, 1-MCP treatment resulted in a reduction of gray mold incidence and severity (Seglie et al., 2012). However, it should be stated that fruit ripening stage at the time of 1-MCP application may be critical, as has been shown in avocado fruits that were 1-MCP treated and artificially inoculated with *Colletotrichum gloeosporioides* (Wang et al., 2006). In that study, early harvested avocado fruits treated with 1-MCP revealed significant inhibition of fruit ripening and the synthesis of (Z,Z)-1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene (AFD), one of the most active antifungal compounds in avocado fruits (Wang et al., 2006).

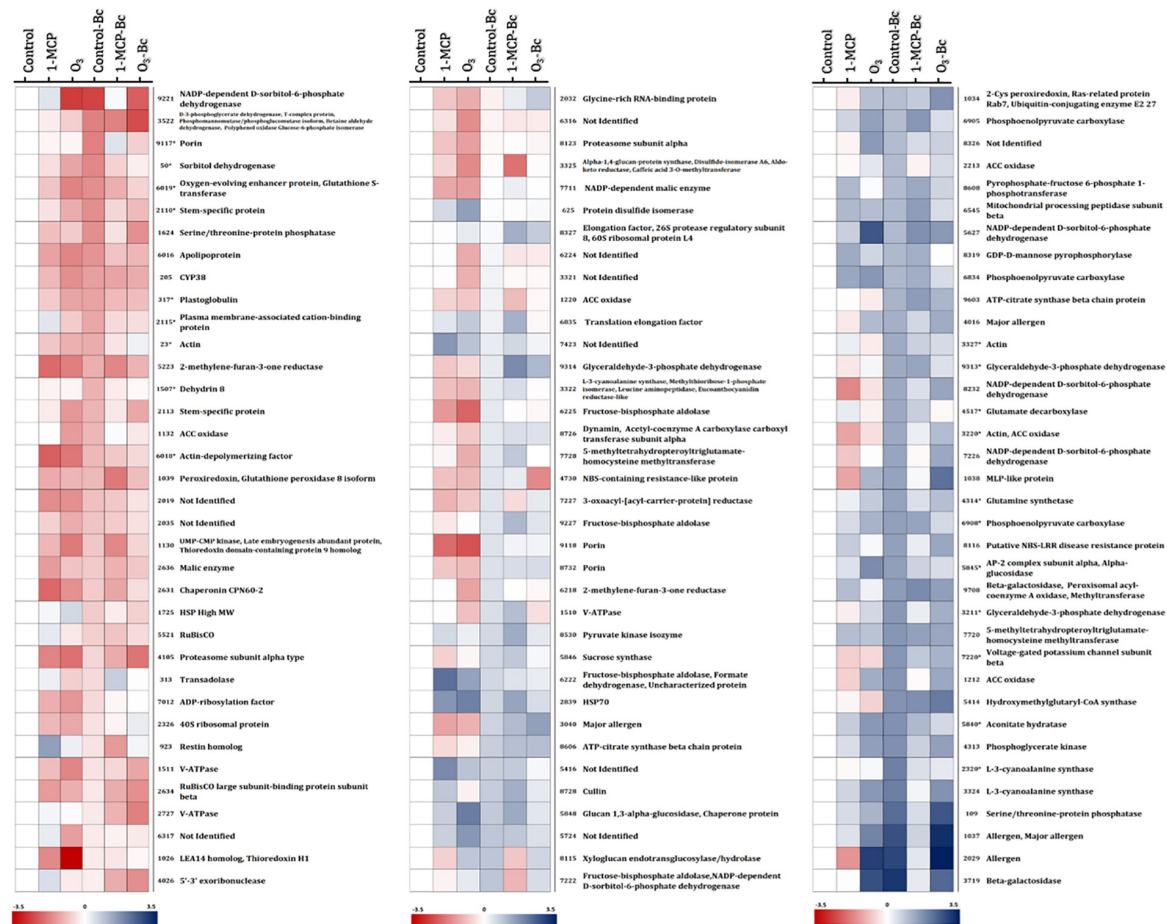


FIGURE 5 | Protein abundance changes among “untreated,” “1-MCP-treated,” and “O₃-treated” apple fruits (cv. “Granny Smith”) inoculated or not with *Botrytis cinerea* compared with the non-inoculated and untreated apple fruits (control). Heat map of proteins that demonstrated statistically significant differences among the different experimental conditions. Color scale shows the relative abundance of each protein across the experimental conditions, as it has been calculated as log₂ of the ratio of the protein abundance of all experimental conditions to the protein abundance to the corresponding control. Blue and red colors indicate, respectively, enhanced and reduced abundance. (*) indicates protein accumulation prevented by O₃ treatment. Proteins correspond to those listed in **Supplementary Tables S1A,B**.

An important result revealed by this study was that ozone exposure strongly reduced gray mold severity compared with control. *In vitro* studies have shown that ozone treatments in kiwifruit exhibited a strong toxic impact on *B. cinerea* conidia viability and a robust mycostatic activity on pathogen's mycelial growth (Minas et al., 2010). However, the observed reduction of disease severity on O₃-treated apple fruits could not be correlated with direct toxic effects of O₃ on the conidia or mycelial growth. The artificially inoculated fruits were transferred to the ozonated chambers after some hours of incubation at 20°C. This is expected to allow the germination of conidia on fruit surface. Notably, previous studies on the effect of ozone exposure on apple fruits revealed an increased spoilage rate in ozone-treated apple fruits by unspecified spoilage agents (Antos et al., 2018). In contrast, Yaseen et al. (2015) reported a reduction in *Penicillium expansum* population densities on fruit surface. However, in the latter report, only surface fungal population was measured and not disease incidence or disease severity on

infected fruits. More recently, in a study aiming to determine the effects of ozone treatments on fruits of different apple cultivars, it was reported that effects on apple fruit spoilage were cultivar dependent (Juhnveica-Radenkova et al., 2019).

To characterize the mechanisms behind the responses of 1-MCP- or O₃-treated apple to *B. cinerea*, a comparative proteomic analysis was conducted on non-inoculated and artificially inoculated fruits. Initially, we performed a direct comparison of the proteome of fruits that were subjected to the two postharvest treatments tested, without artificial inoculation with *B. cinerea*. Using this approach, we determined differences in the protein abundance imposed by 1-MCP and ozone in the absence of pathogen that could be associated with the observed phenotypic response to *B. cinerea*. Data indicated that ozone induced a stronger alteration in non-inoculated fruit proteome compared with protein changes caused by 1-MCP. Such as greater protein impact of ozone treatment compared with that imposed by 1-MCP application has been described in kiwifruit

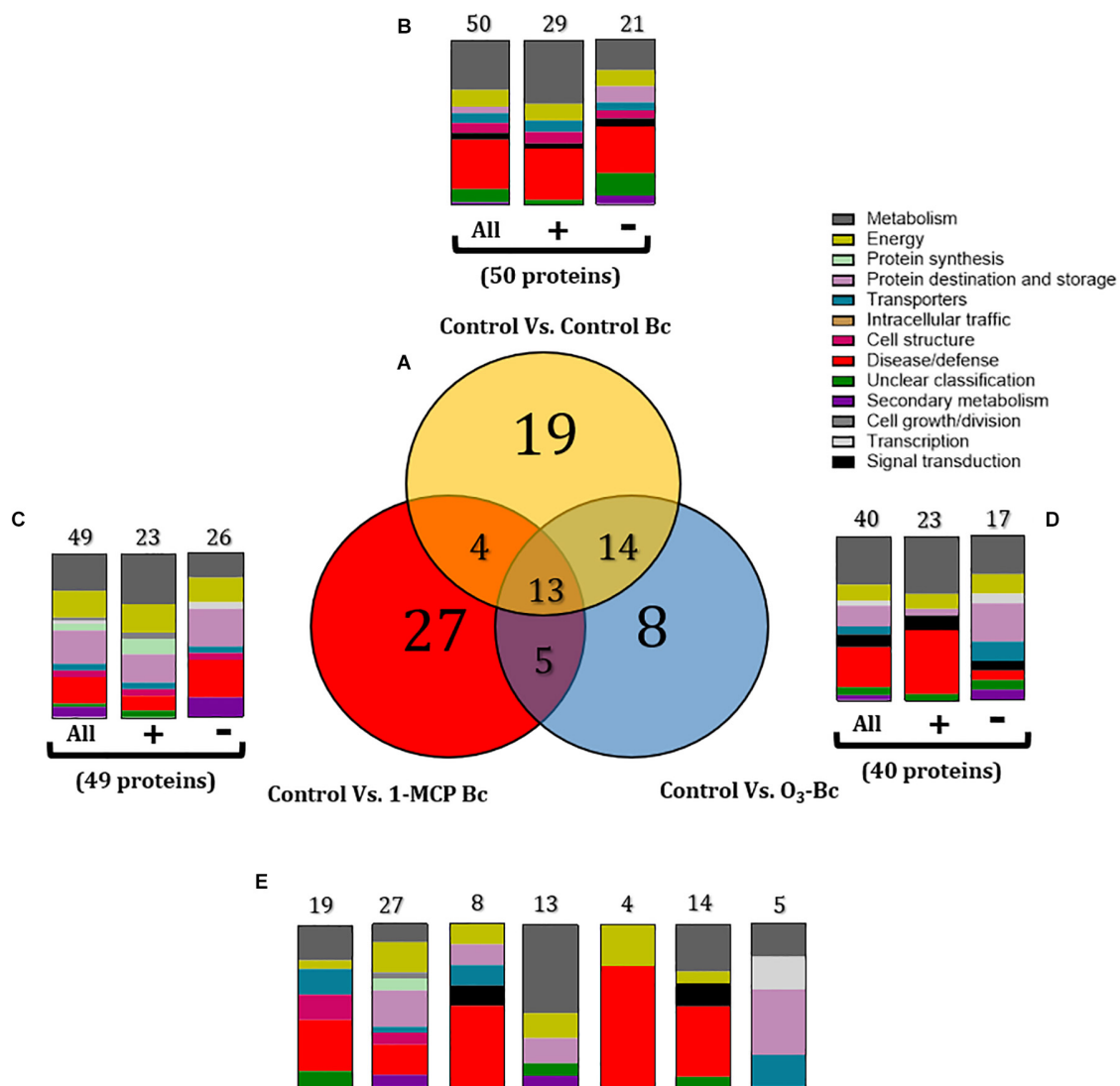


FIGURE 6 | (A) Venn diagram demonstrating the common and distinct differentially expressed proteins of 1-methylcyclopropene (1-MCP) and O₃ application on apple proteome after 60 days of cold storage [1°C, relative humidity (RH) 95%]. Graphical representation of the functional classification of differentially expressed proteins in **(B)** Apple fruits inoculated with *Botrytis cinerea*, **(C)** Apple fruits treated with 1-MCP and inoculated with *B. cinerea*, and **(D)** Apple fruits inoculated with *B. cinerea* and incubated in O₃ enriched-atmosphere chamber (0.3 μl L⁻¹, 1°C, RH 95%) and **(E)** Functional categorization of the unique and overlapping proteins presented in the Venn diagram **(A)**. Symbols (+) and (-) present the up- and downregulated proteins on each treatment. Control: untreated and non-inoculated fruits. Control Bc: untreated, inoculated with *B. cinerea* fruits. 1-MCP Bc: 1-MCP-treated fruits, inoculated with *B. cinerea*. O₃ Bc: O₃-treated fruits, inoculated with *B. cinerea*.

(Minas et al., 2018). Interestingly, most of the changed ozone-responsive proteins were found to be up-accumulated, while, in contrast, most of the proteins changed by 1-MCP were found to be down-accumulated. The majority of the upregulated proteins in the O₃-treated fruits were primarily involved in disease/defense. The obvious absence of defense-related proteins triggering by 1-MCP may explain, therefore, its failure to provide an efficient protection against *B. cinerea*. A similar down-accumulation of defense proteins, such as the PR proteins, has been observed in 1-MCP-treated kiwifruit (Minas et al., 2018) or banana fruits (Kesari et al., 2010). In contrast, in O₃-treated fruits, both in the absence and in the presence of the pathogen,

an upregulation of proteins belonging to the groups of allergen and major allergens was observed.

PR proteins are among the most widespread allergen proteins associated with plants, and they are, currently, organized in 17 distinct families. PR proteins are mainly induced by plant pathogens, but in addition, they can be synthesized in response to abiotic factors such as wounding (Buron-Moles et al., 2014; Sinha et al., 2014). Since ozone is a strong oxidative agent, it is possible that fruit exposure to it may induce the observed upregulation of PR proteins. In apple fruits, allergen proteins, belonging to diverse PR protein families, have been associated with fruit resistance to several fungal and bacterial pathogens, including

B. cinerea (Krebitz et al., 2003; Beuning et al., 2004; Ballester et al., 2017; Banani et al., 2018). For instance, a thaumatin-like (PR-5) protein and a class III chitinase (PR-8) were associated with apple fruit resistance to *B. cinerea* (Banani et al., 2018). Similarly, three Mal d 1 proteins (PR-10 family proteins) were found to be induced in apple fruits in response to stress imposed by abiotic factors such as wounding and biotic factors such as the apple pathogen *P. expansum* or the non-apple pathogen *Penicillium digitatum* (Buron-Moles et al., 2015). Interestingly, some of these were upregulated by both *Penicillium* spp., while for at least one of them, a higher induction rate was observed in fruit challenged with the apple non-pathogen *P. digitatum*, providing evidence for its role in the resistance of apple fruits to *P. digitatum* (Buron-Moles et al., 2015). In another study aimed to determine the proteome profile of apple varieties susceptible and resistant to *Alternaria alternata*, another necrotrophic pathogen of apple, two major allergens, a glucanase (PR-2 family), and a thaumatin-like protein (PR-5 family) were associated with resistance to this disease (Ni et al., 2017). In addition to apple fruits, allergen and major allergen PR proteins have been found to play a major role in the tolerance to *B. cinerea* infections on several other hosts such as strawberry or grape (Monteiro et al., 2003; Haile et al., 2019). Taking into account the variability and complexity that exist within the group of allergen and major allergen proteins of apple fruits, further studies are required to determine precisely the role of each of them in the response of apple fruits to the stress imposed by *B. cinerea* or other spoilage agents and to understand how they regulate the defense responses of the host.

Dynamic changes in fruit proteome were observed in the presence of *B. cinerea* in all the types of fruits tested (control, 1-MCP treated, and O₃ treated). A similar strong modulation of host proteome by pathogen infection has been described in numerous previous reports including the interaction of *B. cinerea* with tomato (Tzortzakakis, 2019). Interestingly, in O₃-treated fruits, a significant number of disease/defense-related proteins were upregulated in comparison with the control fruits. Among these disease/defense-related proteins, the higher accumulation levels were observed for allergen, major allergen, putative nucleotide-binding site leucine-rich repeat (NBS-LRR) disease resistance protein, major latex protein (MLP)-like protein, and 2-Cys peroxiredoxin (2-Cys Prx). All these proteins have been found to play a significant role in the interactions of several plant species including apple with biotic stress factors, and most of them are induced by the ethylene signaling pathway. The critical role of ethylene in the initiation of defense responses of apple fruits against *B. cinerea* had previously been determined (Akagi et al., 2011). Herein, ACO, which catalyzes the last steps of ethylene biosynthesis, was induced by *B. cinerea* in the O₃-treated fruits, while it remained unaffected by *B. cinerea* in 1-MCP-treated fruits. Moreover, ACO was down-accumulated in non-inoculated fruits that were treated with 1-MCP or exposed to ozone, confirming previous observations (Minas et al., 2018). A similar down-accumulation of ACO was observed in O₃-exposed tomato fruits (Tzortzakakis et al., 2013). However, on the same fruits, ACO was also found to be suppressed in O₃-treated fruits challenged with *B. cinerea* (Tzortzakakis et al., 2013; Tzortzakakis, 2019). Our data suggest that ACO was up-accumulated by the pathogen's

presence only in O₃-treated apple fruits. Thus, the observed relative resistance of ozone-exposed fruits to *B. cinerea* infections may be explained by the induction of ethylene signaling that would initiate defense responses.

The current proteomic analysis further indicated that 2-Cys Prx was specifically up-accumulated in the O₃-exposed fruits that had been inoculated with *B. cinerea*. 2-Cys Prx is a distinct subgroup within the family of peroxiredoxins (PRXs) that are associated with resistance of plants to both biotic and abiotic stress, while in apple fruits, their downregulation has been linked to *A. alternata* susceptibility (Borges et al., 2013; Ni et al., 2017; Ojeda et al., 2018). Given that PRXs are highly conserved antioxidant proteins that scavenge reactive oxygen species (ROS) (Zhang et al., 2019), it can be hypothesized that the increased abundance of 2-Cys Prx in O₃-treated fruit reduces ROS level in the host and protects apple cells from the pathogen invasion. It is well established that production of ROS is one of the earliest response of plant tissues to pathogen attacks (Lamb and Dixon, 1997). However, this defense response is primarily effective against biotrophic pathogens, while necrotrophic pathogens such as *B. cinerea* may take advantage in the infection process of host tissues by the generation of ROS produced by the host or even *B. cinerea* itself (Govrin and Levine, 2000; Heller and Tudzynski, 2011). In our study, several ROS-related proteins such as PRX, glutathione peroxidase 8 isoform, oxygen-evolving enhancer protein, and glutathione S-transferase were depressed in 1-MCP-treated fruits that were challenged with *B. cinerea*. These proteins are part of the antioxidant system of the plants participating in their redox homeostasis by suppressing ROS accumulation (Ozyigit et al., 2016; Gullner et al., 2018), while many of them are considered as ripening-induced proteins in apple fruits and other commodities (Nishitani et al., 2010; Zheng et al., 2013; Shi et al., 2014). The observed down-accumulation of these proteins in apple exposed to both 1-MCP and *B. cinerea* suggests that high ROS accumulation occurs in fruits, which facilitates pathogen growth and contributes to the increased susceptibility of 1-MCP-treated apple to pathogen.

The upregulation of the NBS-LRR protein in the O₃-treated fruits in the presence of *B. cinerea* was another interesting finding of this study. Several putative NBS-LRR proteins have been identified from the whole genome data set of apple, although functional characterization was not conducted (Arya et al., 2014). The NBS-LRR proteins are considered R gene products, and their overexpression is usually associated with resistance to biotrophic or hemibiotrophic pathogens (Galli et al., 2010). However, recently, it was demonstrated that an NBS-LRR protein confers resistance of wheat to *Rhizoctonia cerealis* (Zhu et al., 2017). An induction of an NBS-LRR protein encoding gene was observed in ripe strawberry fruits inoculated by *B. cinerea*, while the same gene was found to be downregulated in unripe and pathogen-tolerant fruits (Haile et al., 2019). Therefore, taking into account that NBS-LRR proteins are involved in hypersensitive responses at the site of pathogen entry, it was proposed that their upregulation may assist *B. cinerea* in infecting the ripe strawberry fruits (Haile et al., 2019). Whether a similar response occurs in O₃-treated fruits challenged with *B. cinerea* remains to be further investigated.

The last defense-associated protein found to be upregulated in the O₃-exposed fruits inoculated with *B. cinerea* was the MLP. MLP and the orthologs of MLP (MLP-like protein) belong to the PR10 family and have been found in several plant species, including apple, and most of them are expressed during fruit ripening (He et al., 2020; Song et al., 2020). Although the precise molecular basis of protection of plants by MLPs against plant pathogens currently is unclear, evidence has been provided for their induction on several plant hosts against a wide range of plant pathogens such as fungi, viruses, and phytoplasmas (Segarra et al., 2013; Yang et al., 2015; Gai et al., 2018; Song et al., 2020).

In the current study, we focused on the determination of dynamic changes in apple fruit proteome in response to 1-MCP or ozone treatments. However, the same treatments may also affect the proteome and the secretome of the fungus itself. It is already known from previous studies that the biochemical characteristics of the fungus growth substrate may strongly influence its secretome that plays a crucial role in its pathogenicity (Shah et al., 2009; Li et al., 2012). For instance, it has been shown that in host tissues of lower pH, the fungus preferentially secretes proteins associated with proteolysis rather than cell wall-degrading enzymes (CWDEs), while in host tissues of higher pH, CWDEs are preferentially secreted by *B. cinerea* (Li et al., 2012). Future research on the role of the tested postharvest treatments on the differential induction of fungus secretome could provide further explanations for the observed tolerance of O₃-treated apple fruits to gray mold.

