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ECOFISIOLOGIA DA CEREJEIRA (*Prunus avium* L.), COMPOSIÇÃO FENÓLICA E ACTIVIDADE ANTIOXIDANTE DOS FRUTOS

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Resumo

No contexto da fruticultura moderna, a cerejeira (*Prunus avium* L.) tem uma elevada importância económica, havendo, por isso, a preocupação especial de formar árvores produtoras de cerejas com elevada qualidade, mas com porte mais ananicante devido aos elevados custos da mão-de-obra na colheita e noutras práticas culturais do pomar. Assim, a expansão e a exploração rentável desta cultura implicam a aquisição de conhecimentos, entre outros, nos domínios da ecofisiologia das novas combinações cultivar/porta-enxerto e das características físico-químicas dos frutos. Para isso, traçaram-se três objectivos principais: (1) Estudar a ecofisiologia da cerejeira em 15 combinações cultivar/porta-enxerto, ao nível das características morfo-anatómicas, bioquímicas e fisiológicas de árvores ananicantes *versus* árvores vigorosas; (2) Estudar o efeito da cultivar, do estado de maturação, do ano e da conservação pós-colheita na qualidade sensorial e nutricional da cereja, através de análises físico-químicas (principalmente de compostos fenólicos); (3) Avaliar o potencial antioxidante dos extractos de cereja, à colheita e depois da conservação.

Os estudos no âmbito deste trabalho incidiram sobre quatro cultivares de cerejeira, a Burlat, a Saco, a Summit e a Van, por produzirem frutos com boa aptidão para consumo em fresco e terem elevado potencial produtivo. As cultivares foram enxertadas em cinco porta-enxertos, com efeito ananicante crescente: *Prunus avium* < CAB 11E < Maxma 14 < Gisela 5 < Edabriz. As características gerais dos porta-enxertos e das cultivares encontram-se nos Anexo I e II, respectivamente. A parte experimental deste estudo decorreu basicamente durante 2001–2003, num pomar de cerejeiras instalado em 1999, sito em Abambres, Vila Real. Refere-se, no entanto, que se fez o acompanhamento do ensaio desde a instalação dos porta-enxertos no viveiro em 1997, da sua enxertia em 1998 e posterior plantação em 1999. Os resultados obtidos estão estruturados em oito capítulos, sete dos quais (capítulos 2–8), incluem integralmente a informação publicada ou submetida em revistas indexadas na lista do *Journal Citation Reports*.

O capítulo 2 descreve o estudo cujo principal objectivo foi avaliar os efeitos do atarraque do porta-enxerto e da densidade de plantação no crescimento e nas relações hídricas das cerejeiras. Em 1997, iniciou-se um ensaio com os cinco porta-enxertos sujeitos a diferentes intensidades de atarraque (0, 30, 60 e 90% do crescimento vegetativo foi removido, designando-se por P1, P2, P3 e P4, respectivamente) após terem sido plantados em dois compassos de plantação (S1 = 0,25 x 1,0 m e S2 = 0,45 x 1,5 m). O crescimento vegetativo e o potencial hídrico foliar foram quantificados durante a estação de crescimento. Dos resultados obtidos, concluiu-se que o atarraque à plantação afectou o crescimento do

sistema radicular dos porta-enxertos. O não atarraque (P1) originou plantas com maior comprimento e peso do sistema radicular face às plantas com atarraque mais intenso (P4). Por sua vez, a combinação S1/P1 permitiu a obtenção de uma maior biomassa radicular, enquanto S1/P4 e S2/P4 deram origem às menores biomassas radiculares. O potencial hídrico foliar (Ψ_{folha}) variou significativamente entre os porta-enxertos e com o compasso de plantação, mas não com o atarraque. Os porta-enxertos Maxma 14 e *Prunus avium* atingiram os valores mais negativos de Ψ_{folha} ao meio-dia, mas os maiores valores antes do amanhecer. Em geral, os porta-enxertos apresentaram Ψ_{folha} mais negativos no compasso mais apertado.

No capítulo 3 pretendeu-se estudar a arquitectura hidráulica e as limitações ao transporte da água em combinações cultivar/porta-enxerto. Para tal escolheu-se a cultivar Van enxertada nos cinco porta-enxertos e avaliou-se a anatomia do xilema, a condutividade hidráulica relativa (RC) e o índice de vulnerabilidade (VI) de raízes de diâmetro < 2 mm, 2–5 mm e > 5 mm e de caules. Foram ainda determinadas as relações hídricas, trocas gasosas e variações no crescimento. As raízes apresentaram vasos xilémicos de maior diâmetro (VD), maior RC e VI do que os caules das 5 combinações Van/porta-enxertos. Também se verificou uma maior frequência de vasos xilémicos (VF), mas menor VD, RC e VI das árvores ananicantes, i.e., enxertadas em Edabriz e Gisela 5, do que as árvores nos porta-enxertos mais vigorosos *Prunus avium*, CAB 11E e Maxma 14. Imposições anatómicas provocadas pelos vasos xilémicos de menor diâmetro no estado de hidratação das árvores ananicantes conduzem a uma série de *feedbacks* negativos, tais como uma redução na RC, Ψ, trocas gasosas e crescimento.

No capítulo 4 procurou-se estudar o efeito da arquitectura da copa nas variações morfo-anatómicas (massa foliar por unidade de área – LMA, densidade da folha – LD, espessura dos diferentes tecidos e densidade estomática), composição química (pigmentos fotossintéticos, açúcares solúveis totais – SS, amido – St e fenóis totais – TP), trocas gasosas e potencial hídrico do caule (Ψ_{caule}) verificadas em folhas das cultivares Burlat e Summit (porte erecto, copas muito densas) e Van (porte semi-erecto, copa menos densa). De facto, este estudo indicou que a copa da Van favoreceu uma maior transmitância de luz, com efeitos positivos na taxa fotossintética, especialmente à colheita. No entanto, não se registaram diferenças no estado hídrico das três cultivares. As folhas da Van tinham maior LMA e as variações neste parâmetro estiveram sobretudo associadas à maior espessura do mesófilo e não à respectiva densidade. Anatomicamente, as folhas da Summit e da Van eram mais espessas do que as da Burlat, principalmente devido à maior espessura do mesófilo, embora com menor razão parênquima em paliçada/lacunoso do que a Burlat. A Van tinha a

maior concentração de clorofila total (Chl) por unidade área e a maior razão $Chl_{a/b}$. Por outro lado, a Van apresentou as menores concentrações de SS, St e TP.