CONCLUSION

Data presented in the current study represent a first attempt to investigate the impact of 1-MCP and ozone applications on the development of *Botrytis cinerea*, a major agent of postharvest decay of “Granny Smith” apple fruit. Although 1-MCP was not able to affect the *B. cinerea* development, ozone treatment effectively suppressed the disease severity. Proteomic analysis has allowed the detection of various apple fruit proteins and their specific accumulation pattern in response to 1-MCP and ozone treatments in the presence and the absence of *B. cinerea*, thus allowing an identification of differentially accumulated proteins

in both types of interactions. Fruits treated with ozone exhibited larger proteome programming than 1-MCP exposed apples and possessed a more sensitive defense system when the fruits were challenged with subsequent *B. cinerea* infection. The current study provides a deeper understanding of the interactions among 1-MCP or ozone treatments and *B. cinerea* in apple fruits and underlines the mechanism of action of the two postharvest applications against gray mold.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

GT, IM, AM, and GK designed the experiments. ST performed the experiments. ST, MS, and GT performed analysis and interpretation of the data. ST and GK drafted the manuscript. IM, AM, GT, and GK revised the manuscript. All authors revised the manuscript and approved the final version to be published.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2021.644255/full#supplementary-material>

REFERENCES

- Ainalidou, A., Tanou, G., Belghazi, M., Samiotaki, M., Diamantidis, G., Molassiotis, A., et al. (2016). Integrated analysis of metabolites and proteins reveal aspects of the tissue-specific function of synthetic cytokinin in kiwifruit development and ripening. *J. Proteomics* 143, 318–333. doi: 10.1016/j.jprot.2016.02.013
- Akagi, A., Dandekar, A. M., and Stotz, H. U. (2011). Resistance of *Malus domestica* fruit to *Botrytis cinerea* depends on endogenous ethylene biosynthesis. *Phytopathology* 101, 1311–1321. doi: 10.1094/PHYTO-03-11-0087
- Akagi, A., and Stotz, H. U. (2007). Effects of pathogen polygalacturonase, ethylene, and firmness on interactions between pear fruits and *Botrytis cinerea*. *Plant Dis.* 91, 1337–1344. doi: 10.1094/PDIS-91-10-1337
- Alkan, N., Espeso, E. A., and Prusky, D. (2013). Virulence regulation of phytopathogenic fungi by pH. *Antioxid. Redox Signal.* 19, 1012–1025. doi: 10.1089/ars.2012.5062
- Antos, P., Piechowicz, B., Gorzelany, J., Matlok, N., Migut, D., Józefczyk, R., et al. (2018). Effect of ozone on fruit quality and fungicide residue degradation in apples during cold storage. *Ozone Sci. Eng.* 40, 482–486. doi: 10.1080/01919512.2018.1471389
- Arya, P., Kumar, G., Acharya, V., and Singh, A. K. (2014). Genome-wide identification and expression analysis of NBS-encoding genes in *Malus x domestica* and expansion of NBS genes family in Rosaceae. *PLoS One* 9:9e107987. doi: 10.1371/journal.pone.0107987
- Ballester, A.-R., Norelli, J., Burchard, E., Abdelfattah, A., Levin, E., González-Candelas, L., et al. (2017). Transcriptomic response of resistant (PI613981–*Malus sieversii*) and susceptible (“Royal Gala”) genotypes of apple to Blue Mold (*Penicillium expansum*) infection. *Front. Plant Sci.* 8:1981. doi: 10.3389/fpls.2017.01981
- Banani, H., Olivieri, L., Santoro, K., Garibaldi, A., Gullino, M. L., and Spadaro, D. (2018). Thyme and savory essential oil efficacy and induction of resistance

- against *Botrytis cinerea* through priming of defense responses in apple. *Foods* 7:11. doi: 10.3390/foods7020011
- Beuning, L., Bowen, J., Persson, H., Barraclough, D., Bulley, S., and MacRae, E. (2004). Characterisation of Mal d 1-related genes in *Malus*. *Plant Mol. Biol.* 55, 369–388. doi: 10.1007/s11103-004-0904-9
- Blanco-Ulate, B., Morales-Cruz, A., Amrine, K. C. H., Labavitch, J. M., Powell, A. L. T., and Cantu, D. (2014). Genome-wide transcriptional profiling of *Botrytis cinerea* genes targeting plant cell walls during infections of different hosts. *Front. Plant Sci.* 5:435. doi: 10.3389/fpls.2014.00435
- Borges, L. L., Santana, F. A., Castro, I. S. L., Arruda, K. M. A., Ramos, H. J. d. O., Moreira, M. A., et al. (2013). Differentially expressed proteins during an incompatible interaction between common bean and the fungus *Pseudocercospora griseola*. *Mol. Breed.* 32, 933–942. doi: 10.1007/s11032-013-9922-0
- Buron-Moles, G., Torres, R., Amoako-Andoh, F., Viñas, I., Teixidó, N., Usall, J., et al. (2014). Analysis of changes in protein abundance after wounding in 'Golden Delicious' apples. *Postharvest Biol. Technol.* 87, 51–60. doi: 10.1016/j.postharvbio.2013.07.039
- Buron-Moles, G., Wisniewski, M., Viñas, I., Teixidó, N., Usall, J., Droby, S., et al. (2015). Characterizing the proteome and oxi-proteome of apple in response to a host (*Penicillium expansum*) and a non-host (*Penicillium digitatum*) pathogen. *J. Proteomics* 114, 136–151. doi: 10.1016/j.jprot.2014.11.007
- Díaz, J., ten Have, A., and van Kan, J. A. (2002). The role of ethylene and wound signaling in resistance of tomato to *Botrytis cinerea*. *Plant Physiol.* 129, 1341–1351. doi: 10.1104/pp.001453
- FAO (2018). *Food and Agriculture Organization of the United Nations. FAOSTAT Statistical Database*. Available online at: <http://www.fao.org/faostat/en/#data/QC> (accessed October 15, 2020).
- Gai, Y. P., Yuan, S. S., Liu, Z. Y., Zhao, H. N., Liu, Q., Qin, R. L., et al. (2018). Integrated phloem sap mRNA and protein expression analysis reveals Phytoplasma-infection responses in mulberry. *Mol. Cell. Proteomics* 17, 1702–1719. doi: 10.1074/mcp.RA118.000670
- Galli, P., Patocchi, A., Brogini, G. A. L., and Gessler, C. (2010). The Rvi15 (Vr2) apple scab resistance locus contains three TIR-NBS-LRR genes. *Mol. Plant Microbe Interact.* 23, 608–617. doi: 10.1094/MPMI-23-5-0608
- González-Fernández, R., Valero-Galván, J., Gómez-Gálvez, F. J., and Jorrín-Novo, J. V. (2015). Unraveling the in vitro secretome of the phytopathogen *Botrytis cinerea* to understand the interaction with its hosts. *Front. Plant Sci.* 6:839. doi: 10.3389/fpls.2015.00839
- Govrin, E. M., and Levine, A. (2000). The hypersensitive response facilitates plant infection by the necrotrophic pathogen *Botrytis cinerea*. *Curr. Biol.* 10, 751–757. doi: 10.1016/S0960-9822(00)00560-1
- Gullner, G., Komives, T., Király, L., and Schröder, P. (2018). Glutathione S-transferase enzymes in plant-pathogen interactions. *Front. Plant Sci.* 9:1836. doi: 10.3389/fpls.2018.01836
- Haile, Z. M., Nagpala-De Guzman, E. G., Moretto, M., Sonogo, 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.* 10:1131. doi: 10.3389/fpls.2019.01131
- He, S., Yuan, G., Bian, S., Han, X., Liu, K., Cong, P., et al. (2020). Major latex protein MdMLP423 negatively regulates defense against fungal infections in apple. *Int. J. Mol. Sci.* 21:1879. doi: 10.3390/ijms21051879
- Heller, J., and Tudzynski, P. (2011). Reactive oxygen species in phytopathogenic fungi: signaling, development, and disease. *Annu. Rev. Phytopathol.* 49, 369–390. doi: 10.1146/annurev-phyto-072910-095355
- Janisiewicz, W. J., Leverentz, B., Conway, W. S., Saftner, R. A., Reed, A. N., and Camp, M. J. (2003). Control of bitter rot and blue mold of apples by integrating heat and antagonist treatments on 1-MCP treated fruit stored under controlled atmosphere conditions. *Postharvest Biol. Technol.* 29, 129–143. doi: 10.1016/S0925-5214(03)00040-1
- Jijakli, M. H., and Lepoivre, P. (2004). "State of the art and challenges of post-harvest disease management in apples in Mukerji," in *The Fruit and Vegetable Diseases. Disease Management of Fruits and Vegetables*, ed. K. G. Mukerji (Dordrecht: Kluwer Academic Publishers), 59–94.
- Juhnveica-Radenkova, K., Radenkova, V., Kundzins, K., and Seglina, D. (2019). Effect of ozone treatment on the microstructure, chemical composition and sensory quality of apple fruits. *Food Sci. Technol. Int.* 25, 252–267. doi: 10.1177/1082013218815285
- Karaca, H., and Velioglu, S. Y. (2007). Ozone applications in fruit and vegetable processing. *Food Rev. Int.* 23, 91–106. doi: 10.1080/87559120600998221
- Karagiannis, E., Michailidis, M., Tanou, G., Samiotaki, M., Karamanolis, K., Avramidou, E., et al. (2018). Ethylene-dependent and -independent superficial scald resistance mechanisms in 'Granny Smith' apple fruit. *Sci. Rep.* 8:11436. doi: 10.1038/s41598-018-29706-x
- Karagiannis, E., Tanou, G., Samiotaki, M., Michailidis, M., Diamantidis, G., Minas, I. S., et al. (2016). Comparative physiological and proteomic analysis reveal distinct regulation of peach skin quality traits by altitude. *Front. Plant Sci.* 7:1689. doi: 10.3389/fpls.2016.01689
- Karagiannis, E., Tanou, G., Scossa, F., Samiotaki, M., Michailidis, M., Manioudaki, M., et al. (2020). Systems-Based approaches to unravel networks and individual elements involved in apple superficial scald. *Front. Plant Sci.* 11:8. doi: 10.3389/fpls.2020.00008
- Kesari, R., Trivedi, P. K., and Nath, P. (2010). Gene expression of pathogenesis-related protein during banana ripening and after treatment with 1-MCP. *Postharvest Biol. Technol.* 56, 64–70. doi: 10.1016/j.postharvbio.2009.11.012
- Kolnias-Ostek, J., Wojdylo, A., Markowski, J., and Siucinska, K. (2014). 1-Methylcyclopropene postharvest treatment and their effect on apple quality during long-term storage time. *Eur. Food Res Technol.* 239, 603–612. doi: 10.1007/s00217-014-2256-2
- Konstantinou, S., Karaoglaniadis, G. S., Bardas, G. A., Minas, I. S., Doukas, E., and Markoglou, A. N. (2011). Postharvest fruit rots of apple in Greece: pathogen incidence and relationships between fruit quality parameters, cultivar susceptibility, and patulin production. *Plant Dis.* 95, 666–672. doi: 10.1094/PDIS-11-10-0856
- Krebitz, M., Wagner, B., Ferreira, F., Peterbauer, C., Campillo, N., Witty, M., et al. (2003). Plant-based heterologous expression of Mal d 2, a thaumatin-like protein and allergen of apple (*Malus domestica*), and its characterization as an antifungal protein. *J. Mol. Biol.* 329, 721–730. doi: 10.1016/S0022-2836(03)00403-0
- Lamb, C., and Dixon, R. A. (1997). The oxidative burst in plant disease resistance. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48, 251–275. doi: 10.1146/annurev.arplant.48.1.251
- Li, B., Wang, W., Zong, Y., Qin, G., and Tian, S. (2012). Exploring pathogenic mechanisms of *Botrytis cinerea* secretome under different ambient pH based on comparative proteomic analysis. *J. Proteome Res.* 11, 4249–4260. doi: 10.1021/pr300365f
- Li, L., Lichter, A., Chalupowicz, D., Gamrasni, D., Goldberg, T., Nerya, O., et al. (2016). Effects of the ethylene-action inhibitor 1-methylcyclopropene on postharvest quality of non-climacteric fruit crops. *Postharvest Biol. Technol.* 111, 322–329. doi: 10.1016/j.postharvbio.2015.09.031
- Liñero, E., Chiva, C., Cantoral, J. M., Sabido, E., and Fernández-Acero, F. J. (2016). Phosphoproteome analysis of *B. cinerea* in response to different plant-based elicitors. *J. Proteomics* 139, 84–94. doi: 10.1016/j.jprot.2016.03.019
- Lv, Y., Tahir, I. I., and Olsson, M. E. (2019). Effect of ozone application on bioactive compounds of apple fruit during short-term storage. *Sci. Hortic.* 253, 49–60. doi: 10.1016/j.scienta.2019.04.021
- Ma, L., He, J., Liu, H., and Zhou, H. (2018). The phenylpropanoid pathway affects apple fruit resistance to *Botrytis cinerea*. *J. Phytopathol.* 166, 206–215. doi: 10.1111/JPH.12677
- Marcos, J. F., González-Candelas, L., and Zacarias, L. (2005). Involvement of ethylene biosynthesis and perception in the susceptibility of citrus fruits to *Penicillium digitatum* infection and the accumulation of defence-related mRNAs. *J. Exp. Bot.* 56, 2183–2193. doi: 10.1093/jxb/eri218
- Min, D., Li, F., Zhang, X., Shu, P., Cui, X., Dong, L., et al. (2018). Effect of methyl salicylate in combination with 1-methylcyclopropene on postharvest quality and decay caused by *Botrytis cinerea* in tomato fruit. *J. Sci. Food Agric.* 98, 3815–3822. doi: 10.1002/jsfa.8895
- Minas, I. S., Karaoglaniadis, G. S., Manganaris, G. A., and Vasilakakis, M. (2010). Effect of ozone application during cold storage of the kiwifruit on the development of stem-end rot caused by *Botrytis cinerea*. *Postharvest Biol. Technol.* 58, 203–210. doi: 10.1016/j.postharvbio.2010.07.002
- Minas, I. S., Tanou, G., Karagiannis, E., Belghazi, M., and Molassiotis, A. (2016). Coupling of physiological and proteomic analysis to understand the ethylene- and chilling- Induced kiwifruit ripening syndrome. *Front. Plant Sci.* 7:120. doi: 10.3389/fpls.2016.00120

- Minas, I. S., Tanou, G., Krokida, A., Karagiannis, E., Belghazi, M., Vasilakakis, M., et al. (2018). Ozone-induced inhibition of kiwifruit ripening is amplified by 1-methylcyclopropene and reversed by exogenous ethylene. *BMC Plant Biol.* 18:358. doi: 10.1186/s12870-018-1584-y
- Mir, N. A., Curell, E., Khan, N., Whitaker, M., and Beaudry, R. M. (2001). Harvest maturity, storage temperature, and 1-MCP application frequency alter firmness retention and chlorophyll fluorescence of 'Red chief Delicious' apples. *J. Am. Soc. Hortic. Sci.* 126, 618–624. doi: 10.21273/JASHS.126.5.618
- Monteiro, S., Barakat, M., Piçarra-Pereira, M. A., Teixeira, A. R., and Ferreira, R. B. (2003). Osmotin and thaumatin from grape: a putative general defense mechanism against pathogenic fungi. *Phytopathology* 93, 1505–1512. doi: 10.1094/PHYTO.2003.93.12.1505
- Ni, W., Zhu, L., Sha, R., Tao, J., Cai, B., and Wang, S. (2017). Comparative iTRAQ proteomic profiling of susceptible and resistant apple cultivars infected by *Alternaria alternata* apple pathotype. *Tree Genet. Genomes* 13:23. doi: 10.1007/s11295-017-1104-5
- Nishitani, C., Shimizu, T., Fujii, H., Hosaka, F., Terakami, S., Nakamura, Y., et al. (2010). Oligoarray analysis of gene expression in ripening Japanese pear fruit. *Sci. Hortic.* 124, 195–203. doi: 10.1016/j.scienta.2009.12.032
- Nybom, H., Ahmadi-Afzadi, M., Rumpunen, K., and Tahir, I. (2020). Review of the impact of apple fruit ripening texture and chemical contents on genetically determined susceptibility to storage rots. *Plants* 9:831. doi: 10.3390/plants9070831
- Ojeda, V., Pérez-Ruiz, J. M., and Cejudo, F. J. (2018). 2-Cys peroxiredoxins participate in the oxidation of chloroplast enzymes in the dark. *Mol. Plant* 11, 1377–1388. doi: 10.1016/j.molp.2018.09.005
- Ozyigit, I. I., Filiz, E., Vatansever, R., Kurtoglu, K. Y., Koc, I., Öztürk, M. X., et al. (2016). Identification and comparative analysis of H₂O₂-scavenging enzymes (ascorbate peroxidase and glutathione peroxidase) in selected plants employing bioinformatics approaches. *Front. Plant Sci.* 7:301. doi: 10.3389/fpls.2016.00301
- Palou, L., Crisosto, C. H., Smilanick, J. L., Adaskaveg, J. E., and Zoffoli, J. P. (2002). Effects of continuous 0.3 ppm ozone exposure on decay development and physiological responses of peaches and table grapes in cold storage. *Postharvest Biol. Technol.* 24, 39–48.
- Papavasileiou, A., Tanou, G., Samaras, A., Samiotaki, M., Molassiotis, A., and Karaoglaniadis, G. S. (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
- Rasclé, C., Dieryckx, C., Dupuy, J. W., Muszkieta, L., Souibgui, E., Droux, M., et al. (2018). The pH regulator PacC: a host-dependent virulence factor in *Botrytis cinerea*. *Environ. Microbiol. Rep.* 10, 555–568. doi: 10.1111/1758-2229.12663
- Segarra, G., Santpere, G., Elena, G., and Trillas, I. (2013). Enhanced *Botrytis cinerea* resistance of Arabidopsis plants grown in compost may be explained by increased expression of defense-related genes, as revealed by microarray analysis. *PLoS One* 8:e56075. doi: 10.1371/journal.pone.0056075
- Seglie, L., Spadaro, D., Trotta, F., Devecchi, M., Gullino, M. L., and Scarlot, V. (2012). Use of 1-methylcyclopropene in cyclodextrin-based nanosponges to control grey mould caused by *Botrytis cinerea* on *Dianthus caryophyllus* cut flowers. *Postharvest Biol. Technol.* 64, 55–57. doi: 10.1016/j.postharvbio.2011.09.014
- Shah, P., Atwood, J. A., Orlando, R., El Mubarek, H., Podila, G. K., and Davis, M. R. (2009). Comparative proteomic analysis of *Botrytis cinerea* secretome. *J. Proteome Res.* 8, 1123–1130. doi: 10.1021/pr8003002
- Shi, Y., Jiang, L., Zhang, L., Kang, R., and Yu, Z. (2014). Dynamic changes in proteins during apple (*Malus x domestica*) fruit ripening and storage. *Hortic. Res.* 1:6. doi: 10.1038/hortres.2014.6
- Sinha, M., Singh, R. P., Kushwaha, G. S., Iqbal, N., Singh, A., Kaushik, S., et al. (2014). Current overview of allergens of plant pathogenesis related protein families. *Sci. World J.* 2014:543195. doi: 10.1155/2014/543195
- Song, L., Wang, J., Jia, H., Kamran, A., Qin, Y., Liu, Y., et al. (2020). Identification and functional characterization of NbMLP28, a novel MLP-like protein 28 enhancing Potato virus Y resistance in *Nicotiana benthamiana*. *BMC Microbiol.* 20:55. doi: 10.1186/s12866-020-01725-7
- Spotts, R. A., Cervantes, L. A., and Mielke, E. A. (1999). Variability in postharvest decay among apple cultivars. *Plant Dis.* 83, 1051–1054. doi: 10.1094/PDIS.1999.83.11.1051
- Tanou, G., Minas, I. S., Karagiannis, E., Tsikou, D., Audebert, S., Papadopoulou, K. K., et al. (2015). The impact of sodium nitroprusside and ozone in kiwifruit ripening physiology: a combined gene and protein expression profiling approach. *Ann. Bot.* 116, 649–662. doi: 10.1093/aob/mcv107
- The EU Arabidopsis Genome Project, Bevan, M., Bancroft, I., Bent, E., Love, K., Goodman, H., et al. (1998). Analysis of 1.9 Mb of contiguous sequence from chromosome 4 of *Arabidopsis thaliana*. *Nature* 391, 485–488. doi: 10.1038/35140
- Tzortzakakis, N. (2019). Physiological and proteomic approaches to address the active role of *Botrytis cinerea* inoculation in tomato postharvest ripening. *Microorganisms* 7:681. doi: 10.3390/microorganisms7120681
- Tzortzakakis, N., Taybi, T., Antony, E., Singleton, I., Borland, A., and Barnes, J. (2013). Profiling shifts in protein complement in tomato fruit induced by atmospheric ozone-enrichment and/or wound-inoculation with *Botrytis cinerea*. *Postharvest Biol. Technol.* 78, 67–75. doi: 10.1016/j.postharvbio.2012.12.005
- Wang, X., Kobiler, I., Lichter, A., Leikin-Frenkel, A., Pesis, E., and Prusky, D. (2006). 1-MCP prevents ethylene-induced accumulation of antifungal diene in avocado fruit. *Physiol. Mol. Plant Pathol.* 67, 261–267. doi: 10.1016/j.pmpp.2006.03.002
- Yang, C.-L., Liang, S., Wang, H.-Y., Han, L.-B., Wang, F.-X., Cheng, H.-Q., et al. (2015). Cotton major latex protein 28 functions as a positive regulator of the ethylene responsive factor 6 in defense against *Verticillium dahliae*. *Mol. Plant* 8, 399–411. doi: 10.1016/j.molp.2014.11.023
- Yaseen, T., Ricelli, A., Turan, B., Albanese, P., and D'Onghia, A. M. (2015). Ozone for post-harvest treatment of apple fruits. *Phytopathol. Mediterr.* 54, 94–103.
- Zhang, H.-H., Xu, N., Teng, Z.-Y., Wang, J.-R., Ma, S., Wu, X., et al. (2019). 2-Cys Prx plays a critical role in scavenging H₂O₂ and protecting photosynthetic function in leaves of tobacco seedlings under drought stress. *J. Plant Interact.* 14, 119–128. doi: 10.1080/17429145.2018.1562111
- Zheng, Q., Song, J., Campbell-Palmer, L., Thompson, K., Li, L., Walker, B., et al. (2013). A proteomic investigation of apple fruit during ripening and in response to ethylene treatment. *J. Proteomics* 93, 276–294. doi: 10.1016/j.jprot.2013.02.006
- Zhu, X., Lu, C., Du, L., Ye, X., Liu, X., Coules, A., et al. (2017). The wheat NB-LRR gene TaRCR1 is required for host defence response to the necrotrophic fungal pathogen *Rhizoctonia cerealis*. *J. Plant Biotechnol.* 15, 674–687. doi: 10.1111/pbi.12665

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.

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Table S1A. Identification details of differentiable proteins in the mesocarp of untreated, 1-MCP and O₃ treated apples (cv. Granny Smith) inoculated/or not with the pathogen *B. cinerea* after of 60 days of cold storage.

Spot N° ^a	Accession Number ^b	Suggested Name ^c	Matching criteria ^d	Organism ^e	Subcellular Localization ^f	Functional category ^g	Score	Coverage	# Proteins	# Unique Peptides	# Peptides	# PSMs	# AAs	MW [kDa]	calc. pI	Peptide confidence	Sequence	# PSMs	# Proteins	# Protein Groups	Protein Group Accessions	Modifications	ΔCn	XCorr	Charge	MH+ [Da]	ΔM [ppm]	RT [min]	# Missed Cleavages
23	gi 57976500	Actin		<i>Malus domestica</i>	Cytoskeleton	09.04-Cell structure/Cytoskeleton	37.42	15.12	10	2	6	28	377	41.7	5.49	High	VAPEEHPVLLTEA PLNPK	16	7	1	657976500		0.00	3.82	3	1954.07	0.59	42.01	0
																High	AGFAGDDAPR	3	10	1	657976500		0.00	3.41	2	976.45	-0.19	37.96	0
																Low	GYSFTTTAER	5	8	1	657976500		0.00	2.36	2	1132.53	-0.05	40.02	0
																Low	CDVDIRK	1	10	1	657976500	C1(Carbamidomethyl)	0.03	1.36	2	905.45	-0.34	34.86	1
																Low	LDLAGR	1	10	1	657976500		0.00	1.29	2	644.37	-0.05	38.77	0
																Low	GILTLK	2	10	1	657976500		0.00	0.85	2	644.43	0.31	10.37	0
317	gi 57963783	Plastoglobulin	100% to Prunus mume gi 645231139 E-value:0.0	<i>Malus domestica</i>	Chloroplastic	02.07-Energy/Pentose phosphate	5.44	5.63	1	1	2	2	355	38.0	4.83	High	GDGGLFVLAR	1	1	1	657963783		0.00	3.31	2	1004.55	-0.07	43.00	0
																Low	EGSFQPPEIK	1	1	1	657963783		0.00	2.13	2	1131.57	0.04	38.71	0
923	gi 58013978	Restin		<i>Malus domestica</i>	Nucleus	20.1-Secondary metabolism/Phenylpropanoids/Phenolics	146.30	38.32	2	22	56	113	1378	154.4	4.70	High	DGSHAAETALVE DDKPSVIER	1	1	1	658013978		0.00	3.68	3	2239.09	0.96	39.82	0
																High	LQEALGSFTNR	2	1	1	658013978		0.00	3.67	2	1235.64	0.74	41.93	0
																High	LTEDVSTYESK	2	2	1	658013978		0.00	3.55	2	1271.60	0.73	38.86	0
																High	LQEQTTEAEEK	2	2	1	658013978		0.00	3.30	2	1317.65	0.23	37.44	0
																High	LKSAAEQLEQQGK	3	2	1	658013978		0.00	3.29	3	1487.77	0.06	36.71	1
																High	TSSLEVALQAANEK	2	1	1	658013978		0.00	3.17	2	1460.76	0.68	43.40	0
																High	AVAAQENLAIVTK	2	1	1	658013978		0.00	3.15	2	1327.76	0.68	41.28	0
																High	SAEEQLEQQGK	3	2	1	658013978	Q5(Deamidated)	0.00	3.12	2	1247.58	1.03	36.96	0
																High	LSDLEVEVER	1	1	1	658013978		0.00	3.12	2	1188.61	0.45	41.29	0
																High	ELFEALNVATEEK	1	2	1	658013978		0.00	3.09	2	1492.75	1.27	45.58	0
																High	LEDASSSFTEK	2	2	1	658013978		0.00	3.03	2	1213.56	0.35	38.97	0
																High	LNALQGQVK	2	1	1	658013978	Q5(Deamidated)	0.00	2.91	2	971.55	0.23	38.91	0
																High	QLTGQVQYQEK	2	2	1	658013978		0.00	2.91	2	1450.72	0.95	38.26	0
																High	GDGGSVAGLEVK	1	2	1	658013978		0.00	2.89	2	1088.56	-0.02	39.24	0
																High	NAELESLSK	2	2	1	658013978		0.00	2.85	2	1119.55	0.47	39.43	0
																High	DLEANELLEK	2	1	1	658013978		0.00	2.69	2	1173.60	0.28	43.78	0
																High	FASSESTNEELR	1	1	1	658013978		0.00	2.51	2	1369.62	0.64	37.36	0
																Medium	RLEDASSSFTEK	2	2	1	658013978		0.00	2.48	2	1369.66	0.63	38.09	1
																Medium	VSELEEIK	1	2	1	658013978		0.00	2.46	2	946.51	-0.06	40.06	0
																Medium	ESETAFATATEK	2	1	1	658013978		0.00	2.44	2	1284.60	0.89	39.12	0
																Medium	DAEANSLEK	2	2	1	658013978		0.00	2.43	2	1089.54	1.55	40.00	0
																Medium	ALDLHSASEAR	3	1	1	658013978		0.00	2.43	2	1169.59	0.22	37.39	0
																Low	SAEEQLEQQGK	1	2	1	658013978		0.00	2.33	2	1246.59	0.25	35.86	0
																Low	DIGSTVSTPSK	2	1	1	658013978		0.00	2.25	2	1091.56	0.31	38.21	0
																Low	DLLNEQVTK	1	2	1	658013978		0.00	2.25	2	1059.57	0.05	41.00	0
																Low	LNALQGQVK	2	1	1	658013978		0.00	2.25	2	970.57	0.05	38.65	0
																Low	VSNGEVPPVEK	1	1	1	658013978	N3(Deamidated)	0.00	2.22	2	1155.59	1.82	37.30	0
																Low	KDDLSSQSLNNNAELEQK	1	1	1	658013978		0.00	2.17	3	2032.02	0.87	41.96	1
																Low	NSELQALHETLVR	3	1	1	658013978		0.00	2.14	2	1509.80	1.14	42.60	0
																Low	LQEQTTEAEEK	2	2	1	658013978	Q4(Deamidated)	0.00	2.11	2	1318.64	0.41	38.53	0
																Low	FIAVEQK	1	1	1	658013978		0.00	2.07	2	834.47	0.23	39.19	0
																Low	SASLKEELDNSLAK	3	2	1	658013978		0.00	2.05	2	1504.79	1.25	41.58	1
																Low	TIEDLMQQHSSEGGK	3	1	1	658013978	M6(Oxidation)	0.00	2.02	2	1746.80	1.08	37.33	0
																Low	EKLSDLVEEVER	1	1	1	658013978		0.02	1.98	3	1445.75	-0.50	41.43	1
																Low	NKEIALLDK	3	2	1	658013978		0.00	1.96	2	1043.61	-0.28	39.15	1
																Low	SLEELEAK	2	1	1	658013978		0.00	1.93	2	918.48	-0.21	40.10	0
																Low	LQEAIQR	2	1	1	658013978		0.00	1.91	2	786.45	0.02	37.27	0
																Low	LHSELQLAHATVAEK	3	1	1	658013978		0.00	1.84	3	1646.89	0.09	39.82	0
																Low	LQLADTK	1	1	1	658013978		0.00	1.81	2	788.45	0.23	37.51	0
																Low	STVEELTDKHSR	2	1	1	658013978		0.00	1.79	3	1401.70	-0.15	38.18	1
																Low	VYEEHXAEEAR	3	1	1	658013978	X6(L)	0.00	1.79	2	1345.64	0.02	36.86	0

																		Low	EMEDQMASIQGE LK	2	2	1	658013978	M2(Oxidation); M6(Oxidation)	0.00	1.75	2	1640.72	1.02	38.68	0
																		Low	SQGVLDLEEK	2	1	1	658013978		0.00	1.71	2	1004.49	0.20	37.91	0
																		Low	DETVEQLQAAK	2	2	1	658013978		0.00	1.71	2	1231.62	0.37	38.61	0
																		Low	VSELELMLETEK	1	2	1	658013978	M7(Oxidation)	0.00	1.64	2	1436.72	1.24	42.77	0
																		Low	TSSLEVALQAANE KER	1	1	1	658013978		0.00	1.62	2	1745.91	1.74	41.60	1
																		Low	LYEEQVR	2	2	1	658013978		0.00	1.59	2	936.48	-0.25	37.77	0
																		Low	LKLEENFGK	1	1	1	658013978		0.17	1.59	3	1164.63	0.43	39.09	1
																		Low	FASSESTNEELRK	6	1	1	658013978		0.00	1.57	3	1497.72	-0.01	36.60	1
																		Low	KLQEQITEAEEK	3	2	1	658013978		0.00	1.54	2	1445.75	0.04	38.74	1
																		Low	STVEELTDK	2	1	1	658013978		0.00	1.50	2	1021.51	0.57	39.04	0
																		Low	HSESENSELKHEV LLTK	1	1	1	658013978		0.00	1.48	4	1980.00	-0.19	38.94	1
																		Low	VYEEHXAEAEARK	1	1	1	658013978	X6(L)	0.00	1.41	3	1473.73	0.26	37.25	1
																		Low	SEIENLK	1	1	1	658013978		0.01	1.37	2	832.44	0.04	37.20	0
																		Low	YSTAVVEK	1	2	1	658013978		0.00	1.36	2	896.47	-0.14	36.40	0
																		Low	AEVAEKPLLQNSL K	4	2	1	658013978		0.00	1.36	2	1539.88	0.65	40.35	0
																		Low	ALEFEK	2	2	1	658013978		0.03	1.32	2	736.39	-0.17	40.01	0
																		Low	VSNGEVPPVEK	1	1	1	658013978		0.00	1.32	2	1154.61	0.45	36.40	0
																		Low	VSNGEVPPVEKER	1	1	1	658013978	N3(Deamidated)	0.06	1.29	3	1440.73	-0.02	36.53	1
																		Low	VSELEEIKLK	1	2	1	658013978		0.00	1.04	3	1187.69	0.15	40.49	1
1026	gi657942890	Desiccation protectant protein Lea14 homolog		Malus domestica	Unclear	11.05- Disease/Defense /Stress responses	29.88	28.67	1	4	4	17	150	16.5	5.55			High	VIASGTIPDPGSLK	3	1	1	657942890		0.00	3.83	2	1354.76	1.00	42.39	0
																		High	MGLSHVEYLSK	9	1	1	657942890	M1(Oxidation)	0.00	3.63	2	1279.64	0.88	39.66	0
																		High	ISNVAKPEAEVTD VDFK	3	1	1	657942890		0.00	2.89	3	1861.95	-0.60	40.38	0
																		High	ISNVAKPEAEVTD VDFK	2	1	1	657942890		0.00	2.58	3	1990.05	1.71	39.63	1
1026	gi657991543	Thioredoxin		Malus domestica	Cytosol	02.20- Energy/Electron- transport	18.93	25.60	3	2	4	9	125	13.7	5.55			High	HAAPADVPADVA TASA	2	1	1	657991543		0.00	2.90	2	1463.71	1.08	39.39	0
																		High	KNPEVTFLK	4	3	1	657991543		0.00	2.70	3	1075.61	-0.07	39.82	1
																		Low	VDVDELK	2	3	1	657991543		0.00	2.29	2	817.43	1.12	39.99	0
																		Low	NPEVTFLK	1	3	1	657991543		0.00	1.96	2	947.52	-0.19	42.15	0
1034	gi657999067	2-Cys peroxiredoxin BAS1		Malus domestica	Chloroplast	11.06- Disease/Defense /Detoxification	19.53	17.71	1	1	5	14	271	29.4	7.97			High	EGVIQHSTVNNLA IGR	7	1	1	657999067		0.00	2.85	3	1707.91	0.50	41.16	0
																		Low	GSKEYFSAI	2	1	1	657999067		0.00	1.99	2	1001.49	0.32	43.29	1
																		Low	YPLISDVTK	1	1	1	657999067		0.00	1.85	2	1035.57	-0.05	43.44	0
																		Low	GLFIIDK	2	1	1	657999067		0.00	1.81	2	805.48	0.30	46.00	0
																		Low	LSEYIGK	2	1	1	657999067		0.00	1.54	2	809.44	0.21	39.08	0
1034	gi657999259	Ras-related protein Rab7		Malus domestica	Nucleus	10.04-Signal transduction/Me diators	8.56	27.67	3	2	5	6	206	23.1	5.08			High	TSLMNQYVNK	1	1	1	657999259	M4(Oxidation)	0.00	2.57	2	1213.59	0.50	37.47	0
																		High	VIIIGDSGVGK	2	3	1	657999259		0.00	2.26	2	1057.63	0.27	43.15	0
																		Low	GADCCVLVYDVN SMK	1	1	1	657999259	C4(Carbamidomethyl); C5(Carbamidomethyl); M14(Oxidation)	0.00	1.72	2	1746.75	1.63	42.33	0
																		Low	ATIGADFLTK	1	3	1	657999259		0.00	1.57	2	1036.57	0.39	43.58	0
																		Low	GNIPYYETSAK	1	1	1	657999259		0.00	1.25	2	1242.60	0.51	40.08	0
1034	gi658000539	Ubiquitin- conjugating enzyme E2 27		Malus domestica	Cytosol	06.13-Protein destination and storage/Proteolys is	6.11	19.49	2	1	3	3	195	21.3	5.10			High	DYQTFVG TAR	1	2	1	658000539		0.00	2.23	2	1157.56	-0.39	41.45	0
																		Low	LVEMGFPEAQR	1	2	1	658000539	M4(Oxidation)	0.00	1.98	2	1363.67	0.88	40.08	0
																		Low	STLEAAGGDENLA LEK	1	1	1	658000539		0.00	1.89	2	1617.80	0.28	40.81	0
1038	gi658008681	MLP-like protein 329		Malus domestica	Cytoplasm	11.02- Disease/Defense /Defense-related	29.90	39.24	1	1	8	21	158	17.8	5.40			High	YTIDGNVETYK	2	2	2	658008685;65 8008681		0.00	2.95	2	1302.62	0.76	40.71	0
																		High	LETMEAEVEIK	3	1	1	658008681	M4(Oxidation)	0.00	2.87	2	1307.64	0.62	40.47	0
																		High	DVDAHLIKE	2	2	2	658008685;65 8008681		0.00	2.81	2	1039.54	-0.57	39.28	1
																		Low	YTIDGNVETYKEK	3	2	2	658008685;65 8008681		0.00	2.36	2	1559.76	1.03	39.42	1
																		Low	DVDAHLIK	5	2	2	658008685;65 8008681		0.00	1.74	2	910.50	-0.12	40.56	0

																		Low	IIFQVTPNSEGGSV K	1	1	1	658008681	N8(Deamidated)	0.00	1.61	2	1576.82	0.95	44.34	0
																		Low	ITLEYK	2	1	1	658008681		0.00	1.41	2	766.43	-0.07	39.80	0
																		Low	IIFQVTPNSEGGSV K	2	1	1	658008681		0.00	1.30	2	1575.84	1.11	42.79	0
																		Low	EKVEIDEANK	1	1	1	658008681		0.02	0.50	2	1174.60	0.47	36.22	1
1039	gi657946863	Peroxisredoxin		Malus domestica	Plastid	11.06- Disease/Defense /Detoxification	21.58	19.75	1	2	3	16	162	17.4	5.41			High	DVLFLADGSAK	3	1	1	657946863		0.00	3.28	2	1135.60	-0.02	46.35	0
																		High	VQSVSVHSLAAGK	6	1	1	657946863		0.00	2.72	2	1282.71	0.60	39.47	0
																		Low	HVPGFIEK	7	1	1	657946863		0.00	1.59	2	926.51	-0.09	45.25	0
1039	gi658000239	Glutathione peroxidase		Malus domestica	Nuclear	11.06- Disease/Defense /Detoxification	5.68	24.54	9	1	4	6	163	18.6	5.00			High	GNDVDLSLYK	2	9	1	658000239		0.00	2.20	2	1123.56	0.45	42.70	0
																		Low	EIEDFVCTR	2	9	1	658000239	C7(Carbamidomethyl)	0.00	1.64	2	1168.53	0.08	42.06	0
																		Low	IEVNGDNTAPVYK	1	2	1	658000239		0.00	1.30	2	1419.71	1.19	38.92	0
																		Low	SEFPIDFK	1	4	1	658000239		0.00	1.16	2	982.49	-0.23	45.57	0
1130	gi658023330	UMP-CMP kinase		Malus domestica	Chloroplast	01.03- Metabolism/Nuc leotides	9.31	23.11	2	2	6	11	212	23.6	5.21			High	SGSENGTMISDMI K	2	2	1	658023330	M8(Oxidation); M12(Oxidation)	0.00	2.65	2	1501.65	1.11	38.75	0
																		High	AAFEDVTK	2	2	1	658023330		0.00	2.30	2	880.44	0.24	39.65	0
																		Low	SGSENGTMISDMI K	1	2	1	658023330	N5(Deamidated); M8(Oxidation); M12(Oxidation)	0.00	1.81	2	1502.63	-1.17	39.10	0
																		Low	NQGREDDNIEITIR	2	3	1	658023330		0.00	1.40	2	1559.74	0.93	38.45	1
																		Low	EDDNIETIR	1	3	1	658023330		0.00	1.39	2	1104.52	0.07	39.85	0
																		Low	FLIDGFPR	2	3	1	658023330		0.00	1.35	2	964.53	0.13	47.39	0
																		Low	AIFAPK	1	2	1	658023330		0.00	1.05	2	646.39	0.20	39.74	0
1130	gi657959671	Late embryogenesis abundant protein	99% to Prunus mume gi645237380 Evalua: 2e-79	Malus domestica	Cell wall	11.05- Disease/Defense /Stress responses	6.70	5.00	1	1	2	4	240	26.2	5.33			High	KTEDAVGTLAEK	2	1	1	657959671		0.00	3.15	2	1261.66	0.68	38.29	1
																		Low	TEDAVGTLAEK	2	1	1	657959671		0.00	1.69	2	1133.57	0.33	38.46	0
1130	gi657962934	Thioredoxin domain-containing protein		Malus domestica	Nucleus	02.20- Energy/Electron- transport	5.91	26.32	1	1	6	8	209	23.9	5.45			High	VDDDDLEALR	1	1	1	657962934		0.00	2.53	2	1160.54	0.61	42.14	0
																		Low	AQVIFYEGESSMK	1	1	1	657962934	M12(Oxidation)	0.00	1.61	2	1504.70	1.31	41.35	0
																		Low	SPFLAEK	2	1	1	657962934		0.00	1.60	2	791.43	0.20	40.93	0
																		Low	AVQEILEK	1	1	1	657962934		0.00	1.48	2	929.53	-0.41	39.42	0
																		Low	LDEEIAALDR	2	1	1	657962934		0.00	1.33	2	1144.59	0.97	42.24	0
																		Low	VVCHFYZ	1	1	1	657962934	C3(Carbamidomethyl)	0.00	0.87	3	980.48	0.20	37.77	0
1624	gi658008993	Serine/threonine- protein phosphatase		Malus domestica	Unclear	10.04-Signal transduction/Kin ases	19.06	11.24	5	3	7	18	587	65.5	5.21			High	FAATVEPAHLK	5	3	1	658008993		0.00	2.89	2	1183.65	0.09	39.50	0
																		High	LLRDNEAEVR	6	5	1	658008993		0.00	2.60	3	1214.65	0.10	38.59	1
																		High	NDDIQLR	1	3	1	658008993		0.00	2.18	2	873.44	-0.23	39.55	0
																		Low	TDLVPAYVR	2	3	1	658008993		0.00	1.96	2	1033.57	-0.51	43.43	0
																		Low	LLAVEGCAALGK	1	4	1	658008993	C7(Carbamidomethyl)	0.00	1.39	2	1201.66	0.56	42.05	0
																		Low	LLPVVVNAAK	2	2	1	658008993		0.00	1.37	2	1023.66	-0.21	43.75	0
																		Low	DEFPDVR	1	5	1	658008993		0.00	1.36	2	877.40	-0.55	41.36	0
2113	gi657997261	Stem-specific protein		Malus domestica	Chloroplast	12-Unclear classification	26.59	30.80	2	2	8	15	250	26.9	6.40			High	SPEALQSPQSGSAS ALK	3	1	1	657997261		0.00	3.75	2	1657.84	1.58	39.76	0
																		High	DRGPYPADQVVR	2	2	1	657997261		0.00	3.13	3	1372.70	-0.43	38.72	1
																		Low	GCFFTSSGGLR	1	2	1	657997261	C2(Carbamidomethyl)	0.00	2.55	2	1188.55	0.41	42.76	0
																		Low	GPYPADQVVR	2	2	1	657997261		0.00	2.27	2	1101.57	0.10	39.93	0
																		Low	GCGNSFAFPK	1	2	1	657997261	C2(Carbamidomethyl)	0.00	2.20	2	1181.54	0.48	41.93	0
																		Low	GPYPADQVVR	1	2	1	657997261	Q7(Deamidated)	0.00	2.16	2	1102.56	2.20	40.11	0
																		Low	DGFLAR	2	1	1	657997261		0.00	1.83	2	678.36	0.34	41.23	0
																		Low	VDSSGEMCGANF K	1	2	1	657997261	M7(Oxidation); C8(Carbamidomethyl)	0.00	1.40	2	1417.57	0.94	37.05	0
																		Low	QNPLLPR	1	2	1	657997261		0.00	1.26	2	837.49	0.48	41.00	0
																		Low	VDSSGEMCGANF K	1	2	1	657997261	C8(Carbamidomethyl)	0.00	0.82	2	1401.58	0.98	39.11	0
2631	gi658021595	Chaperonin		Malus domestica	Chloroplast	06.01-Protein destination and storage/Folding and Stability	33.65	34.43	3	1	22	32	575	61.1	6.15			High	NVVLEQSFSGAPK	2	3	1	658021595		0.00	3.16	2	1288.69	0.74	42.91	0
																		High	NVVLEQSFSGAPK	2	3	1	658021595	Q6(Deamidated)	0.00	2.69	2	1289.67	1.02	43.20	0
																		Low	VGIELTGCK	1	2	1	658021595	C9(Carbamidomethyl)	0.00	2.35	2	1077.56	0.15	40.81	0
																		Low	IGGASEAEVSEK	1	2	1	658021595		0.00	2.32	2	1176.57	0.25	37.55	0
																		Low	ITVSKDDTVILDG AGDKK	1	3	1	658021595		0.00	2.18	3	1875.01	0.23	40.68	2