No capítulo 5, cujo objectivo era relacionar diversos parâmetros fisiológicos das folhas (relações hídricas, trocas gasosas, fluorescência da clorofila a, transmitância da luz pela copa, pigmentos fotossintéticos e metabolitos) e da qualidade dos frutos (peso, cor, firmeza, pH, índice refractométrico e acidez titulável) em cultivares (Burlat, Summit e Van) enxertadas nos cinco porta-enxertos, constatou-se que o porta-enxerto afectou significativamente todos os parâmetros fisiológicos. Com efeito, as cerejeiras enxertadas em porta-enxertos mais vigorosos apresentaram maior potencial hídrico ao meio-dia (Ψ_{MD}), taxa de assimilação líquida de CO_2 (A), condutância estomática (g_s), concentração de CO_2 intercelular (C_i) e eficiência fotoquímica máxima do fotossistema II (F_v/F_m). O Ψ_{MD} correlacionou-se positivamente com A, g_s e C_i. Também A foi correlacionada positivamente com g_s e os declives das regressões lineares e os respectivos coeficientes de determinação aumentaram dos porta-enxertos mais vigorosos para os mais ananicantes. O efeito do porta-enxerto foi também significativo para os pigmentos fotossintéticos, enquanto que as concentrações de metabolitos e as características físico-químicas dos frutos mostraram-se mais dependentes do efeito da cultivar. Das três cultivares em estudo, as folhas da Burlat tinham as menores concentrações de pigmentos fotossintéticos, mas eram as mais ricas em SS, St e TP. Comparativamente às outras cultivares, a Summit apresentou frutos mais pesados e maiores, independentemente do porta-enxerto. A Burlat revelou frutos menos firmes e com menor concentração de SS e acidez titulável do que os da Van. No entanto, as cerejas da Van tinham menor luminosidade (L^*), croma (C^*) e tonalidade (H^*), o que é indicativo de frutos mais vermelhos e escuros, comparativamente aos da Summit. De uma maneira geral, o Ψ_{MD} foi correlacionado positivamente com o peso do fruto e A foi correlacionada negativamente com a L^* e C^* do fruto.

O capítulo 6 centrou-se no estudo do efeito da cultivar, do estado de maturação (cerejas parcialmente maduras e no estado óptimo de maturação), da conservação (temperatura ambiente – T1, 15 ± 5 ºC e 55–60% H.R., durante uma semana; câmara frigorífica, em atmosfera normal – T2, 1–2 ºC e 90–95% H.R., durante um mês) e do ano (condições climáticas, nomeadamente temperatura e insolação) no perfil fenólico dos frutos das quatro cultivares de cerejeira. Dos resultados obtidos, constatou-se que os ácidos hidroxicinâmicos mais abundantes em todas as cultivares foram o ácido neoclorogénico e o ácido *p*-cumaroilquínico e, em concentração menos elevada, o ácido clorogénico. A cianidina-3-rutinósido e a cianidina-3-glucósido foram identificadas como sendo as principais antocianinas das cerejas. Em contrapartida, a peonidina e a pelargonidina-3-rutinósido

tiveram pouca expressão. A peonidina-3-glucósido apenas foi identificada nas cultivares Burlat e Van. Foram ainda identificados dois flavanóis, em que os compostos deste grupo fenólico mais quantificados foram a epicatequina e a catequina. O flavonol rutina foi também detectado. A cultivar Saco registou as concentrações mais elevadas de fenóis totais (227 mg 100 g^{-1} de peso fresco), contrastando com a Van (124 mg 100 g^{-1} de peso fresco). As concentrações de compostos fenólicos foram sempre mais altas em cerejas maduras do que em cerejas parcialmente maduras. A conservação provocou variações nos ácidos hidroxicinâmicos, sendo a tendência final uma redução das concentrações nas cerejas armazenadas a T2 e um aumento nas cerejas armazenadas para todas as cultivares a T1, excepto para a Burlat. Os teores em antocianinas aumentaram em ambos os dois estados de conservação, especialmente na Van, em que o aumento quintuplicou no armazenamento a T1 (de 47 para 230 mg 100 g^{-1} de peso fresco). As concentrações de rutina, epicatequina e catequina permaneceram bastante estáveis durante o armazenamento em ambas as temperaturas. Geralmente, registaram-se maiores concentrações de ácidos hidroxicinâmicos em 2001 e de antocianinas em 2002.

No capítulo 7 estudou-se a relação entre os parâmetros cromáticos e a concentração de antocianinas nos frutos das quatro cultivares de cerejeira analisadas à colheita e após conservação em T1 e T2. A cor foi determinada através de medição colorimétrica (sistema CIELAB) directamente nos frutos, enquanto que as antocianinas foram quantificadas por HPLC-DAD. A concentração de antocianinas totais dos frutos das diferentes cultivares variou na seguinte ordem: Burlat > Saco > Van > Summit. As cerejas armazenadas em T1 apresentam maior redução da L^*, C^* e H^* do que os frutos armazenados em T2. Os parâmetros cromáticos (L^*, a^*, b^*, C^* e H^*) correlacionaram-se negativamente com o teor em antocianinas totais e com os fenóis totais. Os resultados permitem concluir que as funções cromáticas do C^* e H^* podem servir para prever a evolução da cor e os níveis de antocianinas durante o período de conservação.