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																	Medium	VLIAGATGFIGR	1	1	1	658009013		0.00	2.38	2	1174.69	0.24	42.10	0
																	Medium	FLPSEFGHDVDR	1	2	1	658009013		0.00	2.14	2	1418.67	0.77	39.10	0
																	Medium	AYFVDGTDIGK	1	2	1	658009013		0.00	2.04	2	1185.58	0.39	40.45	0
3325	gi658050324	Alpha-1,4-glucan-protein synthase		Malus domestica	Chloroplast	09.01-Cell structure/Cell wall	34.30	28.73	7	6	11	22	362	41.1	5.97	High	FVDAVLTIPIK	2	5	1	658050324		0.00	3.30	2	1102.65	0.07	44.61	0	
																High	AVEANGVVAK	1	1	1	658050324	N5(Deamidated)	0.00	2.75	2	958.52	-0.21	37.36	0	
																High	DINALEQHIK	3	4	1	658050324		0.00	2.58	2	1180.63	0.84	41.70	0	
																High	DCTTVQGCYLELSK	1	3	1	658050324	C2(Carbamidomethyl); C8(Carbamidomethyl)	0.00	2.48	2	1673.75	1.70	42.37	0	
																High	VICDHLGLGVK	5	4	1	658050324	C3(Carbamidomethyl)	0.00	2.44	2	1210.66	0.39	42.11	0	
																High	VDDYFIK	2	1	1	658050324		0.00	2.31	2	899.45	-0.57	42.91	0	
																Low	ASNPFVNLIK	2	4	1	658050324		0.00	1.94	2	989.54	0.05	43.03	0	
																Low	ASCISFK	2	3	1	658050324	C3(Carbamidomethyl)	0.00	1.80	2	812.40	0.76	39.68	0	
																Low	VPEGFDYELYNR	1	2	1	658050324		0.00	1.74	2	1501.70	0.26	43.72	0	
																Low	GYPFSLR	1	6	1	658050324		0.00	1.01	2	839.44	0.27	44.14	0	
																Low	EGADFVR	2	4	1	658050324		0.00	0.82	2	793.38	0.02	40.16	0	
3325	gi657957773	Disulfide-isomerase		Malus domestica	Endoplasmatic reticulum	06.01-Protein destination and storage/Folding and Stability	33.75	25.48	1	3	12	26	361	39.7	5.73	High	NAEALAEFVNK	3	1	1	657957773		0.00	4.49	2	1205.62	0.00	43.52	0	
																High	YGVSGYPTLK	3	1	1	657957773		0.00	2.72	2	1084.57	0.06	41.59	0	
																High	YGNLYLK	1	1	1	657957773		0.00	2.64	2	870.47	-0.23	40.29	0	
																Low	IEEEVGKLEGSAA R	5	2	2	657957773;657992452		0.00	1.95	2	1487.77	1.21	38.66	1	
																Low	GADYAKNEIQR	3	1	1	657957773		0.00	1.94	2	1264.63	-0.09	37.84	1	
																Low	KNILYTFASSS	1	1	1	657957773		0.00	1.87	2	1230.64	0.21	44.74	1	
																Low	ADEFTLKK	2	2	2	657957773;657992452		0.00	1.73	2	951.51	-0.05	39.24	1	
																Low	SILIAK	1	2	2	657957773;657992452		0.04	1.33	2	644.43	0.22	40.80	0	
																Low	ADEFTLK	2	2	2	657957773;657992452		0.00	1.28	2	823.42	0.02	41.46	0	
																Low	SLAPTYEK	3	2	2	657957773;657992452		0.00	1.19	2	908.47	-0.20	38.34	0	
																Low	NILYTFASSS	1	1	1	657957773		0.00	1.02	2	1102.54	0.34	46.94	0	
																Low	LAPEYEK	1	2	2	657957773;657992452		0.10	0.46	2	849.44	-0.07	36.54	0	
3325	gi657992826	Aldo-keto reductase		Malus domestica	Chloroplast	11.06-Disease/Defense /Detoxification	15.83	21.78	10	3	9	12	349	38.4	5.85	High	NLDSNIDSLK	2	1	1	657992826		0.00	2.98	2	1118.57	-0.06	41.38	0	
																High	YIGLSEASPDTR	1	10	1	657992826		0.00	2.93	2	1421.73	1.17	42.14	0	
																High	VQLGNCGFEVSK	2	1	1	657992826	C6(Carbamidomethyl)	0.00	2.77	2	1337.65	0.84	41.70	0	
																Low	IKNLDSNIDSLK	1	1	1	657992826		0.00	1.64	2	1359.75	1.32	40.40	1	
																Low	FVGENLDK	1	1	1	657992826		0.00	1.61	2	921.47	0.10	39.05	0	
																Low	IEILGK	1	1	1	657992826		0.00	1.50	2	672.43	1.05	41.64	0	
																Low	FVGENLDKNK	1	1	1	657992826		0.04	1.05	3	1163.61	0.15	38.23	1	
																Low	GFFGGK	2	3	1	657992826		0.04	0.86	2	612.31	0.22	41.20	0	
																Low	EVVESVSANSYVA SHPR	1	1	1	657992826		0.00	0.78	2	1830.90	1.33	39.38	0	
3325	gi657945783	Caffeic acid 3-O-methyltransferase		Malus domestica	Cytoplasm	20.1-Secondary metabolism/Phenylpropanoids/Phenolics	14.24	15.47	11	2	8	10	433	47.1	6.52	Low	TEKEFEALAK	1	8	1	657945783		0.00	2.37	3	1165.61	0.57	39.87	1	
																High	NCYAAPDNGK	1	6	1	657945783	C2(Carbamidomethyl)	0.00	2.34	2	1222.55	0.52	38.22	0	
																Medium	DAVLEGGIPFNK	1	9	1	657945783		0.00	2.18	2	1259.66	0.70	43.93	0	
																Low	GDAIFMK	1	11	1	657945783	M6(Oxidation)	0.00	2.03	2	797.39	0.26	39.52	0	
																Low	NPDAPVMLDR	1	5	1	657945783	M7(Oxidation)	0.00	1.94	2	1143.55	0.39	38.89	0	
																Low	GSGFQGER	2	7	1	657945783		0.00	1.72	2	855.41	-0.37	39.92	0	
																Low	LYGLGPVCK	1	5	1	657945783	C8(Carbamidomethyl)	0.00	1.53	2	1006.54	-0.19	41.77	0	
																Low	NPDAPVMLDR	1	5	1	657945783		0.09	1.07	2	1127.55	-0.54	41.10	0	
																Low	EFEALAK	1	8	1	657945783		0.03	0.97	2	807.43	0.36	40.20	0	
3522	gi657951571	D-3-phosphoglycerate dehydrogenase		Malus domestica	Unclear	01.01-Metabolism/Amino Acid	16.07	15.55	3	4	10	14	598	62.9	7.77	High	LAVQLVAGGSGVK	1	1	1	657951571		0.00	3.10	2	1198.72	0.23	42.04	0	
																High	GGVIDEDALVR	1	3	1	657951571		0.00	3.02	2	1143.60	0.22	41.71	0	
																Medium	LGEPIGNLLK	2	1	1	657951571		0.00	2.42	2	1053.63	-1.08	44.54	0	
																Medium	TLAVMGFGK	2	2	1	657951571	M5(Oxidation)	0.00	2.13	2	939.50	0.00	41.55	0	
																Low	DGVPHLTK	2	1	1	657951571		0.00	1.89	2	866.47	-0.44	38.55	0	
																Low	ISLCDALIVR	1	1	1	657951571	C4(Carbamidomethyl)	0.00	1.82	2	1159.65	0.55	45.71	0	
																Low	VLNDDTFAK	2	1	1	657951571		0.00	1.70	2	1022.52	-0.10	39.94	0	
																Low	FASAISESGEIK	1	1	1	657951571		0.00	1.51	2	1238.63	0.31	39.95	0	

																		Low	VKDGVPHLTK	1	1	1	657951571		0.00	1.35	2	1093.64	0.05	39.14	1
																		Low	YVGVSLVGK	1	2	1	657951571		0.00	1.02	2	921.54	-0.11	41.27	0
3522	gi657943380	Phosphomannomutase/phosphoglucotase isoform	100% to Prunus mume gi645255050 E-value: 0.0	Malus domestica	Chloroplast	01.05-Metabolism/Sugars and polysaccharides	15.46	14.34	3	2	9	14	544	58.3	5.91			High	SAAVDSTGLEFNR	1	3	1	657943380		0.00	3.62	2	1366.66	1.10	40.92	0
																		High	AITQAVLDNK	1	3	1	657943380		0.00	2.76	2	1072.60	0.03	39.30	0
																		Low	LEDAISR	1	3	1	657943380		0.00	1.97	2	803.43	-0.35	37.26	0
																		Low	NVIDEAIR	2	3	1	657943380		0.00	1.80	2	929.51	0.06	40.87	0
																		Low	HLENYIDSDPK	3	3	1	657943380		0.00	1.65	2	1330.63	0.52	39.81	0
																		Low	EFPALDTSALDK	1	3	1	657943380		0.00	1.50	2	1306.65	0.50	44.34	0
																		Low	DYGEAVLK	1	3	1	657943380		0.05	1.39	2	894.46	-0.12	39.82	0
																		Low	KLEDAISR	3	3	1	657943380		0.00	1.35	2	931.52	-0.17	37.59	1
																		Low	LQNGSDIR	1	3	1	657943380	Q2(Deamidated); N3(Deamidated)	0.09	1.10	2	904.44	-0.44	36.90	0
3522	gi658022678	Betaine aldehyde dehydrogenase		Malus domestica	Chloroplast	01.01-Metabolism/Amino Acid	14.83	11.13	3	4	5	8	503	54.8	5.69			High	IAFTGSTMGTGSK	1	2	1	658022678	M8(Oxidation)	0.00	2.79	2	1216.59	0.45	38.49	0
																		Medium	IADPLEEGCR	2	2	1	658022678	C9(Carbamidomethyl)	0.00	2.39	2	1159.54	0.77	41.06	0
																		Medium	LGPVVSQGGQYEK	2	1	1	658022678		0.00	2.28	2	1233.65	1.25	39.70	0
																		Medium	EEVFGPVLGVK	2	3	1	658022678	C9(Carbamidomethyl)	0.00	2.06	2	1276.66	0.60	44.61	0
																		Low	APISLPMEQFK	1	1	1	658022678	M7(Oxidation)	0.00	1.65	2	1276.66	1.37	42.39	0
3522	gi657970680	T-complex protein		Malus domestica	Cytoplasm	06.01-Protein destination and storage/Folding and Stability	14.49	12.33	2	1	7	12	527	57.0	5.88			High	VDEITCAPR	2	2	1	657970680	C7(Carbamidomethyl)	0.00	3.12	2	1173.59	0.53	39.79	0
																		Low	GSTNLEAIQIK	2	2	1	657970680		0.00	1.96	2	1286.73	0.75	44.28	0
																		Low	SHAIEAFSR	3	2	1	657970680		0.00	1.92	2	1017.51	-0.28	40.22	0
																		Low	IFKDEASEEKGER	1	2	1	657970680		0.01	1.91	3	1537.75	-0.24	38.76	2
																		Low	IGLGQPK	1	2	1	657970680		0.00	1.59	2	712.44	0.15	37.64	0
																		Low	VLVDISK	1	2	1	657970680		0.00	1.39	2	773.48	-0.05	39.97	0
																		Low	GISEAFK	1	2	1	657970680		0.00	1.08	2	751.40	0.28	39.13	0
																		Low	GISEAFK	1	2	1	657970680	N-Term(Acetyl)	0.00	0.51	2	793.41	1.64	41.28	0
3522	gi658007487	Glucose-6-phosphate isomerase		Malus domestica	Cytosol	02.01-Energy/Glycolysis	10.89	12.78	3	3	9	11	618	67.4	6.25			High	AIIAEGNCGSPR	1	3	1	658007487	C8(Carbamidomethyl)	0.00	2.93	2	1244.61	0.59	37.48	0
																		High	VNQLTVYGNK	2	3	1	658007487		0.00	2.48	2	1192.63	0.43	39.36	0
																		Medium	FSNDVVSGK	1	2	1	658007487		0.00	2.05	2	952.47	0.02	37.43	0
																		Low	NGLLEVQK	1	3	1	658007487	N1(Deamidated)	0.00	1.63	2	901.50	0.39	41.81	0
																		Low	AAGEVLALQK	1	3	1	658007487		0.00	1.55	2	999.58	-0.20	39.95	0
																		Low	EFDLDGNNR	1	1	1	658007487		0.04	1.50	2	965.43	-0.18	40.42	0
																		Low	EAGLNEAK	1	3	1	658007487		0.00	1.30	2	849.45	0.33	39.70	0
																		Low	FQAFAK	1	2	1	658007487		0.01	1.17	2	711.38	0.12	39.07	0
																		Low	TLEALLK	2	1	1	658007487		0.00	0.97	2	787.49	-0.47	43.53	0
3522	gi657959555	Polyphenol oxidase		Malus domestica	Chloroplast	20.1-Secondary metabolism/Phenylpropanoids/Phenolics	10.62	10.73	7	2	8	10	587	65.1	6.71			High	LGYYVDEK	2	4	1	657959555		0.00	2.77	2	986.48	-0.37	39.28	0
																		High	AQDEVLVK	1	5	1	657959555		0.00	2.56	2	1014.58	0.07	41.06	0
																		Low	KAQDEVLVK	1	5	1	657959555		0.00	1.91	2	1142.68	0.33	40.22	1
																		Low	LGTTDIEK	1	1	1	657959555		0.00	1.63	2	832.44	-0.04	36.85	0
																		Low	ALPDDDPK	2	6	1	657959555		0.00	1.25	2	898.43	-0.11	36.69	0
																		Low	AIELMR	1	3	1	657959555	M5(Oxidation)	0.04	1.24	2	748.40	0.29	38.28	0
																		Low	VKVCDSLDTK	1	1	1	657959555	C4(Carbamidomethyl)	0.00	1.14	2	1164.59	-1.48	37.85	1
																		Low	ENLTTMYQQMYSK	1	3	1	657959555	M6(Oxidation); M10(Oxidation)	0.00	1.14	2	1604.73	0.84	38.53	0
5414	gi657979903	Hydroxymethylglutaryl-CoA synthase		Malus domestica	Cytoplasm	01.06-Metabolism/Lipid and sterol	24.53	10.75	2	3	5	12	465	51.0	6.40			High	DAAATTNANGVV ANGH	2	1	1	657979903	N9(Deamidated); N14(Deamidated)	0.00	4.56	2	1484.66	-0.55	36.70	0
																		High	DAAATTNANGVV ANGH	2	1	1	657979903	N9(Deamidated)	0.00	4.18	2	1483.68	-1.12	35.91	0
																		High	DAAATTNANGVV ANGH	1	1	1	657979903		0.00	3.22	2	1482.69	0.86	35.25	0
																		High	ASQQVAKPLYDAK	4	2	1	657979903	Q3(Deamidated)	0.00	2.96	3	1419.75	0.51	35.11	0
																		High	LEVGETVIDK	1	2	1	657979903		0.00	2.70	2	1189.63	-0.08	38.29	0
																		Low	LEVGETVIDKSK	1	2	1	657979903		0.00	2.15	2	1404.76	0.16	36.58	1
																		Low	LSQTCYLK	1	2	1	657979903	Q3(Deamidated); C5(Carbamidomethyl)	0.00	2.06	2	1013.50	-0.11	36.80	0
5845	gi657952945	AP-2 complex subunit alpha		Malus domestica	Chloroplast	07.99-Transporters/Others	9.69	3.24	3	3	4	4	1019	113.5	6.11			High	MAQLLDER	1	2	1	657952945	M1(Oxidation)	0.00	2.69	2	991.49	0.38	36.91	0

																		High	KAEDTEVDTAEQS AIK	1	3	1	657952945		0.00	2.43	3	1734.84	0.23	35.54	1
																		High	AAEYLALSR	1	3	1	657952945		0.00	2.41	2	993.54	0.38	39.06	0
																		Low	AEDTEVDTAEQSA IK	1	3	1	657952945		0.00	2.16	2	1606.75	1.66	37.39	0
5845	gi658027395	Alpha-glucosidase		<i>Malus domestica</i>	Cytoplasm	01.05- Metabolism/Sug ars and polysaccharides	6.95	3.78	1	2	3	3	793	88.1	6.00			High	GANLNANSDIR	1	1	1	658027395		0.00	2.56	2	1144.57	0.34	34.87	0
																		High	DNYETYIR	1	1	1	658027395		0.00	2.37	2	1073.49	0.08	39.39	0
																		Low	SSIFGLGEHTK	1	1	1	658027395		0.00	2.02	2	1175.61	0.69	38.13	0
5848	gi658003341	Glucan 1,3-alpha- glucosidase		<i>Malus domestica</i>	Vacuole	09.01-Cell structure/Cell wall	18.61	6.75	2	5	6	7	934	106.7	6.37			High	DALHVEGVEHR	2	2	1	658003341		0.00	3.26	3	1261.63	-0.15	34.69	0
																		High	GPGVEVSEPYR	1	2	1	658003341		0.00	3.01	2	1189.59	0.66	36.82	0
																		High	DEEDVEQVDSK	1	2	1	658003341		0.00	2.58	2	1292.55	1.20	35.89	0
																		High	GALIEPANQK	1	2	1	658003341		0.00	2.40	2	1040.57	0.58	35.47	0
																		High	HASVYLPQK	1	2	1	658003341		0.00	2.32	2	971.53	-0.24	34.69	0
																		Low	MLFPHPPEEMQR	1	2	1	658003341	M1(Oxidation); M9(Oxidation)	0.00	2.01	2	1446.65	0.20	36.37	0
5848	gi658001510	Chaperone protein		<i>Malus domestica</i>	Chloroplast	06.01-Protein destination and storage/Folding and Stability	16.70	5.48	7	5	5	6	912	100.9	6.15			High	TAVVEGLAQR	1	3	1	658001510	Q9(Deamidated)	0.00	3.20	2	1044.57	-0.40	37.61	0
5848																		High	TAVVEGLAQR	1	3	1	658001510		0.00	3.17	2	1043.58	-0.04	36.85	0
5848																		High	VESASGDTTFQAL K	1	3	1	658001510		0.00	2.93	2	1453.72	1.00	38.46	0
5848																		High	TKNNPVLIGEPGV GK	1	7	1	658001510		0.00	2.69	3	1522.86	0.25	36.60	1
5848																		High	NNPVLIGEPGVGK	1	7	1	658001510		0.00	2.56	2	1293.72	0.78	38.91	0
5848																		Medium	ALVVAQLSSR	1	2	1	658001510		0.00	2.16	2	1114.66	-1.04	38.87	0
6019	gi657976562	Oxygen-evolving enhancer protein		<i>Malus domestica</i>	Chloroplast	11.02- Disease/defense/ Defense-related	7.46	15.67	2	2	3	3	268	28.7	8.59			High	TADGDEGGKHQLI TATVK	1	2	1	657976562		0.00	3.01	3	1840.94	0.37	34.67	1
6019																		High	EVEFPQGVLRL	1	1	1	657976562		0.00	2.44	2	1173.63	0.17	42.02	0
6019																		Low	SIADYGSPEEFLAK	1	1	1	657976562		0.00	2.01	2	1526.74	1.82	42.99	0
6019	gi657955010	Glutathione S- transferase		<i>Malus domestica</i>	Cytosol	11.06- Disease/Defense /Detoxification	5.40	6.57	3	1	1	2	213	24.0	6.65			High	MTTDAAVVEENE AK	1	3	1	657955010	M1(Oxidation)	0.00	2.81	2	1523.69	1.38	35.25	0
6019																		High	MTTDAAVVEENE AK	1	3	1	657955010		0.00	2.60	2	1507.70	0.94	36.54	0
6222	gi657993900	Fructose- biphosphate aldolase		<i>Malus domestica</i>	Chloroplast	02.01- Energy/Glycolys is	19.66	11.45	3	4	4	6	393	42.5	8.02			High	GILAIDESNATCG K	1	2	1	657993900	C12(Carbamidomethyl)	0.00	4.34	2	1448.71	1.24	39.82	0
																		High	GILAIDESNATCG K	1	2	1	657993900	N9(Deamidated); C12(Carbamidomethyl)	0.00	4.06	2	1449.69	-0.71	40.74	0
																		High	LDSIGLDNTEVNR	1	2	1	657993900		0.00	3.26	2	1445.73	1.31	39.61	0
																		High	FVDVLCDDQK	1	2	1	657993900	C6(Carbamidomethyl)	0.00	2.78	2	1123.55	-0.05	40.00	0
																		High	FVDVLCDDQK	1	2	1	657993900	C6(Carbamidomethyl); Q8(Deamidated)	0.00	2.72	2	1124.53	0.70	41.11	0
																		High	ANSLAQLGK	1	3	1	657993900		0.00	2.50	2	901.51	-0.57	36.10	0
6222	gi658009454	Uncharacterized protein			Cell membrane	12-Unclear classification	12.93	10.36	1	3	4	6	386	42.7	7.72			High	DGFEVGNADK	1	1	1	658009454		0.00	3.24	2	1051.47	0.14	38.01	0
																		High	TFGVNGYCIDSM AK	1	1	1	658009454	M12(Oxidation)	0.00	2.66	2	1475.69	1.28	39.83	0
																		High	SLNQFIEK	2	1	1	658009454	Q4(Deamidated)	0.00	2.64	2	979.51	-0.14	39.45	0
																		Low	SLNQFIEK	1	1	1	658009454		0.00	2.27	2	978.53	-0.51	38.67	0
																		Low	EELRFPK	1	1	1	658009454		0.00	2.13	2	989.54	-0.58	36.36	1
6222	gi658012400	Formate dehydrogenase		<i>Malus domestica</i>	Mitochondrion	11.06- Disease/Defense /Detoxification	7.45	6.49	1	2	3	3	385	42.1	6.99			High	IDPELEQQTGAK	1	1	1	658012400		0.00	2.63	2	1328.67	0.69	36.42	0
																		High	VKIDPELEQQTGA K	1	1	1	658012400		0.00	2.51	2	1555.83	1.41	36.34	1
																		Low	FEEDLDAMLPK	1	1	1	658012400	M8(Oxidation)	0.00	2.31	2	1323.61	0.68	40.58	0
7220	gi658062690	Voltage-gated potassium channel subunit beta		<i>Malus domestica</i>	Chloroplast	07.01- Transporters/Ion s	8.77	7.76	3	1	2	3	232	26.3	5.62			High	AEEIMGQAIR	1	3	1	658062690	M5(Oxidation)	0.00	3.47	2	1133.56	0.05	35.35	0
																		High	AEEIMGQAIR	1	3	1	658062690	M5(Oxidation); Q7(Deamidated)	0.00	3.12	2	1134.55	1.44	36.14	0

																Low	AMNYVIDK	1	3	1	658062690	M2(Oxidation)	0.00	2.19	2	969.47	0.59	35.60	0
7222	gi657989479	Fructose-bisphosphate aldolase cytoplasmic isozyme		<i>Malus domestica</i>	Cytosol	02.01-Energy/Glycolysis	10.01	7.26	9	2	2	3	358	38.4	8.31	High	GILAADESTGTIGK	1	9	1	657989479		0.00	4.07	2	1332.70	0.91	39.12	0
																High	LGEAAESLHVK	2	3	1	657989479		0.00	3.18	2	1210.64	0.35	36.15	0
7222	gi657980729	NADP-dependent D-sorbitol-6-phosphate dehydrogenase		<i>Malus domestica</i>	Cytoplasm	02.10-Energy/TCA pathway	9.95	8.39	2	3	3	4	310	34.9	7.23	High	REELFITTK	1	2	1	657980729		0.00	2.89	2	1136.63	-0.23	37.39	1
																High	TASLLGEDK	2	2	1	657980729		0.00	2.44	2	933.49	-0.44	37.10	0
																High	SVAQICLR	1	2	1	657980729	C6(Carbamidomethyl)	0.00	2.43	2	946.51	-0.13	37.66	0
7226	gi658057933	NADP-dependent D-sorbitol-6-phosphate dehydrogenase		<i>Malus domestica</i>	Cytoplasm	02.10-Energy/TCA pathway	5.93	5.82	5	2	2	2	292	32.9	6.29	High	REDLFITTK	1	5	1	658057933		0.00	2.97	2	1122.62	0.33	37.98	1
																High	TVAQVVLR	1	2	1	658057933		0.00	2.95	2	885.55	-0.26	37.73	0
8123	gi657948234	Proteasome subunit alpha		<i>Malus domestica</i>	Cytosol	06.13-Protein destination and storage/Proteolysis	22.53	16.06	4	3	5	9	249	27.1	7.43	High	ALLEVVESGGK	2	4	1	657948234		0.00	3.53	2	1101.62	0.29	41.72	0
																High	GTDITVLGVEKK	1	2	1	657948234		0.00	2.90	2	1259.72	0.79	37.03	1
																High	GTDITVLGVEK	2	2	1	657948234		0.00	2.75	2	1131.63	0.23	40.54	0
																Low	NIEVAVMTK	1	2	1	657948234	M7(Oxidation)	0.00	2.59	2	1020.54	-0.77	36.35	0
																Low	YIAGLQKK	2	2	1	657948234	Q7(Deamidated)	0.00	2.40	2	921.50	0.57	36.83	0
																Low	NIEVAVMTK	1	2	1	657948234		0.00	2.15	2	1004.54	0.05	38.62	0
8327	gi657977276	Elongation factor		<i>Malus domestica</i>	Ribosome	05.04-Protein synthesis/Translation factors	16.50	13.87	6	1	6	7	447	49.3	9.07	High	YYCTVIDAPGHR	2	6	1	657977276	C3(Carbamidomethyl)	0.00	2.94	3	1451.67	-0.17	36.70	0
																Low	GFVASNSKDDPAK	1	3	1	657977276		0.00	2.45	2	1335.65	0.63	33.11	1
																Low	ARYEEIVK	1	2	1	657977276		0.00	2.33	3	1007.55	0.49	34.50	1
																Low	IGGIGTVPVGR	1	6	1	657977276		0.00	2.32	2	1025.61	-0.23	38.82	0
																Low	STTGHLIYK	1	6	1	657977276		0.00	2.28	2	1120.60	0.05	34.11	0
																Low	LPLQDVYK	1	6	1	657977276	Q4(Deamidated)	0.00	2.04	2	976.54	0.26	40.17	0
8327	gi657949619	26S protease regulatory subunit 8 homolog A		<i>Malus domestica</i>	Cytoplasm/Nucleus	06.13-Protein destination and storage/Proteolysis	15.71	11.99	2	2	5	6	417	47.0	8.60	High	AVCTEAGMFALR	1	2	1	657949619	C3(Carbamidomethyl); M8(Oxidation)	0.00	3.00	2	1341.63	0.84	39.21	0
																High	VHVTQEDFEMAVAK	1	2	1	657949619	M10(Oxidation)	0.00	2.93	2	1619.77	0.54	37.68	0
																Low	KIEFPNPSEESR	1	2	1	657949619		0.00	2.79	2	1432.71	1.00	37.34	1
																Low	GVLLYGGPGTGK	2	4	2	657949619;657980610		0.00	2.64	2	1158.65	0.41	40.01	0
																Low	IEFPNPSEESR	1	2	1	657949619		0.00	2.11	2	1304.61	0.62	39.54	0
8327	gi658029902	60S ribosomal protein L4		<i>Malus domestica</i>	Cytosol	05.01-Protein synthesis/Ribosomal proteins	8.60	5.43	3	2	3	3	405	44.8	10.32	High	MSLLAAEQR	1	2	1	658029902	M1(Oxidation)	0.00	3.21	2	1092.54	0.11	36.66	0
																High	GPLIVYGTEGAK	1	3	1	658029902		0.00	3.15	2	1204.66	0.21	39.17	0
																Low	KGPLIVYGTEGAK	1	3	1	658029902		0.00	2.24	2	1332.75	1.01	36.78	1
8726	gi657979411	Dynamin		<i>Malus domestica</i>	Cytoplasm	03.22-Cell growth/division/Cell Cycle	40.24	16.74	2	4	13	17	926	100.3	9.06	High	SAFDAAANGPVD R	1	2	1	657979411		0.00	4.05	2	1290.61	0.26	37.65	0
																High	IEELLQEDQNVK	1	2	1	657979411		0.00	3.51	2	1457.75	1.46	39.15	0
																High	SAFDAAANGPVD R	1	2	1	657979411	N8(Deamidated)	0.00	3.27	2	1291.59	-0.13	38.44	0
																High	HYSDPAQNGDVN SGPNSGSR	3	2	1	657979411	N12(Deamidated)	0.00	3.04	3	2059.87	-1.09	34.07	0
																High	MVVALVDMER	1	2	1	657979411	M1(Oxidation); M8(Oxidation)	0.00	2.98	2	1194.59	1.08	38.24	0
																Low	RPADPEEELR	1	2	1	657979411		0.00	2.69	3	1211.60	-0.02	34.64	0
																Low	HYSDPAQNGDVN SGPNSGSR	1	2	1	657979411		0.00	2.60	3	2058.89	1.04	33.50	0
																Low	SQIVQDELVK	1	2	1	657979411		0.00	2.49	2	1158.64	-0.11	37.91	0
																Low	SQQVSASALR	1	2	1	657979411		0.00	2.42	2	1046.56	-0.05	34.48	0
																Low	AAAASSYSGGGG AQESISR	1	1	1	657979411		0.00	2.28	2	1726.80	1.46	34.87	0
																Low	SLALELCR	1	2	1	657979411	C7(Carbamidomethyl)	0.00	2.25	2	961.51	-0.36	40.17	0
																Low	LIDLPGLDQR	1	2	1	657979411		0.00	2.24	2	1139.64	0.58	43.34	0
																Low	APISIDLQR	1	2	1	657979411		0.00	2.24	2	1012.58	0.04	39.69	0
																Low	GQDAEQAIMNR	1	1	1	657979411	M9(Oxidation)	0.00	2.16	2	1248.56	0.17	34.75	0
																Low	QSLSDGSLDTMTR	1	2	1	657979411	M11(Oxidation)	0.00	2.02	2	1426.65	1.17	36.57	0

8726	gi657991756	Acetyl-coenzyme A carboxylase transferase subunit alpha		Malus domestica	Mitochondrion	01.06-Metabolism/Lipid and sterol	15.08	9.37	1	1	6	6	758	84.3	8.87	High	IEDLNGEINER	1	1	1	657991756		0.00	2.97	2	1301.64	1.58	37.88	0
																Low	QLSAAAVESSNLK	1	1	1	657991756		0.00	2.79	2	1317.70	0.50	38.79	0
																Low	TPVADLEGEVEK	1	1	1	657991756		0.00	2.70	2	1286.65	1.33	39.62	0
																Low	KPVEQTLPEIK	1	1	1	657991756		0.00	2.28	2	1368.77	1.06	35.83	0
																Low	DDEDPYSFDASR	1	1	1	657991756		0.00	2.27	2	1416.56	1.50	41.42	0
																Low	NFGMPTPHGYR	1	1	1	657991756	M4(Oxidation)	0.00	2.07	2	1292.58	0.50	34.55	0
8728	gi657988901	Cullin		Malus domestica	Nucleus	03.22-Cell growth/division/Cell Cycle	13.80	7.86	1	2	6	7	738	85.9	7.06	High	LDKVDDLRS	1	1	1	657988901		0.00	2.72	3	1060.56	0.48	35.17	1
																High	GGAGTQEQLIK	2	1	1	657988901	Q6(Deamidated)	0.00	2.49	2	1201.64	0.32	37.29	0
																Low	IAVIDLINK	1	1	1	657988901		0.00	2.26	2	998.62	-0.38	43.30	0
																Low	MEGMVTDLTAR	1	1	1	657988901	M1(Oxidation); M4(Oxidation)	0.00	2.23	2	1368.65	1.14	38.89	0
																Low	YADAAIVR	1	1	1	657988901		0.00	2.05	2	991.56	0.15	40.12	0
																Low	IEDLITR	1	1	1	657988901		0.00	2.04	2	859.49	-0.30	38.42	0
8732	gi658038306	Porin		Malus domestica	Mitochondrion	11.02-Disease/defense/Defense-related	27.18	23.91	2	3	7	10	276	29.5	8.79	High	GPGLFTDIGK	2	2	1	658038306		0.00	3.45	2	1004.54	-0.30	42.57	0
																High	SVLTISGEVDTK	1	2	1	658038306		0.00	2.99	2	1248.67	0.51	39.78	0
																High	GGLSTGDILTQYK	1	1	1	658038306		0.00	2.97	2	1352.71	0.95	41.10	0
																Low	YKNTVVDVK	1	2	1	658038306		0.00	2.78	2	1065.59	-0.12	34.13	1
																Low	LGALLQHEVIPK	1	1	1	658038306		0.00	2.71	3	1317.79	-0.13	38.91	0
																Low	SAAVGEITR	3	1	1	658038306		0.00	2.56	2	903.49	-0.32	35.86	0
																Low	GPGLFTDIGKK	1	2	1	658038306		0.00	2.18	2	1132.64	0.44	38.59	1
9603	gi657996330	ATP-citrate synthase beta chain protein		Malus domestica	Mitochondrion	07.22-Transporters/Transport ATPases	34.64	16.94	2	9	9	15	608	65.9	7.65	High	TTQALFYNYK	1	2	1	657996330		0.00	3.08	2	1248.63	0.66	39.73	0
																High	TTQALFYNYK	2	2	1	657996330	N8(Deamidated)	0.00	3.02	2	1249.61	1.63	40.63	0
																High	VELLQAFAR	1	2	1	657996330		0.00	2.98	2	1046.60	-0.42	43.17	0
																High	SGGMSNELYNTV AR	2	2	1	657996330	M4(Oxidation); N10(Deamidated)	0.00	2.90	2	1515.67	-1.19	38.57	0
																High	DEYSLVEALK	1	2	1	657996330		0.00	2.76	2	1166.59	0.57	44.38	0
																High	SGGMSNELYNTV AR	1	2	1	657996330	M4(Oxidation)	0.00	2.73	2	1514.69	0.75	37.48	0
																High	LYRPGSVGFVSK	1	2	1	657996330		0.00	2.68	3	1309.72	-3.19	37.07	0
																High	FGGAVDDAAR	2	2	1	657996330		0.00	2.62	2	978.46	-0.09	35.70	0
																High	SIGLIGHTFDQK	1	2	1	657996330		0.00	2.45	2	1315.70	0.80	39.30	0
																High	VVAIIAEGVPESDT K	1	2	1	657996330		0.00	2.45	2	1527.83	1.30	40.68	0
																High	LIVVLGELGGR	1	2	1	657996330		0.00	2.37	2	1125.70	-0.39	44.01	0
																Low	VELLQAFAR	1	2	1	657996330	Q5(Deamidated)	0.00	2.16	2	1047.58	0.62	44.22	0
9708	gi657947113	Beta-galactosidase		Malus domestica	Secreted	01.05-Metabolism/Sugars and polysaccharides	23.86	11.86	7	5	7	12	725	81.1	8.03	High	SGCAAFLANYDT K	1	1	1	657947113	C3(Carbamidomethyl)	0.00	3.47	2	1417.64	0.74	39.91	0
																High	LGNNQEAHVNT K	4	1	1	657947113	N11(Deamidated)	0.00	2.73	2	1472.71	-0.79	36.33	0
																High	ATFNAPPGHAPLA LDMGSMGK	1	1	1	657947113	M16(Oxidation); M19(Oxidation)	0.00	2.71	3	2115.00	0.47	38.61	0
																High	LGNNQEAHVNT K	1	1	1	657947113		0.00	2.69	2	1471.73	0.95	35.23	0
																High	ILISGSIHYPR	1	7	1	657947113		0.00	2.64	2	1255.72	0.76	38.74	0
																High	LVQQAGLFVNLK	1	1	1	657947113		0.00	2.63	2	1357.80	1.06	42.78	0
																Low	LTFSQNVK	2	1	1	657947113	N6(Deamidated)	0.00	2.27	2	937.50	1.42	37.79	0
																Low	AIINGQR	1	3	1	657947113		0.00	2.15	2	884.53	-0.04	36.29	0
9708	gi657955906	Peroxisomal acyl-coenzyme A oxidase 1		Malus domestica	Proxisome	01.06-Metabolism/Lipid and sterol	9.56	8.13	1	2	4	4	664	74.2	7.87	High	FGSGAYNSMDNGVL R	1	1	1	657955906	M9(Oxidation)	0.00	2.68	2	1603.72	1.42	38.36	0
																High	QTIVADASSALSR	1	1	1	657955906		0.00	2.44	2	1318.70	1.32	39.81	0
																Low	TDEFVINSPTLTSS K	1	1	1	657955906		0.00	2.28	2	1638.83	3.66	41.66	0
																Low	MSIACAQDLK	1	1	1	657955906	M1(Oxidation); C5(Carbamidomethyl)	0.00	2.17	2	1239.57	0.39	36.26	0
9708	gi657968408	Methyltransferase		Malus domestica	Chloroplast	12-Unclear classification	6.85	5.06	1	1	3	3	632	71.8	7.93	High	SIMDAMQYDVR	1	1	1	657968408	M3(Oxidation); M6(Oxidation)	0.00	2.49	2	1360.59	0.97	37.19	0
																Low	LINLGDGSR	1	1	1	657968408		0.00	2.24	2	1043.58	-0.28	39.44	0
																Low	TMDYQLAEPGR	1	1	1	657968408	M2(Oxidation)	0.00	2.13	2	1296.59	1.21	37.45	0

Table S1B. Identification details of differentiable proteins in the mesocarp of untreated, 1-MCP and O₃ treated apples (cv. Granny Smith) inoculated/or not with the pathogen *B. cinerea* after of 60 days of cold storage.