No capítulo 8 procurou-se avaliar a actividade antioxidante dos extractos de cerejas das quatro cultivares, à colheita e depois da conservação em T1 e T2. Os ácidos hidroxicinâmicos dominaram em todos os extractos e representaram 60–74% dos compostos fenólicos das cerejas frescas ou armazenadas das cultivares Saco, Summit e Van, e apenas 45% dos compostos fenólicos da Burlat. Os compostos fenólicos dos extractos das cerejas inibiram a oxidação das lipoproteínas de baixa densidade (LDL) *in vitro*. À medida que se aumentava a concentração de compostos fenólicos aumentava a actividade antioxidante. A adição de 20 μM de equivalentes de ácido gálico (GAE) bloqueou integralmente a oxidação das LDL *in vitro*. Quando comparados a 12,5 μM GAE, os extractos das cerejas frescas

tiveram maior actividade antioxidante. Os frutos das cultivares Summit e Van tiveram a maior e a menor actividade antioxidante, respectivamente. As cerejas Van conservadas em T1 e T2 manifestaram actividade prooxidante. As diferenças no efeito antioxidante foram positivamente correlacionadas com as concentrações do ácido *p*-cumaroilquínico, mas negativamente correlacionadas com a cianidina-3-rutinósido.

Finalmente, no capítulo 9 apresentam-se as conclusões mais relevantes e sugerem-se temas para trabalho futuro.

Abstract

In modern horticulture, sweet cherry tree (*Prunus avium* L.) is economically very important. The actual trends of sweet cherry tree culture are to obtain dwarf trees and high fruit quality for fresh market. Therefore, the cherry tree management involves the knowledge about the ecophysiology of the new scion-rootstock combinations and the physicochemical fruit characteristics. So, three main objectives were considered: (1) To study the ecophysiology of fifteen scion-rootstock combinations at morpho-anatomical, biochemical and physiological level of dwarfed trees *versus* invigorating trees; (2) To study the effect of cultivar, ripeness stage, postharvest storage and year in the nutritional fruit quality, by physicochemical analysis (mainly of phenolic compounds); (3) To evaluate the antioxidant activity of cherry extracts at harvest and after storage.

The study was carried out with four high quality sweet cherry cultivars Burlat, Saco, Summit and Van grafted onto five rootstocks with contrasting size-controlling potentials, which increase in the order: *Prunus avium* < CAB 11E < Maxma 14 < Gisela 5 < Edabriz. The experimental study was conducted essentially during 2001–2003 in a sweet cherry tree orchard installed in 1999, in Abambres, Vila Real. However, a preliminary study was done with the rootstocks in the nursery in 1997, during grafting in 1998 and after plantation in 1999. The experimental work is divided in eight chapters, seven of them (chapters 2–8) include the information published or submitted to journals belonging to the *Journal Citation Reports*.

In chapter 2, the aim of the first study was to describe the effects of pruning and plant spacing on growth and water relations of sweet cherry trees. In the nursery, a trial with the five cherry rootstocks was begun in 1997. Pruning severities were applied on the rootstocks (0, 30, 60 and 90% of the vegetative growth was removed corresponding to P1, P2, P3 and P4 treatments, respectively) after planting to two plant spacings (S1 = 0.25 x 1.0 m and S2 = 0.45 x 1.5 m). Canopy, root growth and leaf water potential (Ψ_{leaf}) were quantified throughout the growing season. Pruning significantly affected root length and root weight of the rootstocks. Uncut plants (P1) showed a heavier and expanded root biomass than the intensively pruned plants (P4). The greater root biomass was obtained with the spacing/pruning combination, S1/P1, and the smaller with S1/P4 and S2/P4. Ψ_{leaf} varied significantly between the rootstocks and plant spacing but not with pruning. Maxma 14 and *Prunus avium* attained the lowest values of midday Ψ_{leaf}, but the highest values of predawn Ψ_{leaf}. Generally, with high density (S1), the rootstocks exhibited lower predawn and midday Ψ_{leaf}.

In chapter 3, to assess hydraulic architecture and limitations to water transport across scion-rootstock combinations (*Prunus avium* L. cultivar Van grafted on the five rootstocks), we compared xylem anatomy, relative hydraulic conductivity (RC) and vulnerability index (VI) of roots (small diameter, < 2 mm; medium, 2–5 mm; and large, > 5 mm) and stems. Water relations, leaf gas exchange and variations in growth were also determined. Roots exhibited larger-diameter xylem conduits (VD), greater RC and VI than stems in all Van-rootstock combinations. Moreover, the data obtained here showed significantly higher vessel frequency (VF), lower VD, RC and VI in dwarfed trees, i.e., grafted on Edabriz and Gisela 5 than trees on the invigorating rootstocks *Prunus avium*, CAB 11E and Maxma 14. Anatomical constraints on turgor imposed by the smaller VD of dwarfed trees imply a series of negative feedbacks, like a decrease in RC, stem water potential (Ψ_{stem}), leaf gas exchange and growth.

In chapter 4, canopy architecture effects on morpho-anatomy (leaf mass per unit area – LMA, leaf density – LD, leaf tissue thickness and stomatal density), chemical composition (photosynthetic pigments, total soluble sugars – SS, starch – St and total phenols – TP concentrations), gas exchange rates and Ψ_{stem} were studied in leaves of *Prunus avium* L. cultivars Burlat, Summit (upright, high dense canopies) and Van (spreading, low dense canopy). Van presented the highest value of canopy light transmittance, which improves photosynthetic rates, especially at harvest, compared to Summit and Burlat. However, no differences in Ψ_{stem} were found among cultivars. Van showed the highest LMA and variations in this parameter were mainly associated with alterations in leaf thickness than in LD. Anatomically, leaves of Summit and Van were thicker than those of Burlat, mainly due to increased mesophyll thickness, but they had lower palisade/spongy ratio. Total chlorophyll (Chl) concentration per area and the $Chl_{a/b}$ ratio were always higher in Van leaves than in the other two cultivars. On the other hand, Van leaves had the lowest concentrations of SS, St and TP.