Spot N° ^a	Accession Number ^b	Suggested Name ^c	Matching criteria ^d	Organism ^e	Subcellular localization ^f	Functional category ^g	Mascot Score	MS Coverage	Protein MW	pI-Value	Sequence	# Peptides
50	tr Q8W2C8 Q8W2C	Sorbitol dehydrogenase		<i>Malus domestica</i>	Cytoplasm	11.05-Disease/Defense/Stress responses	40	21	40152	8.9	ENMAAWLVDVNTIK	9
											ENMAAWLVDVNTIK	
											AVGICGSDVHYLK	
											AVGICGSDVHYLKTMK	
											SLGADGTVKVSR	
											EVDVVGVFRCK	
											CKNTWPLCLEFLR	
											NTWPLCLEFLRSGK	
											GGDAIKVMFNL	
109	tr M5Y2Z8 M5Y2Z	Serine/threonine-protein phosphatase		<i>Prunus persica</i>	Cytoplasm	10.04-Signal transduction/Kinases	72	32	35442	4.8	YPAHITLLR	8
											QLTQVYGFYDECQR	
											YGNANAWR	
											GAGWLFGSR	
											VTSEFNHINNLDLVCR	
											GLVTVWSAPNYCYR	
											CGNVASILSFNDNMER	
											FFTETEENNQMR	
205	tr A0A0D4CZ16 A	CYP38		<i>Malus domestica</i>	Chloroplast	06.01-Protein destination and storage/Folding and Stability	45	19	48569	4.8	LEAGMNELQQIVEDR	7
											VKDNPSLTDCVFR	
											DNPSLTDCVFR	
											IVLDGYNAPVTAGNFVDL VER	
											HFYDGMETIQR	
											TIPLEIMVEGEK	
											ESELTSPSNSNILDGR	

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											NTSHYHTK	
1132	tr R4I6H0 R4I6H	ACC oxidase		<i>Malus domestica</i>	Cytoplasm	11.05- Disease/Defense/Stress responses	41	23	34274	5	GLDDVQSEIHDLDWESTFF LR	5
											HLPSSNISEIPDLEEEYR	
											HLPSSNISEIPDLEEEYRK	
											VSNYPCKPKPDLIK	
											AHSDAGGIILLFQDDK	
1212	tr R4I6H0 R4I6H	ACC oxidase		<i>Malus domestica</i>	Cytoplasm	11.05- Disease/Defense/Stress responses	83	41	34274	5	GLDDVQSEIHDLDWESTFF LR	9
											HLPSSNISEIPDLEEEYR	
											HLPSSNISEIPDLEEEYRK	
											LLDLLCENLGLEK	
											VSNYPCKPKPDLIK	
											AHSDAGGIILLFQDDK	
											VIAQSDGTR	
											MSIASFYNPGNDSFISPAPA VLEK Oxidation (M)	
											FVFDYMK Oxidation (M)	
1220	tr R4I6H0 R4I6H	ACC oxidase		<i>Malus domestica</i>	Cytoplasm	11.05- Disease/Defense/Stress responses	60	35	34274	5	GLDDVQSEIHDLDWESTFF LR	7
											HLPSSNISEIPDLEEEYR	
											HLPSSNISEIPDLEEEYRK	
											LLDLLCENLGLEK	
											VSNYPCKPKPDLIK	
											AHSDAGGIILLFQDDK	
											MSIASFYNPGNDSFISPAPA VLEK	
1507	tr J9PZL0 J9PZL	Dehydrin 8		<i>Malus domestica</i>	Nucleus	11.05- Disease/Defense/Stress responses	52	25	32802	5.1	SQEHEYER	19
											GLFDLGLK	
											ISDHHEALAPDQYTSSYSK	
											VYEEHHHQAPAPVHHH EEPTDYPTEEK	
											LPGYHSK	
1510	tr A0A0B4S3Q7 A	V-ATPase		<i>Eriobotrya japonica</i>	Chloroplast	07.22- Transporters/Transport ATPases	70	41	54667	4.89	TVSGVAGPLVILEK	14

											AIVQVFEGTSGIDNK	
											YTTVQFTGEVLK	
											IFNGSGKPIDNGPPILPEAY LDISGSSINPSER	
											TYPEEMIQTGISTIDVMNSI AR	
											TYPEEMIQTGISTIDVMNSI AR Oxidation (M)	
											IPLFSAAGLPHNEIAAQICR	
											VTLFLNLANDPTIER	
											IITPRIALTTAEYLAYECGK	
											GYPGYMYTDLAQIYER	
											GYPGYMYTDLAQIYER Oxidation (M)	
											QIYPPINVLP SLR	
											RDHSDVSNQLYANYAIGK	
											DHSDVSNQLYANYAIGK	
1511	tr M5VIV9 M5VIV	V-ATPase	100% to Prunus mume gi 645260938 E-value: 0.0	<i>Prunus persica</i>	Chloroplast	07.22- Transporters/Transport ATPases	109	48	54622	4.8	TVSGVAGPLVILEK	17
											AIVQVFEGTSGIDNK	
											TVQFTGEVLK	
											IFNGSGKPIDNGPPILPEAY LDISGSSINPSER	
											TYPEEMIQTGISTIDVMNSI AR	
											TYPEEMIQTGISTIDVMNSI AR Oxidation (M)	
											IPLFSAAGLPHNEIAAQICR	
											VTLFLNLANDPTIER	
											IITPRIALTTAEYLAYECGK	
											IALTTAEYLAYECGK	
											HVLVILTDMSYADALR Oxidation (M)	
											GYPGYMYTDLAQIYER	

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2320	sp Q1KLZ2 CAS1_	L-3-cyanoalanine synthase		<i>Malus domestica</i>	Mitochondrion	01.01-Metabolism/Amino Acid	102	39	41085	8.7	TQVSQLIGR	11
											DRPALSMINDAEK	
											GYKMVLTMPSYTSLER	
											MVLTMPSYTSLER	
											CFGADLILTDPTK	
											AYDLLESTPNAYMLQQFS NPANTK	
											VIEVTSEDAVNMAR	
											EGLMVGISSGANTVAAME LAK	
											GKLIVTVHPSFGER	
											LIVTVHPSFGER	
											YLSSVLFQELRQEAENMQ PVAVDYP Oxidation (M)	
2326	tr M5W4J8 M5W4J	40S ribosomal protein		<i>Malus domestica</i>	Cytosol	05.01-Protein synthesis/Ribosomal proteins	63	22	32506	5.2	NCDFQMER	5
											FAQHTGANAIAGR	
											HTPGTFTNQLQTSFNEPR	
											LLILTDPR	
											EAALGNIPTIAFCDTDSMP R	
2634	tr M5W737 M5W73	RuBisCO large subunit-binding protein subunit beta	100% to Prunus mume gi 645215194 E-value: 0.0	<i>Prunus mume</i>	Chloroplast	06.01-Protein destination and storage/Folding and Stabillity	51	13	64778	5.7	MASTFAAMTSVGSLAAPS SR	5
											SAENSLYVVEGMQFDR	
											DLINILEEAIK	
											SQYLDDIAILTGGTVIR	
											AAVEEGIVVGGGCTLLR	
2636	tr A0A023IRP7 A	Malic enzyme		<i>Malus domestica</i>	Mitochondrion	02.10-Energy/TCA pathway	74	25	66495	5.1	GTSFSFTER	12
											GLLPPNVMSTEHQIER	
											RLEEQVR	
											GMYFSAEDR	
											LDLYVAAAGINPQR	
											MFNDDVQGTAGVALAGL LGAVR	
											IVVAGAGSAGIGVLNAAR	

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											LAYVALDYEQELETAK	
											SSSSVEKNYELPDGQVITIG AER	
											NYELPDGQVITIGAER	
											KDLYGNIVLSGGSTMFPGI ADR	
											DLYGNIVLSGGSTMFPGIA DR	
											DLYGNIVLSGGSTMFPGIA DR Oxidation (M)	
											GEYDESGPSIVHR	
3220	tr R4I6H0 R4I6H	ACC oxidase		Malus domestica	Cytoplasm	11.05- Disease/Defense/Stress responses	67	35	34274	4.98	EMVAAKGLDDVQSEIHDLDWESTFFLR	8
											GLDDVQSEIHDLDWESTFFLR	
											HLPSSNISEIPDLEEEYR	
											HLPSSNISEIPDLEEEYRK	
											VSNYPPCPKPDLIK	
											VSNYPPCPKPDLIKGLR	
											AHSDAGGIILLFQDDK	
											MSIASFYNPNGNDSFISPAPAVLEKK Oxidation (M)	
3324	sp Q1KLZ1 CAS2_	L-3-cyanoalanine synthase		Malus domestica	Mytochondrion	01.01- Metabolism/Amino Acid	87	31	40688	8.8	TQVSQLIGR	9
											VTEGCGAFIAVK	
											QEMFQPTASIK	
											DRPALSMINDAEEK	
											MVLTMPSYTSLER	
											CFGADLILTDPTK	
											AYDLLESTPNAFMLQQFS NPANTK	
											LIVTVHASFGER	
											YLSSVLFQDLR	
3327	tr G5ELM7 G5ELM	Actin		Malus domestica	Cytoskeleton	09.04-Cell structure/Cytoskeleton	65	34	40369	5.6	AGFAGDDAPR	10
											HTGVMVGMGQK 2 Oxidation (M)	
											VAPEEHPVLLTEAPLNPK	
											EIVRDMK Oxidation (M)	

											LAYVALDYEQELETAK	
											SSSSVEKNYELPDGQVITIG AER	
											NYELPDGQVITIGAER	
											DLYGNIVLSGGSTMFPGIA DR	
											VVAPPER	
											GEYDESGPSIVHR	
3719	sp P48981 BGAL_	Beta-galactosidase		Malus domestica	Apoplast	01.05- Metabolism/Sugars and polysaccharides	57	11	81628	5.6	ILISGSIHYPR	8
											LVQQEGLFVNLR	
											YVPGIAFR	
											SESDCAAFLANYDAK	
											LSFSQNVNLR	
											GQIWINGQSVGR	
											HWPGYIAR	
											WYHIPR	
4016	sp Q40280 MAL12	Major allergen		Malus domestica	Unclear	11.02- Disease/defense/Defens e-related	85	49	17528	5.6	GVYTFENEYTSEIPPPR	8
											GVYTFENEYTSEIPPPRLFK	
											HAEILEGDGGPGTIK	
											HAEILEGDGGPGTIKK	
											KITFGEQSQYGYVK	
											ITFGEQSQYGYVK	
											HKIDSVDEANYSYAYTLIE GDALTDTIEK	
											IDSVDEANYSYAYTLIEGD ALTDTIEK	
4026	gi 657979046	5'-3' exoribonuclease		Malus domestica	Nucleus	04.99- transcription/Others	51	34	22112	5.7	YSETTAAYGSTTTTHESELD YK	5
											YSETTAAYGSTTTTHESELD YKK	
											HLEHLGEAGVAAAGAFAL HEK	
											HKIEEEIAAAAAVGSGGFA FHEHHEK	
											IEEEIAAAAAVGSGGFAFH EHHEK	

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											HETADINTFK	
											HETADINTFKWGVANR	
4517	tr V9SI88 V9SI8	Glutamate decarboxylase		<i>Malus domestica</i>	Unclear	01.01-Metabolism/Amino acid	95	33	56941	5.5	GEQVNCTFASR	16
											DSAYQIINDELMLDGNPR	
											DSAYQIINDELMLDGNPR Oxidation (M)	
											LNLASFVTTWMEPECDR	
											NYVDMDEYPVTTELQNR	
											LSEGYYVMDPAK	
											GSSQIIAQYYQFIR	
											NVMENCMENTR	
											DIGVPLVAFSLK	
											HTVFEVADSLR	
											HTVFEVADSLRK	
											FGWTVPAYTMPANAEHV AVLR	
											FGWTVPAYTMPANAEHV AVLR Oxidation (M)	
											VVIREDFSR	
											STVHKSETEAEQEIVSR	
											SETEAEQEIVSR	
4730	tr Q0GE69 Q0GE6	NBS-containing resistance		<i>Prunus cerasus</i> <i>var. caproniana</i>	Unclear	11.02-Disease/defense/Defense-related	52	46	18538	5.7	EVYATHGLVPLQK	6
											QLLSNLR	
											KVLLILDDVDQSDQLEMLIR	
											DCFGLGSRHIITR	
											VYKVMPLTQDEALYLFSR	
											VMPLTQDEALYLFSR Oxidation (M)	
5223	sp Q84V25 ENOXE	2-methylene-furan-3-one reductase		<i>Fragaria ananassa</i>	Chloroplast	02.20-Energy/Electron-transport	52	30	34205	5.5	AWVYSEYGK	8
											FDPSVAVPEVK	
											FDPSVAVPEVKEDQVLIK	
											VVAASLNPVDFK	
											VVAASLNPVDFKR	
											DTDSPLPTVPGYDVAGVV VKVGSQVTK	

											FKVGDEVYGDLINEAALVN PTR	
											LKPYLESGK	
5521	tr A0A0F7EVJ8 A	RuBisCO		Malus domestica	Chloroplast	02.30- Energy/Photosynthesis	72	34	50013	6.5	DYKLTYYPDPYETK	15
											LTYYPDPYETK	
											LTYYPDPYETKDTDILAAF R	
											LEDLRIPVAYVK	
											TFQGPPHGIQVER	
											YGRPLLGCTIKPK	
											AVYECLRGGLDFTK	
											GGLDFTKDDENVNSQPFM R	
											GGLDFTKDDENVNSQPFM R Oxidation (M)	
											DRFLFCAEAIYK	
											FLFCAEAIYK	
											ELGVPIVMHDYLTGGFTA NTTLAHYCR	
											EITLGFVDLLR	
											EITLGFVDLLRDDFVEK	
											DLAREGNEIIR	
5627	sp P28475 S6PD_	NADP-dependent D-sorbitol-6- phosphate dehydrogenase		Malus domestica	Chloroplast/Cy toplasm/ Peroxisome.	01.05- Metabolism/Sugars and polysaccharides	46	35	35168	6.9	STVTLSSGYEMPVIGLGLW R	8
											EVILNAIK	
											LQIDYLDLYLVHYPMP TK Oxidation (M)	
											VLDIDVTISLQQTWEGME K Oxidation (M)	
											TVSLGLVR	
											HGVLPTAHTPLGGAAANK	
											DMFGSVSPLDDPVLNDVA K	
											DMFGSVSPLDDPVLNDVA KK	
5840	tr Q6YNR9 Q6YNR	Aconitate hydratase		Prunus avium	Cytosol	02.10-Energy/TCA pathway	51	24	99444	6	ILTALEKPDGGEFGK	15

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6018	tr Q6V8T2 Q6V8T	Actin-depolymerizing factor		<i>Malus domestica</i>	Unclear	09.04-Cell structure/Cytoskeleton	74	64	11189	9.5	SIVFKIEEK	6
											QKQVVVEHVGEPAETYEQ FTEK	
											QVVVEHVGEPAETYEQFT EK	
											YAIQDFDFLTPEGVQK	
											SRIFFIWSPDTSR	
											IFFIAWSPDTSR	
6218	gi 657974830	2-methylene-furan-3-one reductase		<i>Malus domestica</i>	Chloroplast	02.20-Energy/Electron-transport		25	36974	7.6	VVAASLNPVDFK	6
											IGSLAEYTAAEER	
											NLSFVEAASLPLAIETAYE GLER	
											FDVVYDAVGQSDR	
											VKPVLDPGYPYFSK	
											TVEAFAYLETSR	
6225	tr M5XFH2 M5XFH	Fructose-bisphosphate aldolase		<i>Prunus persica</i>	Cytosol	02.01-Energy/Glycolysis	59	25	42973	9	GILAIDESNATCGK	8
											GILAIDESNATCGKR	
											RLDSIGLDNTEVNR	
											LDSIGLDNTEVNR	
											GLVPLPGSNNESWCQGLD GLASR	
											TVVSIPCGPSALAVK	
											EAAWGLAR	
											YAAISQDNGLVPIVEPEILL DGDHPIER	
6545	gi 657975136	Mitochondrial-processing peptidase subunit beta		<i>Malus domestica</i>	Mitochondrion	06.04-Protein destination and storage/Targeting	115	31	59284	6.3	YGSPHPSLTDHSHILTAPET R	13
											VTTLPNGLR	
											TATVGWIDAGSR	
											DLEEEVENMGGHLNAYTS R	
											DLEEEVENMGGHLNAYTS R Oxidation (M)	

											LSGDPATASQLVAEEPSFF TGSEVR	
											HMGSELAQR	
											VAINDIAENYMAFNTNYK	
											DTGLFGVFATAK	
											SSLLHLHDGTSAVAEDIGR	
											QLLTYGR	
											IPLAEMCAR	
											LPDYNWFR	
6834	tr B7SKM8 B7SKM	Phosphoenolpyruvate carboxylase		<i>Malus domestica</i>	Cytosol	02.10-Energy/TCA pathway	46	16	110632	7.4	KSPQEVFDALK	16
											NQTVDLVLTAHPTQSVR	
											EIQAAFR	
											HYIEFWK	
											LYHTRER	
											AIADGSLLDFLR	
											QESDRHTDVIDAITK	
											FSAAWQLYK	
											FGVKLTMFHGR Oxidation (M)	
											LTMFHGR	
											GGGPHTHLAILSQPPDTIHGSLR	
											RALMDEMAYVSTEDYR Oxidation (M)	
											SIVFQEPR	
											FVEYFR	
											MNIGSRPSK	
6835	tr M5WQE7 M5WQE	Translation elongation factor	100% to Prunus persica gi 388540218 E-value: 0.0	<i>Prunus persica</i>	Unclear	05.04-Protein synthesis/Translation factors	190	47	91248	5.5	KSTLTDSLVAAGIHAQEVAGDVR	32
											STLTDSLVAAGIHAQEVAGDVR	
											NGNEYLINLIDSPGHVDFS SEVTAALR	
											ITDGALVVVDCIEGVVCVQETVLR	
											IRPVLTVNK	
											CFLELQVDGEEAYQTFQR	

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7227	tr M5W2W1 M5W2W	3-oxoacyl-[acyl-carrier-protein] reductase	96% to Prunus mume gi 645270925 E-value: 1e-180	<i>Prunus persica</i>	Chloroplast	01.06-Metabolism/Lipid and sterol	61	23	31340	8.9	MANQVADHLEPWR	6
											VVMVTGASSGLGR	
											TNLTGSWLVSK	
											VNAISPGLFR	
											DWLHNVAMK	
											TFGTSDPALTSLVR	
7711	tr M5VVJ1 M5VVJ	NADP-dependent malic enzyme		<i>Prunus persica</i>	Chloroplast	02.10-Energy/TCA pathway	52	17	55230	7.7	QYQVPLQK	8
											YMALTELQER	
											RPQGLYISLK	
											TVQVIVVTDGER	
											ILGLGDLGCQGMGIPVGK	
											IWLVDSK	
											AIKPTVLIGSSGVGR	
											LPRPENLVK	
7720	tr M5WFB9 M5WFB	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase	100% to Prunus mume gi 645263470 E-value: 0.0	<i>Prunus persica</i>	Chloroplast	01.01-Metabolism/Amino Acid	69	18	84901	6.1	YGWNGGEIGFDTYFSMAR	13
			98% to Prunus mume gi 645263470 E-value: 0.0								YGWNGGEIGFDTYFSMAR Oxidation (M)	
											TLTSLKGVTAYGFDLVR	
											GVTAYGFDLVR	
											SWLAFAAQK	
											ALAGHKDEAFFSANAAAQ ASR	
											DEAFFSANAAAQASR	
											AAAALKGSDHR	
											KLNLPILPPTTTIGSFQTIEL R	
											LNLPILPTTTIGSFQTIELR	
											LQEELDIDVLVHGEPER	
											SDEKLLSVFR	
											YGAGIGPGVYDIHSPR	

[illegible]

											INHMIYDYCTDR	
											INHMIYDYCTDR Oxidation (M)	
											SRYPVAPPECTAGF	
											YPVAPPECTAGF	
8116	tr Q154Z0 Q154Z	Putative NBS-LRR disease resistance protein		<i>Malus domestica</i>	Unclear	11.02- Disease/defense/Defense-related	43	31	16498	5.7	IFHHFEVHCFLDSPMLK	3
											ILTQQWGINYTK	
											NMPEDGFLELSECFIK	
8232	sp P28475 S6PD_	NADP-dependent D-sorbitol-6-phosphate dehydrogenase		<i>Malus domestica</i>	Chloroplast	01.05- Metabolism/Sugars and polysaccharides	130	60	35168	6.9	HFDCAAHYK	15
											SEADVGEALAEAFK	
											REELFITTK	
											IWNSDHGHVVEACK	
											VLDIDVTISLQQTWEGME K Oxidation (M)	
											TVSLGLVR	
											SIGLSNYELFLTR	
											IKPAVSQFETHPYFQR	
											HGVLPTAHTPLGGAAANK	
											DMFGSVSPDDPVLNDVAK	
											DMFGSVSPDDPVLNDVAK Oxidation (M)	
											SVAQICLR	
											SVAQICLRWGIQR	
											ENLEVLEFQLSDEDMQLIY SIDR	
											TSLPSKTWGLDVYA	
8319	tr C0LQA0 C0LQA	GDP-D-mannose pyrophosphorylase		<i>Malus domestica</i>	Chloroplast	01.05- Metabolism/Sugars and polysaccharides	43	22	36849	5.9	ITCSQETEPGLGTAGPLALAR	4
											LIDDSGEPFFVLNSDVISEY PFK	
											LYLDSLR	
											GEGCLIGPDVAIGPGCHIES GVR	

											AVSLCVEDQDYMGR Oxidation (M)	
											TIVKPGCSQEVLK	
9117	gi 658038306	Porin		<i>Malus domestica</i>	Mitochondrion	11.02- Disease/defense/Defense-related	86	35	29460	9.2	FTVSTYSDAGVALTSTAV K	6
											GGLSTGDILTQYK	
											YTAGISITKPDQSASIILGD K	
											ASYVHYMDLLK	
											FSTNENTFTVGGQYAVDIY TIVK	
											LGALLQHEVIPK	
9118	gi 658038306	Porin		<i>Malus domestica</i>	Mitochondrion	11.02- Disease/defense/Defense-related	76	34	29460	9.2	FTVSTYSDAGVALTSTAV K	5
											VDTESNISTTFTINEIVPSTK	
											YTAGISITKPDQSASIILGD K	
											FSTNENTFTVGGQYAVDIY TIVK	
											LGALLQHEVIPK	
9221	sp P28475 S6PD_	NADP-dependent D-sorbitol-6- phosphate dehydrogenase		<i>Malus domestica</i>	Chloroplast/Cy toplasm/ Peroxisome	01.05- Metabolism/Sugars and polysaccharides	45	25	35168	6.9	HFDCAAHYK	7
											VLDIDVTISLQQTWEGME KTVSLGLVR	
											TVSLGLVR	
											IKPAVSQFETHPYFQR	
											HGVLPTAHTPLGGAAANK	
											SVAQICLR	
											GKYADELIANAAYIGTPGK	
9227	tr M5W2H9 M5W2H	Fructose- bisphosphate aldolase		<i>Prunus persica</i>	Mitochondrion	02.01- Energy/Glycolysis	63	30	38617	7.6	FASINVENVESNR	9
											FASINVENVESNRR	
											VDKGTVELAGTNGETTTQ GLDGLAQR	

											GTVELAGTNGETTTQGLD GLAQR	
											GTVELAGTNGETTTQGLD GLAQRCQK	
											YYEAGAR	
											YAIICQENGLVPIVEPEILV DGPHDIEK	
											KPWSLSFSFGR	
9313	tr X5D281 X5D28	Glyceraldehyde-3-phosphate dehydrogenase		Malus baccata	Cytoplasm/Mitochondrion	11.05-Disease/Defense/Stress responses	59	24	37104	9.2	TLLFGKEKPVTVF GIR	5
											FGIVEGLMTTVHSITATQK Oxidation (M)	
											VPTVDVS VVDL TVR	
											GILGYTEDDVSTDFIGDS R	
											LVS W Y D N E W G Y S T R	
9314	tr X5D281 X5D28	Glyceraldehyde-3-phosphate dehydrogenase		Malus baccata	Cytosol	11.05-Disease/Defense/Stress responses	112	59	37104	9.2	DDVELVAVNDPFITTDYM TYMFK	16
											TLLFGKEKPVTVF GIR	
											DAPMFVVGVNEK	
											EYKPDIHILSNASCTTNC L APLAK	
											FGIVEGLMTTVHSITATQK	
											FGIVEGLMTTVHSITATQK Oxidation (M)	
											TVDGPSQKDWR	
											AASFNIIPSSTGA AK	
											VLPALNGK	
											LTGMAFR	
											LTGMAFRVP TVDVSVVDL TVR	
											VPTVDVS VVDL TVR	
											LAKPAS YD Q I K	
											GILGYTEDDVSTDFIGDS R	
											AGIALNDTFVK	
											LVS W Y D N E W G Y S T R	

^a Spot N^o, protein spot number of the reference gel maps presented in **Figure S1**;

^b Accession number, UNIPROT accession number;

^c Suggested name, Suggested protein name according to database identification or manual blast against current databases;

^d Matching criteria adopted criteria for the valuation of similarity for proteins identified after manual blast against current databases. Blank cells correspond to proteins readily identified without further blast;

^e Organism, organism in which the protein has been matched to by BLAST search;

^f Subcellular localization, Subcellular localization of proteins was assigned based on database searches;

^g Functional category, proteins ontologically classified into functional categories proposed by Bevan et al., 1998;

Table S2. Percentage (%) of modulated proteins by 1-MCP and O₃ treatments in fruit artificially inoculated with *Botrytis cinerea* based on their functional category.

Treatments ^a									
^b Functional classification	Control vs. Control-Bc (n=50)			Control vs. 1-MCP-Bc (n=49)			Control vs. O ₃ -Bc (n=40)		
	^c Modulation (%)								
	Total	Up	Down	Total	Up	Down	Total	Up	Down
Metabolism	30	38	19	22	30	15	30	35	24
Energy	10	10	10	16	17	15	10	9	12
Cell growth/division	0	0	0	2	4	0	0	0	0
Transcription	0	0	0	2	0	4	3	0	6
Protein synthesis	0	0	0	4	9	0	0	0	0
Protein destination and storage	4	0	10	20	17	23	13	4	24
Transporters	6	7	5	4	4	4	5	0	12
Intracellular traffic	0	0	0	0	0	0	0	0	0
Cell structure	6	7	5	4	4	4	0	0	0
Signal transduction	4	3	5	0	0	0	8	9	6
Disease/defense	30	31	29	16	9	23	25	39	6
Unclear classification	8	3	14	2	4	0	5	4	6
Secondary metabolism	2	0	5	6	0	12	3	0	6

^a Control: untreated and non-inoculated fruit, Control-Bc: Untreated fruit, inoculated with *B. cinerea*, 1-MCP-Bc: fruit treated with 1-MCP and inoculated with *B. cinerea*, O₃-Bc: fruit incubated in O₃ enriched-atmosphere chamber and inoculated with *B. cinerea*

^b Functional category, proteins ontologically classified into functional categories proposed by Bevan et al. (1998).

^c Percentage of total, up- or down-regulated proteins to each functional category

Table S3. Quantitative data for apple protein spot volumes on 2DE-gels. Protein extracts have been analyzed by two-dimensional electrophoresis and 2D-gels were submitted to image analysis as described in experimental procedures

Spot N°	Control Mean	Control CV (%)	Control-Bc Mean	Control-Bc CV (%)	Ratio Control Mean/Control-Bc Mean	p-value Control/Control-Bc	1-MCP-Bc Mean	1-MCP-Bc CV (%)	Ratio 1-MCP-Bc Mean/Control Mean	p-value 1-MCP-Bc/Control	O ₃ -Bc Mean	O ₃ -Bc CV (%)	Ratio O ₃ -Bc Mean/Control Mean	p-value O ₃ -Bc/Control	1-MCP Mean	1-MCP CV (%)	Ratio 1-MCP Mean/Control Mean	p-value 1-MCP/Control	O ₃ Mean	O ₃ CV (%)	Ratio O ₃ Mean/Control Mean	p-value O ₃ /Control	Ratio O ₃ Mean/1-MCP Mean	p-value O ₃ /1-MCP
23	1233.1	23.60%	573	16.20%	0.465	0.02	966.2	18.90%	0.784	0.25	1398.4	14.80%	1.13	0.47	725	18.90%	0.59	0.052	594.8	35.60%	0.48	0.037	0.82	0.422
50	3382.3	33.90%	1124.9	31.40%	0.333	0.031	2236.1	15.60%	0.661	0.17	2909.4	13.90%	0.86	0.54	2501.6	7.70%	0.74	0.26	1467.8	21.10%	0.43	0.049	0.59	0.008
109	276.5	26.40%	977.4	30.90%	3.535	0.017	376.3	28.40%	1.361	0.25	1189.8	10.00%	4.30	0.00	376.9	8.80%	1.36	0.095	529.3	31.00%	1.91	0.071	1.40	0.19
205	704.6	8.00%	293.3	26.00%	0.416	0.002	303.1	21.30%	0.430	0.00	325.5	12.60%	0.46	0.00	368.4	17.60%	0.52	0.002	249.9	63.30%	0.35	0.009	0.68	0.296
313	1030.8	1.20%	733.3	46.30%	0.711	0.204	1583.2	22.80%	1.536	0.06	1050.2	18.70%	1.02	0.87	1184.4	12.90%	1.15	0.157	661	47.80%	0.64	0.113	0.56	0.061
317	823.3	9.00%	351.8	15.50%	0.427	0.001	438.9	28.30%	0.533	0.01	449.3	7.10%	0.55	0.00	512.6	14.20%	0.62	0.007	357.2	33.50%	0.43	0.005	0.70	0.127
625	1895.5	15.60%	1956.8	10.30%	1.032	0.782	1949.8	9.20%	1.029	0.80	2004.6	0.40%	1.06	0.56	2631.3	6.30%	1.39	0.02	4341.2	3.90%	2.29	0	1.65	0
923	240.1	17.40%	185.2	14.90%	0.771	0.131	94.3	26.50%	0.393	0.01	263	15.60%	1.10	0.54	547.1	116.60%	2.28	0.452	276.4	7.00%	1.15	0.244	0.51	0.503
1026	1540.8	21.90%	1279.1	28.70%	0.830	0.416	1221.1	20.50%	0.793	0.26	1438.7	1.90%	0.93	0.63	521.7	9.10%	0.34	0.007	143.1	14.30%	0.09	0.002	0.27	0
1034	665.6	5.70%	1162.9	4.60%	1.747	0	1047.4	25.30%	1.574	0.07	1707.7	20.50%	2.57	0.01	567.9	13.10%	0.85	0.112	1151.3	66.90%	1.73	0.337	2.03	0.262
1037	536.7	11.70%	2422.3	13.80%	4.513	0.001	790.2	34.70%	1.472	0.19	3244.2	12.10%	6.04	0.00	696.4	18.50%	1.30	0.126	1559.9	36.60%	2.91	0.037	2.24	0.063
1038	510.8	17.40%	1131.5	20.10%	2.215	0.012	544.7	57.90%	1.066	0.87	1751	16.90%	3.43	0.00	221.3	67.40%	0.43	0.045	973.7	14.10%	1.91	0.008	4.40	0.003
1039	1550.5	3.70%	808.4	16.10%	0.521	0.001	445.4	53.00%	0.287	0.00	847.7	9.70%	0.55	0.00	709.9	28.60%	0.46	0.002	785	29.30%	0.51	0.005	1.11	0.694
1130	2276.6	1.30%	1350	17.80%	0.593	0.003	752.4	29.70%	0.330	0.00	1541.2	13.60%	0.68	0.00	1158.3	17.00%	0.51	0.001	662.4	43.10%	0.29	0.001	0.57	0.069
1132	1258.5	7.20%	645.9	19.50%	0.513	0.002	1306.9	17.70%	1.038	0.75	1013.6	9.90%	0.81	0.03	1244.4	6.00%	0.99	0.844	499.9	14.80%	0.40	0	0.40	0
1212	1745	19.60%	4360.4	10.30%	2.499	0.001	1670.5	17.10%	0.957	0.79	3710	3.20%	2.13	0.00	1129.2	10.10%	0.65	0.041	3543.7	55.10%	2.03	0.191	3.14	0.099
1220	1204.4	3.70%	1349.3	11.50%	1.120	0.196	648.6	13.80%	0.539	0.00	1129.7	12.90%	0.94	0.44	799.3	25.20%	0.66	0.027	715.3	55.50%	0.59	0.101	0.89	0.76
1507	2080.1	8.50%	1023.8	7.20%	0.492	0.001	1738.3	12.00%	0.836	0.10	1965.2	5.90%	0.94	0.40	1845.4	3.50%	0.89	0.097	1890.5	13.10%	0.91	0.341	1.02	0.775
1510	967.7	16.30%	1292.5	43.40%	1.336	0.389	1703	16.20%	1.760	0.02	718.5	7.70%	0.74	0.06	957.2	80.80%	0.99	0.983	541.9	32.20%	0.56	0.035	0.57	0.416
1511	1189.8	13.10%	920.7	24.20%	0.774	0.162	826.5	13.50%	0.695	0.03	520.9	11.60%	0.44	0.00	645.5	8.60%	0.54	0.005	385.3	24.00%	0.32	0.002	0.60	0.014
1624	1827.7	24.10%	646.6	14.30%	0.354	0.01	1401.8	3.50%	0.767	0.17	654	4.60%	0.36	0.01	1009.4	7.90%	0.55	0.034	1137.9	8.00%	0.62	0.056	1.13	0.141
1725	907.1	16.20%	563.1	18.50%	0.621	0.03	764.7	9.60%	0.843	0.21	590.6	23.30%	0.65	0.05	984.3	9.20%	1.09	0.483	1277.3	19.70%	1.41	0.092	1.30	0.13
2029	533.4	19.90%	2619.6	14.20%	4.911	0.001	665.3	40.80%	1.247	0.48	3644.6	15.40%	6.83	0.00	197.6	27.00%	0.37	0.008	2789.6	10.00%	5.23	0	14.12	0
2032	2565.2	6.20%	2240.3	20.40%	0.873	0.309	3032.7	23.40%	1.182	0.33	4055.4	19.00%	1.58	0.03	1510.5	12.30%	0.59	0.002	1200.8	33.40%	0.47	0.005	0.79	0.292
2110	1355.5	12.50%	478.2	17.90%	0.353	0.001	929.5	7.40%	0.686	0.02	720.1	6.40%	0.53	0.00	1038.1	15.50%	0.77	0.078	636.5	47.70%	0.47	0.023	0.61	0.113
2113	823.2	4.70%	411.1	9.20%	0.499	0	658.5	23.00%	0.800	0.14	363.6	11.70%	0.44	0.00	685.2	22.80%	0.83	0.211	318.1	62.30%	0.39	0.012	0.46	0.065
2115	3563.3	3.40%	1576.2	6.20%	0.442	0	2542.4	18.60%	0.713	0.02	2632.6	12.40%	0.74	0.01	4462.7	9.70%	1.25	0.026	2199.3	13.50%	0.62	0.002	0.49	0.002
2213	6234	8.40%	11211.6	1.80%	1.798	0	5544	6.60%	0.889	0.13	8510.1	7.70%	1.37	0.01	5906.7	10.70%	0.95	0.528	7733.3	11.90%	1.24	0.07	1.31	0.047
2320	1350.7	2.00%	4056	20.60%	3.003	0.005	1792.2	23.00%	1.327	0.14	1648	2.30%	1.22	0.00	1284.8	15.90%	0.95	0.609	1444.4	18.90%	1.07	0.585	1.12	0.462
2326	668.5	15.40%	509.6	11.00%	0.762	0.079	599.9	2.30%	0.897	0.32	792.1	15.40%	1.18	0.25	347.2	2.20%	0.52	0.006	298.2	9.20%	0.45	0.004	0.86	0.041
2631	1178	17.70%	727.3	9.10%	0.617	0.023	516.2	4.20%	0.438	0.01	864.3	12.30%	0.73	0.08	292.8	16.10%	0.25	0.002	422.8	11.50%	0.36	0.004	1.44	0.029
2634	1357.5	1.70%	1089.2	37.80%	0.802	0.323	633.5	2.90%	0.467	0.00	486.5	2.30%	0.36	0.00	535.1	8.60%	0.39	0	648.4	3.90%	0.48	0	1.21	0.02
2636	1317.7	20.60%	809.2	7.80%	0.614	0.034	644.3	1.50%	0.489	0.01	784.4	0.80%	0.60	0.03	527.9	1.40%	0.40	0.007	757.8	18.90%	0.58	0.034	1.44	0.05
2727	756	20.40%	617.4	5.30%	0.817	0.202	374.1	10.70%	0.495	0.01	234.4	1.90%	0.31	0.00	830.4	7.80%	1.10	0.484	726.7	6.70%	0.96	0.769	0.88	0.091
2839	225.7	9.50%	325.9	5.50%	1.444	0.003	496.6	14.00%	2.200	0.00	306.7	6.50%	1.36	0.01	562.8	4.20%	2.49	0	670.3	6.40%	2.97	0	1.19	0.019
3040	1324.9	20.30%	1941.1	17.30%	1.465	0.068	2384.3	16.60%	1.800	0.02	3017.8	39.00%	2.28	0.07	572.5	11.60%	0.43	0.009	651.9	65.50%	0.49	0.082	1.14	0.766
3211	109.9	10.70%	267.3	9.30%	2.432	0.001	129	14.80%	1.174	0.21	219.4	9.80%	2.00	0.00	93.6	15.90%	0.85	0.21	155.3	17.00%	1.41	0.053	1.66	0.024
3220	1232.3	17.40%	2597.9	19.00%	2.108	0.012	1435.5	18.20%	1.165	0.36	2200	10.10%	1.79	0.01	532	6.40%	0.43	0.005	913.4	10.70%	0.74	0.079	1.72	0.003
3322	1240.5	13.20%	1506.4	22.20%	1.214	0.284	1676.3	1.00%	1.351	0.01	1213.4	4.80%	0.98	0.80	687.5	8.30%	0.55	0.005	626.2	49.10%	0.50	0.038	0.91	0.752
3324	815.1	14.80%	2476.1	11.90%	3.038	0.001	897.4	4.80%	1.101	0.33	2101.8	19.10%	2.58	0.01	1179.3	10.00%	1.45	0.02	1989.1	44.60%	2.44	0.086	1.69	0.192
3325	1507.9	5.10%	1462.8	15.70%	0.970	0.764	405.8	10.60%	0.269	0.00	1549.5	2.40%	1.03	0.44	1026.1	9.20%	0.68	0.002	509.1	30.80%	0.34	0.001	0.50	0.008
3327	705.1	22.60%	1455	12.30%	2.064	0.006	1060.2	7.00%	1.504	0.02	973.6	21.40%	1.38	0.15	820.1	15.90%	1.16	0.388	588.5	7.70%	0.83	0.29	0.72	0.044
3522	1433.9	4.60%	443.5	20.80%	0.309	0	447.9	23.30%	0.312	0.00	273	8.10%	0.19	0.00	1201.9	19.30%	0.84	0.17	924.7	15.40%	0.64	0.005	0.77	0.152
3719	131.2	10.40%	738.3	3.70%	5.627	0	158.8	4.20%	1.210	0.03	483	15.70%	3.68	0.00	128.8	10.10%	0.98	0.841	597.6	46.30%	4.55	0.043	4.64	0.043
4016	6123.1	10.40%	12124.4	8.50%	1.980	0.001	8677.2	12.50%	1.417	0.02	12580.9	12.90%	2.05	0.00	5000.1	9.70%	0.82	0.072	11064.9	9.80%	1.81	0.002	2.21	0.001
4026	5944.4	10.80%	5078.3	6.10%	0.854	0.103	2782.7	14.50%	0.468	0.00	2090.6	8.50%	0.35	0.00	7916.8	6.40%	1.33	0.014	4971	31.70%	0.84	0.377	0.63	0.037
4105	1006.1	4.10%	689	20.10%	0.685	0.019	463	16.60%	0.460	0.00	279.8	7.40%	0.28	0.00	320.4	14.70%	0.32	0	269.5	65.80%	0.27	0.002	0.84	0.656
4313	454.8	18.30%	1187.7	7.50%	2.611	0	682.4	22.00%	1.500	0.08	1032.3	13.70%	2.27	0.00	611.6	4.70%	1.34	0.037	1042.1	15.20%	2.29	0.005	1.70	0.01
4314	246	7.40%	545.4	21.60%	2.217	0.012	324.1	18.10%	1.318	0.09	430.6	16.40%	1.75	0.01	284.6	18.20%	1.16	0.291	388.1					