In chapter 5, water relations, leaf gas exchange, chlorophyll a fluorescence, canopy light transmittance, leaf photosynthetic pigments and metabolites and fruit quality indices (weight, colour, firmness, pH, soluble solids and titratable acidity) of cherries from cultivars Burlat, Summit and Van growing on the five rootstocks were studied. Rootstock genotype affected all physiological parameters. Cherry cultivars grafted on invigorating rootstocks had higher values of midday stem water potential (Ψ_{MD}), net CO_2 assimilation rate (A), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and maximum photochemical efficiency of photosystem II (PSII) (F_v/F_m) than cultivars grafted on dwarfing rootstocks. The Ψ_{MD} was positively correlated with A, g_s and C_i. Moreover, A was positively correlated with g_s, and the

slopes of the linear regression and the respective coefficients of determination increased from invigorating to dwarfing rootstocks. The effect of rootstock genotype was also statistically significant for leaf photosynthetic pigments, whereas metabolite concentrations and fruit physicochemical characteristics were more dependent on cultivar genotype. Among cultivars, Burlat leaves had the lowest concentrations of photosynthetic pigments, but were richest in SS, St and TP. Compared with the other cultivars, Summit had heavier fruits, independent of the rootstock. Burlat cherries were less firm and had lower concentrations of soluble sugars and lower titratable acidity than Van cherries. Nevertheless, Van cherries had lower lightness (L^*), chroma (C^*) and hue angle (H^*), representing redder and darker cherries, compared with Summit fruits. In general, Ψ_{MD} was positively correlated with fruit mass and A was negatively correlated with L^* and C^*.

In chapter 6 the main objective was to study the effect of cultivar, ripeness stage (partially ripe and ripe), postharvest storage (room temperature – T1, 15 ± 5 °C and 55–60% R.H., for one week; and cool temperature – T2, 1–2 °C and 90–95% R.H., for one month) and year (climatic conditions, namely, temperature and sunshine hours) in the phenolic profile of the cherries from the four sweet cherry cultivars. Neochlorogenic and *p*-coumaroylquinic acids were the main hydroxycinnamic acid derivatives, but chlorogenic acid was also identified in all cultivars. The 3-glucoside and 3-rutinoside of cyanidin were the major anthocyanins. Peonidin and pelargonidin 3-rutinosides were the minor anthocyanins, and peonidin-3-glucoside was also present in Burlat and Van. Epicatechin was the main monomeric flavan-3-ol with catechin present in smaller amounts in all cultivars. The flavonol rutin was also detected. Saco contained the highest amounts of phenolics [227 mg 100 g^{-1} of fresh weight (fw)] and Van the lowest (124 mg 100 g^{-1} of fw). Levels of phenolics were always higher in ripe than in partially ripe cherries. Phenolic acid contents generally decreased with storage at T2 and increased with storage at T1. Anthocyanin levels increased at both storage temperatures. In Van the anthocyanins increased up to 5-fold during storage at 15 ± 5 °C (from 47 to 230 mg 100 g^{-1} of fw). Rutin, epicatechin and catechin contents remained quite constant at both storage conditions. In general, for all cultivars the levels of phenolic acids were higher in 2001 and the anthocyanin levels were higher in 2002.

In chapter 7, the relationship between colour parameters and anthocyanins of the four sweet cherry cultivars was studied at harvest, and after storage at T1 and T2. The colour was measured by tristimulus colourimetry (CIELAB system) directly on the fruits, while anthocyanins were quantified by HPLC-DAD analysis on methanolic extracts of freeze-dried samples of the fresh cherries and on the differently stored cherries. The total anthocyanin content in fruits of the different cultivars varied in the order Burlat > Saco > Van > Summit.

Cherries stored at T1 showed higher reduction of *L**, *C** and *H** than fruits stored at T2. Chromatic parameters (*L**, *a**, *b**, *C** and *H**) correlated negatively with the total anthocyanins levels and with the total phenols. The results show that chromatic functions of *C** and *H** can be used to predict the evolution of colour and anthocyanins levels during storage of sweet cherries.

In chapter 8, the antioxidant activity of the phenolic extracts of the four sweet cherry cultivars were analysed at harvest and after storage at T1 and T2. Hydroxycinnamates dominated in all samples and represented 60–74% by weight of the phenols in the fresh and stored samples of the cultivars Saco, Summit and Van, and 45% by weight of the phenols in the cultivar Burlat samples, which were richer in anthocyanins. Phenolic cherry extracts inhibited low-density lipoprotein (LDL) oxidation *in vitro* in a dose-dependent manner. Addition of 20 μM gallic acid equivalents (GAE) completely blocked the oxidation in the LDL *in vitro*. When compared at equimolar addition levels of 12.5 μM GAE the extracts of freshly harvested cherries exhibited significantly higher antioxidant activities than extracts of stored samples. Summit samples had the highest antioxidant activity. Van storage at both T1 and T2 led to a prooxidant activity of the extracts. Differences in the antioxidant effects of the cherry samples were positively correlated with their levels of *p*-coumaroylquinic acid but negatively correlated with their cyanidin-3-rutinoside levels.

Finally, in chapter 9 is presented the main conclusions of the study and proposals for future research.

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Símbolos e Abreviaturas / Symbols and Abbreviations

A..... Taxa de assimilação líquida de CO_2 / Net CO_2 assimilation rate

AAC..... Coeficiente da actividade antioxidante / Antioxidant activity coefficient

ABA..... Ácido abscísico / Abscisic acid

A/g_s..... Eficiência intrínseca do uso da água / Intrinsic water-use efficiency

BSE..... Extensão da bainha do feixe / Bundle sheath extension

C^*..... Croma / Chroma

Cab ..... Porta-enxerto CAB 11E / CAB 11E rootstock

Car..... Carotenóides totais / Total carotenoids

CFW..... Peso fresco da copa / Canopy fresh weight

Chl..... Clorofila total / Total chlorophyll

C_i..... Concentração de CO_2 intercelular / Intercellular CO_2 concentration

cy-3-glu..... Cianidina-3-*O*-β-glucósido / Cyanidin-3-*O*-β-glucoside

cy-3-ru ..... Cianidina-3-*O*-β-rutinósido / Cyanidin-3-*O*-β-rutinoside

DAD..... Detector de díodos / Diode array detector

DCA..... Análise canónica discriminante / Discriminant cannonical analysis

E..... Taxa de transpiração / Transpiration rate

Edb..... Porta-enxerto Edabriz / Edabriz rootstock

F..... Firmeza do fruto / Fruit firmness

F_0..... Fluorescência mínima / Minimal fluorescence

F_m..... Fluorescência máxima / Maximal fluorescence

F_v..... Fluorescência variável / Variable fluorescence

F_v/F_m..... Eficiência fotoquímica máxima do PSII / Maximal photochemical efficiency of PSII

GAE..... Equivalentes de ácido gálico / Gallic acid equivalents

g_m..... Condutância para o CO_2 no mesófilo clorofilino / Liquid phase diffusive conductance to CO_2

g_s..... Condutância estomática para o vapor de água / Stomatal conductance

Gsl..... Porta-enxerto Gisela 5 / Gisela 5 rootstock

H^*..... Ângulo hue (ou tonalidade) / Hue angle (or tonality)

HPLC..... Cromatografia líquida de alta performance / High performance liquid chromatography

K_s..... Condutividade hidráulica específica / Specific hydraulic conductivity

L^*..... Luminosidade / Luminosity

LA..... Área foliar / Leaf area

LC_t..... Luz transmitida pela copa / Canopy light transmittance

LD..... Densidade foliar / Leaf density

LDL..... Lipoproteínas de baixa densidade / Low-density lipoproteins

LMA..... Massa foliar específica / Leaf mass per unit area

LTh..... Espessura da lâmina foliar/ Lamina thickness

Mxm ..... Porta-enxerto Maxma 14 / Maxma 14 rootstock

ORAC..... Capacidade de absorção de radicais de oxigénio / Oxygen radical absorption capacity

Pav..... Porta-enxerto *Prunus avium* / *Prunus avium* rootstock

PCA ..... Análise por componentes principais / Principal component analysis

PPFD ..... Densidade de fluxo fotónico fotossinteticamente activo / Photosynthetic photon flux density

PSII..... Fotossistema II / Photosystem II

P1 ..... 0% remoção do crescimento vegetativo / 0% of the vegetative growth was removed

P2 ..... 30% remoção do crescimento vegetativo /30% of the vegetative growth was removed

P3 ..... 60% remoção do crescimento vegetativo / 60% of the vegetative growth was removed

P4 ..... 90% remoção do crescimento vegetativo / 90% of the vegetative growth was removed

R..... Precipitação / Rainfall

RC..... Condutividade hidráulica relativa / Relative hydraulic conductivity

SS ..... Açúcares solúveis totais / Total soluble sugars

St..... Amido / Starch

S1 ..... S1 = 0,25 x 1,0 m, compasso de plantação 1 / plant spacing 1

S2 ..... S2 = 0,45 x 1,5 m, compasso de plantação 2 / plant spacing 2

T ..... Temperatura do ar / Air temperature

T1 ..... Temperatura ambiente (15 ± 5 ºC) / Room temperature

T2 ..... Atmosfera refrigerada (1–2 ºC) / Cool temperature

TA ..... Acidez titulável / Titratable acidity

TAS ..... Actividade antioxidante total / Total antioxidant activity

TEAC ..... Actividade antioxidante em equivalentes trolox / Trolox equivalent antioxidant capacity

TP ..... Compostos fenólicos totais / Total phenolics

UV..... Ultravioleta / Ultraviolet

VD..... Diâmetro dos vasos xilémicos / Vessel diameter

VF ..... Frequência dos vasos xilémicos / Vessel frequency

VI..... Índice de vulnerabilidade / Vulnerability index

VPD ..... Défice de pressão de vapor de água do ar / Vapour pressure deficit

W ..... Peso do fruto / Fruit weight

WC..... Conteúdo de água foliar / Water content

Ψ ..... Potencial hídrico / Water potential

CAPÍTULO 1

INTRODUÇÃO GERAL

1.1. INTRODUÇÃO E OBJECTIVOS

A cerejeira, *Prunus avium* L., cujas características particulares estão apresentadas no Quadro 1.1 e Figura 1.1, é indígena do Norte do Irão, da Ucrânia e de outros países a Sul das Montanhas do Cáucaso. É, ainda, nativa em regiões que vão desde o Sul da Suécia até à Grécia, Itália e Espanha (De Candolle, 1883). A disseminação da espécie, feita principalmente pelas aves, levou a que aparecesse desde o Norte da Índia às planícies do Sul da Europa (Webster, 1996). A partir do segundo quartel do século XVII foi levada para as Américas em barcos de exploradores e colonizadores europeus (Scorza e Hammerschlag, 1992). Actualmente, a cerejeira é cultivada em mais de 40 países. No Hemisfério Sul, a cerejeira é cultivada até 46–47º S na Patagónia (Chile e Argentina) e até 45–46º S em Otago, na Nova Zelândia. No Hemisfério Norte, a Península da Escandinávia, a 61º N, é o extremo em termos de produção (Predieri *et al.*, 2003). Na Europa, e, segundo Webster (1996), a espécie *Prunus avium* domina nas zonas meridionais (Portugal, Espanha, Itália e Sul de França). Em climas de zonas setentrionais (Norte de França, Alemanha e Países de Leste), a ginjeira (*Prunus cerasus* L.), mais resistente ao frio, tem maior expressão. Estas duas espécies são as mais importantes na produção mundial de cereja. De acordo com a FAO (2006), a produção no ano de 2005 foi de 1 858 673 t e teve origem maioritariamente na Turquia (14%) e nos EUA (13,5%). A área mundial está estimada em 406 594 ha. Em Portugal, na campanha de 2005, a produção de cereja foi de 16 500 t numa área de 6 250 ha. Os pomares de cerejeiras situam-se, essencialmente, a norte do rio Tejo, com excepção da região de Portalegre (Serra de S. Mamede), sendo na maioria provenientes de enxertias em *Prunus avium* (GPPAA, 2002). A zona de maior expressão é a região da Cova da Beira (Covilhã, Fundão e Belmonte) com cerca de 55% da produção nacional. A zona do Douro Sul (Resende, Lamego e Penajóia) e Alfândega da Fé, em Trás-os-Montes, perfazem cerca de 40%. Os restantes 5% distribuem-se por Proença-a-Nova, Ferreira do Zêzere e Portalegre. Cerca de 40% da cereja destina-se às grandes superfícies de venda em fresco, 45% aos mercados abastecedores dos grandes centros urbanos e a restante tem como destino a indústria de transformação.