6218	2461.5	11.60%	3229.9	6.20%	1.312	0.019	2487.8	13.80%	1.011	0.92	2285.5	16.40%	0.93	0.55	2457.1	11.80%	1.00	0.986	1053.7	56.40%	0.43	0.021	0.43	0.021
6222	331.5	17.50%	470.2	6.00%	1.418	0.02	428.9	18.40%	1.294	0.16	393.1	17.80%	1.19	0.31	1140.1	15.90%	3.44	0.002	743.5	65.50%	2.24	0.219	0.65	0.257
6225	1567.4	10.80%	1906.7	13.40%	1.216	0.128	1526.1	14.10%	0.974	0.81	1463.9	14.40%	0.93	0.54	603.3	16.30%	0.38	0.001	373.5	52.30%	0.24	0.00	0.62	0.143
6545	252.5	9.40%	461	10.60%	1.826	0.003	590.1	7.90%	2.337	0.00	341.6	10.60%	1.35	0.02	463.6	18.30%	1.84	0.014	447.1	12.10%	1.77	0.00	0.96	0.791
6834	271.9	7.30%	506.9	11.80%	1.864	0.003	549.1	28.90%	2.019	0.04	368.5	8.80%	1.36	0.01	583.1	7.20%	2.14	0	671.9	23.50%	2.47	0.01	1.15	0.4
6835	934.8	8.90%	1057.3	19.20%	1.131	0.389	1838	7.10%	1.966	0.00	869.2	8.20%	0.93	0.36	1173.6	6.40%	1.26	0.021	1486.9	59.70%	1.59	0.34	1.27	0.576
6905	209.5	8.40%	371.5	4.30%	1.773	0	515	2.50%	2.458	0.00	280.4	9.70%	1.34	0.02	303.2	1.80%	1.45	0.001	473.3	18.90%	2.26	0.00	1.56	0.03
6908	259.4	12.90%	589.3	13.30%	2.272	0.003	568.3	14.50%	2.191	0.00	361.7	16.30%	1.39	0.06	355.1	7.70%	1.37	0.019	501.3	88.80%	1.93	0.40	1.41	0.601
7012	2364.1	9.30%	1684.3	17.20%	0.712	0.032	2177.7	17.00%	0.921	0.49	2349.6	21.30%	0.99	0.97	1122.1	20.70%	0.47	0.003	890.2	10.60%	0.38	0	0.79	0.184
7220	397.7	6.60%	989.9	18.80%	2.489	0.006	640.8	17.40%	1.611	0.02	770.4	3.70%	1.94	0.00	255.5	12.50%	0.64	0.004	278.8	19.00%	0.70	0.02	1.09	0.552
7222	1017.7	5.70%	1684.7	2.10%	1.655	0	511.3	0.60%	0.502	0.00	1385.3	7.90%	1.36	0.01	910	16.40%	0.89	0.31	1240.6	16.90%	1.22	0.15	1.36	0.091
7226	1025.6	14.50%	2240.5	7.50%	2.185	0.001	978.4	1.50%	0.954	0.61	2171.7	31.00%	2.12	0.04	603.5	16.90%	0.59	0.015	1014.6	52.10%	0.99	0.97	1.68	0.257
7227	355.5	15.30%	452	13.70%	1.271	0.113	256.4	3.30%	0.721	0.04	431.6	6.90%	1.21	0.10	177.5	13.90%	0.50	0.007	211.9	57.20%	0.60	0.13	1.19	0.656
7711	6097.3	20.20%	6236.9	10.00%	1.023	0.869	7059.6	13.10%	1.158	0.34	6176.7	7.70%	1.01	0.92	2685.2	28.00%	0.44	0.015	2472.8	12.90%	0.41	0.00	0.92	0.675
7720	1773.2	8.40%	4388.7	11.20%	2.475	0.001	3989.7	11.40%	2.250	0.00	3823.7	12.20%	2.16	0.00	3127	10.90%	1.76	0.003	2992.1	18.40%	1.69	0.02	0.96	0.736
7728	745.5	9.10%	925.2	12.50%	1.241	0.081	1201.2	9.40%	1.611	0.00	747.9	5.10%	1.00	0.96	685	50.20%	0.92	0.78	349.8	20.10%	0.47	0.00	0.51	0.174
8115	842.6	6.10%	1391.8	21.90%	1.652	0.037	496.2	3.80%	0.589	0.00	1214.3	9.90%	1.44	0.01	547.9	14.90%	0.65	0.006	1379.7	13.30%	1.64	0.00	2.52	0.002
8116	139.9	21.90%	319.4	16.70%	2.283	0.007	164.9	3.40%	1.179	0.24	299.9	20.00%	2.14	0.01	218.2	11.90%	1.56	0.028	149.1	14.40%	1.07	0.69	0.68	0.024
8123	571.8	5.80%	543.3	10.40%	0.950	0.494	556.8	2.10%	0.974	0.50	521.4	8.20%	0.91	0.18	328.9	9.90%	0.58	0.001	237.7	4.80%	0.42	0	0.72	0.01
8232	533.8	18.30%	1122.7	3.80%	2.103	0.001	704.6	13.30%	1.320	0.09	1160.1	4.70%	2.17	0.00	177.2	3.60%	0.33	0.003	414	6.80%	0.78	0.11	2.34	0
8319	354.4	6.40%	650	8.10%	1.834	0.001	711.6	8.60%	2.008	0.00	357.5	8.90%	1.01	0.90	732.5	12.50%	2.07	0.002	511.9	10.80%	1.44	0.01	0.70	0.023
8327	389.5	12.80%	411.1	2.80%	1.055	0.508	784	19.00%	2.013	0.01	611.5	15.90%	1.57	0.02	384.3	14.70%	0.99	0.91	465.2	2.30%	1.19	0.06	1.21	0.071
8530	350.7	27.90%	494.4	9.80%	1.410	0.085	720.3	20.30%	2.054	0.02	428.2	17.00%	1.22	0.33	489.2	14.30%	1.39	0.117	408.5	14.40%	1.16	0.43	0.84	0.201
8606	420	19.50%	641.5	16.30%	1.527	0.045	731.5	13.10%	1.742	0.01	735.3	15.10%	1.75	0.02	299.1	14.40%	0.71	0.086	364.7	17.40%	0.87	0.40	1.22	0.213
8608	340	6.70%	612.6	16.90%	1.802	0.011	719.2	1.50%	2.115	0.00	496.6	6.30%	1.46	0.00	625.2	18.40%	1.84	0.013	351	5.10%	1.03	0.54	0.56	0.015
8726	201.5	9.10%	247	12.60%	1.226	0.094	268.3	3.30%	1.332	0.00	263.2	6.50%	1.31	0.01	170	9.90%	0.84	0.094	122.1	8.00%	0.61	0.00	0.72	0.013
8728	446.7	9.90%	694.6	5.00%	1.555	0.002	897.5	11.00%	2.009	0.00	542.3	6.70%	1.21	0.04	725.2	17.80%	1.62	0.024	390.9	9.40%	0.88	0.16	0.54	0.013
8732	811.5	18.00%	1060.3	20.10%	1.307	0.171	1100.2	2.80%	1.356	0.03	1107.9	5.40%	1.37	0.03	478.9	13.00%	0.59	0.022	438.3	39.60%	0.54	0.04	0.92	0.723
9117	850.1	14.60%	264.9	30.90%	0.312	0.002	1085.4	42.10%	1.277	0.44	548.4	4.60%	0.65	0.01	769.7	36.10%	0.91	0.671	776.3	78.80%	0.91	0.84	1.01	0.987
9118	1025.6	7.50%	1323	2.00%	1.290	0.003	1139.6	31.20%	1.111	0.62	1137.9	26.50%	1.11	0.57	261.1	20.40%	0.25	0	206.5	12.40%	0.20	0	0.79	0.185
9221	1461.4	16.40%	257	18.30%	0.176	0.001	1548.7	9.00%	1.060	0.62	332	19.10%	0.23	0.00	1849.7	8.20%	1.27	0.077	238.6	9.20%	0.16	0.00	0.13	0
9227	594.6	5.50%	756.8	10.20%	1.273	0.028	1100.4	15.50%	1.851	0.01	755.6	38.00%	1.27	0.39	484.2	38.60%	0.81	0.37	584	57.70%	0.98	0.96	1.21	0.677
9313	626.7	34.80%	1304.2	5.30%	2.081	0.007	1368.8	19.80%	2.184	0.02	820.6	30.10%	1.31	0.37	503	30.60%	0.80	0.467	682.5	16.50%	1.09	0.71	1.36	0.178
9314	452.2	45.80%	543.3	14.30%	1.201	0.515	1232.4	12.50%	2.725	0.01	837.2	57.60%	1.85	0.27	269.4	46.30%	0.60	0.261	316	64.50%	0.70	0.46	1.17	0.752
9603	429.4	14.70%	826.6	13.10%	1.925	0.005	1010.4	13.50%	2.353	0.00	809.4	15.20%	1.88	0.01	438.8	4.10%	1.02	0.816	362.7	34.70%	0.84	0.45	0.83	0.359
9708	168.9	15.20%	399.2	11.80%	2.364	0.002	462.8	19.80%	2.740	0.01	386.8	15.60%	2.29	0.00	300.4	15.20%	1.78	0.012	190.7	38.30%	1.13	0.65	0.63	0.092

Spot N°, spot label on the reference 2DE-map presented in **Figure S1**;

Control Mean, average of normalized spot volumes of untreated apple fruit as obtained from densitometric analysis of individual spots from proteins in 2D gels stained with silver nitrate from three different gels and independent extractions

Control Coef. Var %, Coefficient of variation of normalized spot volumes of untreated fruit as obtained from densitometric analysis of individual spots from proteins in 2D gels stained with silver nitrate from three different gels and independent extractions

Control-Bc Mean, average of normalized spot volumes of untreated fruit inoculated with *B. cinerea*, as obtained from densitometric analysis of individual spots from proteins in 2D gels stained with silver nitrate from three different gels and independent extractions

Control-Bc Coef. Var %, Coefficient of variation of normalized spot volumes of untreated apple fruit inoculated with *B. cinerea* as obtained from densitometric analysis of individual spots from proteins in 2D gels stained with silver nitrate from three different gels and independent extractions

Ratio Control-Bc/Control Mean, The Control-Bc (Mean) spot volume divided by the Control (Mean) spot volume

P value Control-Bc/Control, p value between normalized spot volumes of individual spots from three different gels and independent extractions of untreated apple fruit and normalized spot volumes of individual spots from three different gels and independent extractions of apple fruit inoculated with *B. cinerea*, as obtained from densitometric analysis of individual spots from proteins in 2D gels stained with silver nitrate from three different gels and independent extractions

1-MCP-Bc Mean, average of normalized spot volumes of 1-MCP treated apple fruit inoculated with *B. cinerea* as obtained from densitometric analysis of individual spots from proteins in 2D gels stained with silver nitrate from three different gels and independent extractions

1-MCP-Bc Coef. Var %, Coefficient of variation of normalized spot volumes of 1-MCP treated apple fruit inoculated with *B. cinerea* as obtained from densitometric analysis of individual spots from proteins in 2D gels stained with silver nitrate from three different gels and independent extractions

Ratio 1-MCP-Bc Mean/Control Mean, the 1-MCP-Bc (Mean) spot volume divided by the Control (Mean) spot volume

P value 1-MCP-Bc / Control, p value between normalized spot volumes of individual spots from three different gels and independent extractions of inoculated 1-MCP treated apple fruit and normalized spot volumes of individual spots from three different gels and independent extractions of untreated apple fruit as obtained from densitometric analysis of individual spots from proteins in 2D gels stained with silver nitrate from three different gels and independent extractions

O₃-Bc Mean, average of normalized spot volumes of O₃ treated apple fruit inoculated with *B. cinerea* as obtained from densitometric analysis of individual spots from proteins in 2D gels stained with silver nitrate from three different gels and independent extractions

O₃-Bc Coef. Var %, Coefficient of variation of normalized spot volumes of O₃ treated apple fruit inoculated with *B. cinerea* as obtained from densitometric analysis of individual spots from proteins in 2D gels stained with silver nitrate from three different gels and independent extractions

Ratio O₃-Bc Mean/Control Mean, the O₃-Bc (Mean) spot volume divided by the Control (Mean) spot volume

P value O₃-Bc / Control, p value between normalized spot volumes of individual spots from three different gels and independent extractions of inoculated O₃ treated apple fruit and normalized spot volumes of individual spots from three different gels and independent extractions of untreated apple fruit as obtained from densitometric analysis of individual spots from proteins in 2D gels stained with silver nitrate from three different gels and independent extractions

1-MCP Mean, average of normalized spot volumes of 1-MCP apple fruit as obtained from densitometric analysis of individual spots from proteins in 2D gels stained with silver nitrate from three different gels and independent extractions

1-MCP Coef. Var %, Coefficient of variation of normalized spot volumes of 1-MCP treated fruit as obtained from densitometric analysis of individual spots from proteins in 2D gels stained with silver nitrate from three different gels and independent extractions

Ratio 1-MCP Mean/Control Mean, the 1-MCP (Mean) spot volume divided by the Control (Mean) spot volume

P value 1-MCP/Control, p value between normalized spot volumes of individual spots from three different gels and independent extractions of 1-MCP treated apple fruit and normalized spot volumes of individual spots from three different gels and independent extractions of untreated apple fruit as obtained from densitometric analysis of individual spots from proteins in 2D gels stained with silver nitrate from three different gels and independent extractions

O₃ Mean, average of normalized spot volumes of O₃ apple fruit as obtained from densitometric analysis of individual spots from proteins in 2D gels stained with silver nitrate from three different gels and independent extractions

O₃ Coef. Var %, Coefficient of variation of normalized spot volumes of O₃ treated fruit as obtained from densitometric analysis of individual spots from proteins in 2D gels stained with silver nitrate from three different gels and independent extractions

Ratio O₃ Mean/Control Mean, the O₃ (Mean) spot volume divided by the Control (Mean) spot volume

P value O₃/Control, p value between normalized spot volumes of individual spots from three different gels and independent extractions of O₃ treated apple fruit and normalized spot volumes of individual spots from three different gels and independent extractions of untreated apple fruit as obtained from densitometric analysis of individual spots from proteins in 2D gels stained with silver nitrate from three different gels and independent extractions

Ratio O₃ Mean/1-MCP Mean, The O₃ (Mean) spot volume divided by the 1-MCP (Mean) spot volume

P value O₃/1-MCP, p value between normalized spot volumes of individual spots from three different gels and independent extractions of O₃ treated apple fruit and normalized spot volumes of individual spots from three different gels and independent extractions of 1-MCP treated apple fruit as obtained from densitometric analysis of individual spots from proteins in 2D gels stained with silver nitrate from three different gels and independent extractions

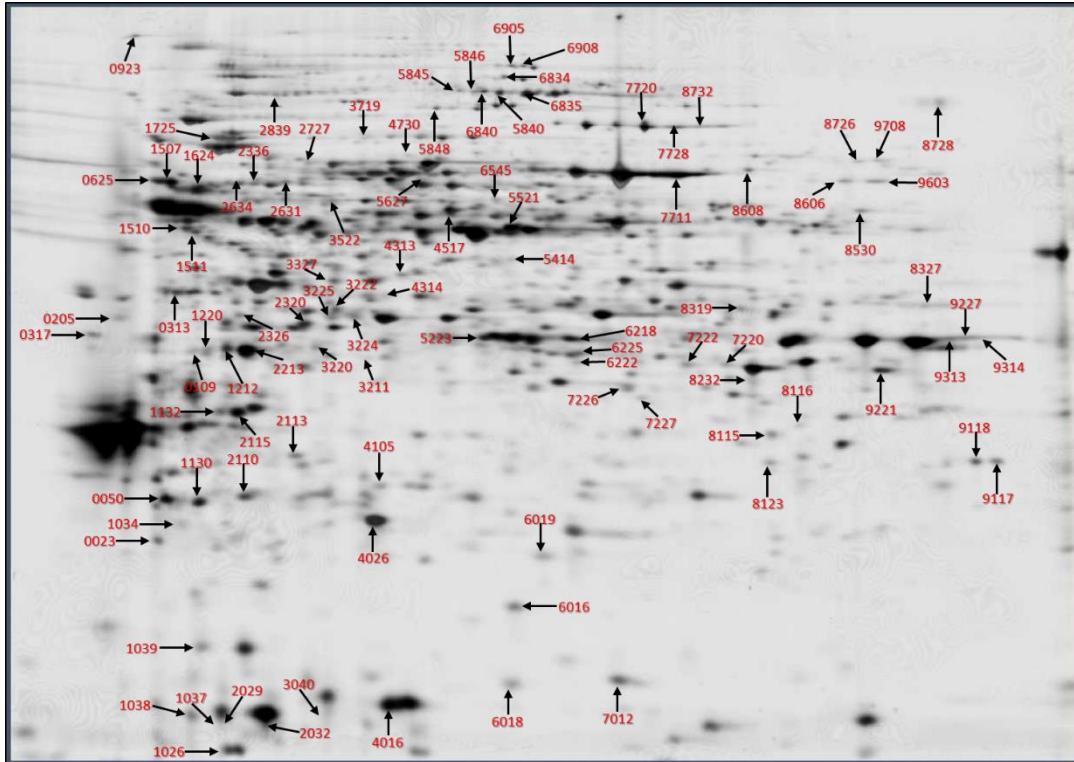


Figure S1. Reference map for apple fruit proteins. The specific proteins spots labelled with the arrows correspond to proteins listed in the Supplementary Data **Table S1A & B**.

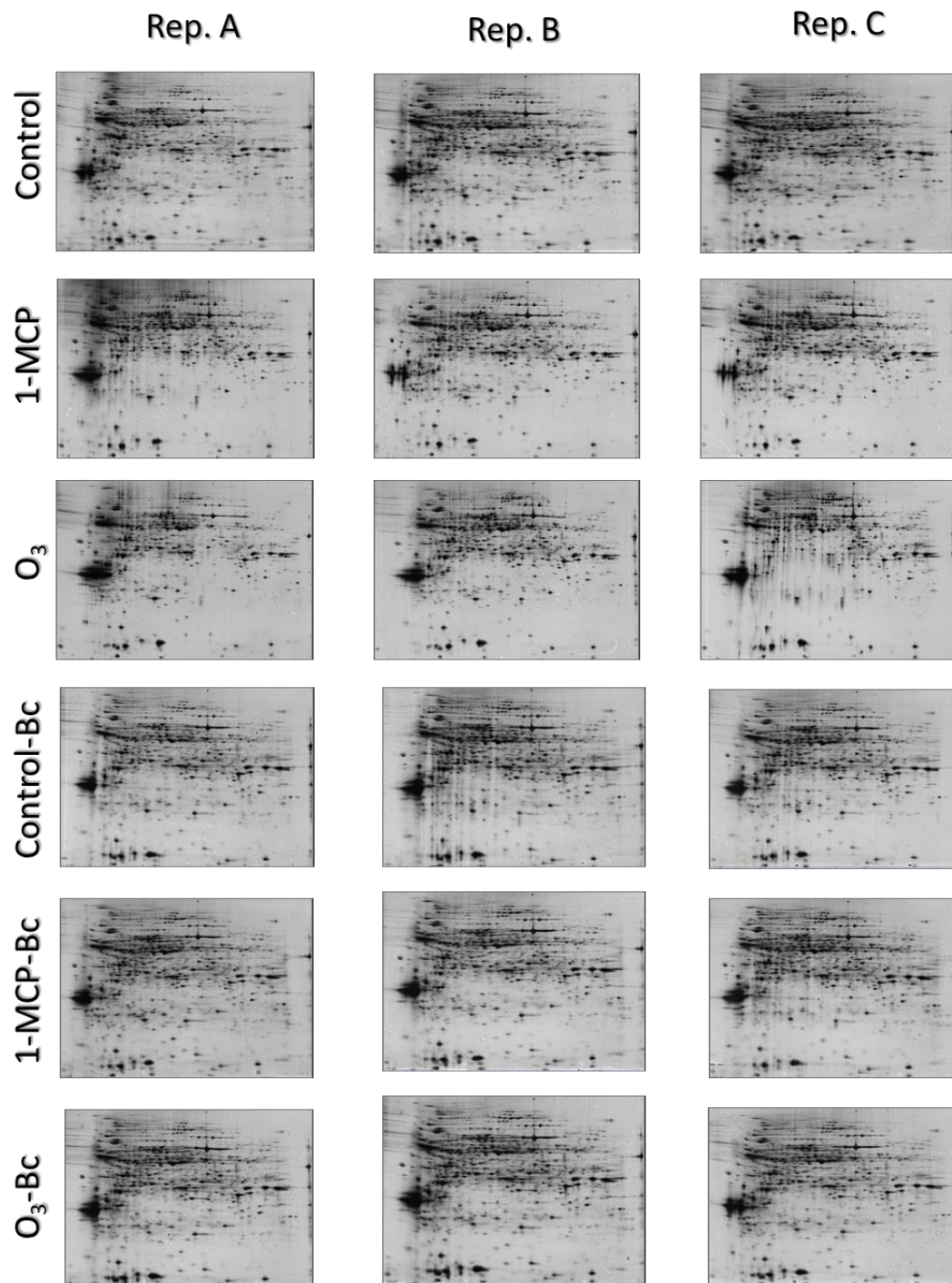


Figure S2. Full-length gels of apple fruit proteins of non-inoculated ('Control,' '1-MCP' and 'O₃')/or inoculated with *B. cinerea* pathogen ('Control-Bc', 1-MCP-Bc' and 'O₃-Bc') of untreated or 1-MCP and O₃ treated apple fruit in which zoomed in views of selected areas are presented in **Figure 3**.