No passado, a cerejeira foi cultivada sistematicamente em porta-enxertos vigorosos que, além de conferirem elevada envergadura às árvores e consequente má acessibilidade aos frutos, atrasavam a entrada em produção. Para colmatar estes entraves e fazer face às actuais exigências de mercado, tem havido uma activa reconversão dos antigos cerejais com recurso a porta-enxertos ananicantes e semi-ananicantes e a cultivares autoférteis de frutos de maior calibre. Desta forma, são assim obtidas árvores menos frondosas, que permitem uma maior facilidade de colheita dos frutos e possibilitam a plantação de pomares com maior densidade,

precocidade e diversidade de cultivares. Para além disso, a actual gama de cultivares permite alargar a época de produção, um uso mais racional da mão-de-obra para a colheita e uma maior rentabilidade para o produtor.

Quadro 1.1
Sistemática, descrição botânica, distribuição e habitat da cerejeira ([a]Crespi *et al.*, 2005a; [b]Franco, 1971; [c]Crespi *et al.*, 2005b).

Divisão[a]	Spermatophyta
Subdivisão[a]	Magnoliophytina (Angiospermae)
Classe[a]	Magnoliatae (Dicotyledoneae)
Subclasse[a]	Rosidae
Ordem[a]	Rosales
Família[a]	Rosaceae
Género[a]	*Prunus*
Espécie[a]	*Prunus avium* L.
Nome comum[a]	Cerejeira; Cerdeira; Cerejeira-brava; Cereja (fruto).
Descrição botânica:	
Planta[b]	Árvore com pernadas subpatentes e sem rebentos de raiz; raminhos castanho-avermelhados, glabros; gomos com 7-10 catáfilos.
Tipo fisionómico[b]	Mesofanerófito.
Folhas[b]	Com 8-15 x 4-7 cm, obovado-oblongas, acuminadas, crenado-serradas, glabras mas baças na página superior, pendentes em novas; pecíolo com 2-5 cm e 2 glândulas na base do limbo.
Flores[b]	Cimeiras 2-6 flores, sésseis, rodeadas na base pelos numerosos catáfilos escariosos do gomo; hipanto urceolado; pétalas com 9-15 mm, brancas.
Fruto[b]	Drupa com 9-12 mm, globosa, vermelho-escura (também amarelada, vermelho vivo ou negra conforme as cultivares), doce ou ácida; endocarpo liso.
Distribuição[c]	Quase toda a Europa, Oeste da Ásia e Noroeste de África.
Habitat[c]	Ruderal e matos.

Embora a nível nacional as cultivares estejam bem adaptadas às regiões atrás referidas, existem poucos estudos sobre o efeito dos novos porta-enxertos ananicantes na performance frutícola. No âmbito deste estudo lida-se principalmente com cultivares de frutos de elevada qualidade enxertadas em porta-enxertos de diferente vigor. Devido à elevada interacção das combinações cultivar/porta-enxerto com o meio ambiente, é de primordial interesse avaliar os comportamentos das árvores em determinadas especificidades locais, dando particular destaque à valorização qualitativa dos frutos.

Figura 1.1
A espécie *Prunus avium* L..

A qualidade dos frutos colhidos no pomar é determinante para a viabilidade económica da exploração, para o seu consumo imediato e também para a sua conservação. A cereja é um fruto particularmente atractivo para o consumidor pelos seus atributos cromáticos e aromáticos, bem como pela riqueza em alguns nutrientes com um forte impacto no bem-estar humano. De facto, o consumo de frutos e outros vegetais parece estar associado a uma redução do risco de várias patologias, como o cancro, doenças cardiovasculares e aterosclerose (Steinmtez e Potter, 1991; Criqui e Ringel, 1994), atribuído à existência de múltiplos constituintes antioxidantes, tais como vitaminas, carotenóides e compostos fenólicos, entre outros.

Segundo Gao e Mazza (1995), a cereja é rica em compostos fenólicos, nomeadamente em ácidos fenólicos (ácidos hidroxicinâmicos) e flavonóides (antocianinas). Segundo Yilmaz e Toledo (2004), os compostos fenólicos actuam como agentes terapêuticos num elevado número de patologias, tendo um papel importante na inibição da carcinogénese, mutagénese e doenças cardiovasculares, estando esta inibição relacionada com a sua actividade antioxidante. Estes compostos têm ainda actividade anti-inflamatória, evitam a aglomeração das plaquetas sanguíneas e a acção dos radicais livres no organismo, protegendo desde o DNA aos lípidos e proteínas (Halliwell, 1990). Na realidade, a actividade antioxidante apresentada por frutos, folhas, sementes e plantas medicinas foi correlacionada com a concentração de compostos fenólicos totais (Velioglu *et al.,* 1998; Kähkönen *et al.*, 1999). Uma vez que os compostos fenólicos não são sintetizados pelo organismo, sendo representativos da parte não energética da dieta humana, têm que ser obtidos através da ingestão de certos alimentos ou através da introdução na dieta alimentar de suplementos nutritivos (Aherne e O'Brein, 2002).

A cereja é também uma excelente fonte de boro (Pillow *et al.*, 1999), que contribui para uma boa constituição óssea (Volpe *et al.*, 1993) e pode ainda alterar favoravelmente os níveis

das hormonas esteróides (Samman *et al*., 1996). Adicionalmente, a cereja tem um baixo índice glicémico (Brand-Miller e Foster-Powell, 1999), o que pode constituir uma vantagem relativamente a outros frutos e vegetais. Sendo a cereja um fruto nutricionalmente muito interessante, são necessários estudos para determinar as diferenças entre cultivares a nível da composição dos frutos assim como quais as condições de conservação ideais para assegurar essas qualidades.

Face ao exposto, a expansão da área de cultura da cerejeira e a sua exploração rentável implica a aquisição de conhecimentos nos domínios do comportamento das novas combinações cultivar/porta-enxerto, fisiologia da árvore, características e conservação dos frutos, entre outros. Tendo consciência que no contexto da fruticultura moderna a cerejeira é uma espécie economicamente interessante e que as tendências actuais são cada vez mais a procura de árvores ananicantes produtoras de frutos de elevada qualidade, principalmente para consumo em fresco, traçaram-se três objectivos principais:

(1) Estudar a ecofisiologia de diferentes combinações cultivar/porta-enxerto de cerejeira, ao nível das características morfo-anatómicas, bioquímicas e fisiológicas de árvores ananicantes *versus* árvores vigorosas;

(2) Estudar o efeito da cultivar, do estado de maturação, do ano (condições climáticas, nomeadamente temperatura e insolação) e da conservação pós-colheita na qualidade sensorial e nutricional da cereja, através de análises físico-químicas (principalmente a identificação e quantificação de compostos fenólicos);

(3) Avaliar o potencial antioxidante dos extractos de cerejas de quatro cultivares, à colheita e após conservação.

1.2. ECOFISIOLOGIA DA CEREJEIRA

Segundo Kozlowski e Pallardy (1997), a regularização do crescimento e o desenvolvimento reprodutivo de qualquer espécie frutícola podem ser controlados com o recurso a diversas práticas culturais. Entre outras destacam-se a disposição e espaçamento das árvores no pomar, a utilização de porta-enxertos com diferentes vigores, a aplicação de fertilizantes, a rega, a poda de ramos e/ou raízes e a aplicação de reguladores de crescimento. Ranney *et al.* (1989) referem que o regulador de crescimento paclobutrazol quando aplicado ao solo em pomares de cerejeira e pelo facto de ser um inibidor da biossíntese de ácido giberélico, controla efectivamente a expansão vegetativa desta espécie. Contudo, preocupações com o ambiente e com a saúde dos consumidores restrigem a sua utilização (Predieri *et al.*, 2003). Também a poda, pelos benefícios que produz a nível do arejamento e do microclima luminoso da copa, pode ajudar no controlo do tamanho da árvore e favorecer uma maturação mais equilibrada dos frutos (Edin *et al.*, 1997). Whiting e Lang (2004) verificaram que árvores da cultivar Bing enxertadas em Gisela 5, com 7–8 anos, podadas a 20 frutos m^{-2} de área foliar apresentaram uma redução de 68% na produtividade, mas um aumento do peso do fruto (+ 25%), da firmeza (+ 25 %), do teor em sólidos solúveis (+ 20%) e do diâmetro (+ 14%) comparativamente às não podadas (84 frutos m^{-2}).

A fertilização e a rega são determinantes na gestão de um cerejal de qualidade. Estudos em nutrição com várias espécies, permitiram concluir que uma deficiente nutrição azotada provocou um aumento da concentração de compostos fenólicos (Andrew e Beech, 1978; Tan, 1980; Bongue-Bartelsman *et al.*, 1994). Em cerejeira, uma fertilização azotada elevada provocou redução no tamanho dos frutos, mas aumentou a sua firmeza e diminuiu a acidez (Nielsen *et al.*, 2002). De um modo geral, as deficiências em potássio são mais frequentes do que as de fósforo, magnésio, cálcio e cobre (Putnam, 1999). No entanto, as deficiências em magnésio têm sido referidas em cerejeiras plantadas em solos calcários, provavelmente devido à insolubilidade deste elemento naquele tipo de solos (Moreno *et al.*, 1996; Belkhodja *et al.*, 1997). A concentração em cálcio tem sido relacionada com a sensibilidade do fruto ao rachamento provocado pela ocorrência de precipitação no período próximo da colheita e na capacidade de resistir ao armazenamento (Lang *et al.*, 1998). A deficiência em ferro é corrente em cerejeiras plantadas em solos com pH elevado. Para colmatar este problema, recomenda-se a correcção do pH ou a adição de quelatos de ferro (Predieri *et al.*, 2003). Esta espécie é também susceptível à deficiência em zinco, causando uma redução no tamanho da folha e a sua queda prematura, redução do tamanho do fruto e da concentração em sólidos solúveis (Putnam, 1999).

Uma vez que os frutos grandes são valorizados pelo mercado, as cerejeiras são geralmente regadas para minimizar os efeitos do défice hídrico no crescimento dos frutos, sendo a fase III do desenvolvimento do fruto (*vide* ponto 1.3) aquela que é particularmente mais sensível (Flore e Layne, 1999).

Todas as técnicas culturais anteriormente referidas são importantes para controlar o crescimento das árvores. No entanto, a escolha do porta-enxerto mais adequado assume primordial importância nesse objectivo.

1.2.1. Porta-enxertos

Os porta-enxertos embora essencialmente utilizados para controlar o tamanho da árvore, também o são para modificar o seu hábito de crescimento, aumentar a resistência ao frio e a doenças, antecipar a entrada em produção, melhorar o tamanho, a qualidade e a maturação do fruto e, por fim, aumentar a produtividade (Wutsher e Dube, 1977; Fallahi *et al.*, 1989; Hartmann *et al.*, 1990; Webster, 1995). Há mais de 20 séculos que se utilizam espécies fruteiras enxertadas (Webster, 1995; Kozlowski e Pallardy, 1997). Já Theophrastus e, mais tarde, os horticultores romanos escolhiam porta-enxertos ananicantes para controlar o crescimento das árvores (Bunyard, 1920). Em cerejeira, o seu efeito é conhecido desde o início do século XVIII (Hesse, 1710). Actualmente, alguns dos porta-enxertos de cerejeira mais utilizados são: *Prunus avium*, *Prunus mahaleb*, *Prunus cerasus* (Edabriz, Weiroot, Vladimir, CAB), *Prunus fruticosa* (série Oppenheim) e os híbridos Maxma, Gisela, Piku, P-HL, Prob e IP-C1 (Webster e Schmidt, 1996).

Outra técnica extremamente dependente do porta-enxerto escolhido que se utiliza para diminuir o vigor das árvores poderá ser a elevação da altura de enxertia (Santos *et al.*, 2004). A altura em que é feita a enxertia tem efeito ténue em porta-enxertos vigorosos, mas em porta-enxertos ananicantes parece ter uma influência marcada no vigor e, portanto, na condução da plantação. Se o ponto de enxertia fica muito próximo do colo da planta, a possibilidade de afrancamento é elevada e a árvore vigoriza, tornando-se então apertado o compasso inicial, com problemas de sombreamento e controlo do vigor, de diminuição da produção e de perda de qualidade do fruto.

1.2.1.1. Mecanismo Ananicante dos Porta-enxertos

O mecanismo fisiológico que provoca o efeito ananicante ainda não está completamente esclarecido, existindo todavia diversas teorias. Segundo Scorza e Hammerschlag (1992), o nanismo em *Prunus avium* parece ser controlado por vários genes, que estão implicados no

crescimento mais reduzido das árvores, devido à diminuição do diâmetro dos vasos xilémicos (Baas *et al*., 1984), ao surgimento de uma zona de descontinuidade no ponto de enxertia que aumenta a resistência ao transporte de água (Sekse, 1998), à menor concentração de solutos na seiva (Jones, 1984), à menor capacidade de transporte de nutrientes (Simons e Swiader, 1985; Tagliavini *et al*., 1992; Nielsen e Kappel, 1996; Rosati *et al*., 1997; Ebel *et al*., 2000) e a uma menor produção de hormonas de crescimento (Chen *et al*., 1985; Sorce *et al*., 2002). Beakbane (1956) refere que este fenómeno está relacionado com a influência dos porta-enxertos nas relações hídricas das árvores. Esta hipótese baseou-se no estudo anatómico de Beakbane e Thompson (1939), que indicava serem os porta-enxertos ananicantes de macieira portadores de raízes com menor número e menor diâmetro de vasos xilémicos do que os porta-enxertos vigorosos. Também Baas *et al.* (1984) encontraram uma correlação positiva entre a altura da planta e o tamanho dos vasos xilémicos, sendo estes de menor diâmetro e mais curtos em árvores ananicantes do que em árvores vigorosas.

As variações no diâmetro dos vasos xilémicos podem afectar radicalmente a condutividade hidráulica (Zimmermann, 1983; Tyree e Ewers, 1991). Vários estudos demonstraram que a condutividade hidráulica é maior em raízes do que em caules (Alder *et al*., 1996; Kavanaugh *et al*., 1999; Martínez-Vilalta *et al*., 2002; McElrone *et al*., 2004). Alguns autores registaram também uma diminuição da condutividade hidráulica com a altura da planta (Mencuccini e Grace, 1996; Ryan *et al*., 2000; McDowell *et al*., 2002), uma vez que o diâmetro dos vasos lenhosos e traqueídos tem tendência a decrescer na direcção acrópeta (Tyree e Zimmermann, 2002). Contudo, o movimento de água é igualmente influenciado pelo gradiente de potencial hídrico no *continuum* solo-planta-atmosfera e pela regulação estomática, sendo esta especialmente sensível quando a quantidade de água no solo é limitada (Reyes-Santamaría *et al*., 2002). Outro factor que pode aumentar a resistência ao transporte de água é a existência de uma descontinuidade parcial no xilema do ponto de enxertia (Sekse, 1998). Isso mesmo foi constatado em macieira, por Olien e Lakso (1986) e Atkinson *et al.* (2001), e em citrinos, por Syvertsen (1981). Alguns investigadores observaram várias alterações morfológicas nesta região, nomeadamente, a presença de vasos xilémicos com menor diâmetro e uma maior percentagem de xilema e floema inactivo ou necrótico (Simons e Chu, 1984; Ussahatanonta e Simons, 1988; Soumelidou *et al*., 1994a; Salvatierra *et al*., 1998).

Em geral, as árvores enxertadas em porta-enxertos ananicantes distribuem prioritariamente a matéria seca pelas raízes e folhas, enquanto as árvores enxertadas em porta-enxertos mais vigorosos privilegiam também o crescimento do caule (DeJong e Doyle, 1984; Glenn e Scorza, 1992; Basile *et al*., 2003a).

O porta-enxerto determina a adaptação das cerejeiras a diferentes condições de fertilidade do solo. Ystaas e Froynes (1998) registaram concentrações mais baixas de potássio e azoto e mais elevadas de cálcio e magnésio em cultivares enxertadas em Colt do que em árvores enxertadas em porta-enxertos Charger e F 12.1. Resultados similares foram obtidos por Moreno *et al.* (1996) para o porta-enxerto Colt, quando comparado com o Adara (*Prunus cerasifera* L.), entre outros. Nielsen e Kappel (1996) também observaram maiores concentrações de magnésio e cálcio na cultivar Bing em Colt. Uma concentração mais alta de cálcio foi ainda obtida na cultivar Van (Betrán *et al*., 1997) e na cultivar Sunburst (Jiménez *et al*., 2004), ambas enxertadas em Colt. De uma maneira geral, as cultivares de cerejeira enxertadas em porta-enxertos ananicantes apresentaram folhas com menores concentrações de cálcio e magnésio do que as árvores em porta-enxertos vigorosos (Sitarek *et al*., 1998). A concentração em azoto foi mais elevada em cerejeiras enxertadas em clones de *Prunus cerasus*, incluindo o CAB 11E, comparativamente a outros sete porta-enxertos (Moreno *et al*., 1996, 2001). Moreno *et al.* (1996) observaram diferenças de sensibilidade dos porta-enxertos em condições de solo calcário (pH 8), com o Adara a ser o mais resistente, o Colt com sensibilidade intermédia e o SL 64 o mais susceptível.

As hormonas vegetais parecem estar envolvidas na regulação das relações complexas que se estabelecem entre o porta-enxerto e a cultivar. Alguns autores (Gersani *et al*., 1980; Muday e Haworth, 1994) sugeriram que a auxina ácido indolacético (IAA), produzida activamente nos ápices caulinares, tem influência determinante nessa relação. Do mesmo modo, também outras hormonas como as citocininas, que são activamente produzidas nas raízes (Chen *et al*., 1985) e exportadas via xilema (Van Staden e Davey, 1979), podem ser determinantes. A translocação acrópeta das citocininas no xilema é dependente da quantidade de auxinas sintetizadas nos lançamentos e que atingem as raízes (Lockard e Schneider, 1981). As citocininas, por sua vez, influenciam o crescimento dos ramos e, consequentemente, a síntese de auxinas e a sua translocação para as raízes (Lockard e Schneider, 1981; Jones, 1986). Vários estudos referem que os porta-enxertos ananicantes limitam o crescimento da cultivar devido à redução na produção de hormonas (auxinas e giberelinas) (Hartmann *et al*., 1990) ou por dificultarem o transporte basípeto das auxinas nos seus tecidos (Lockard e Schneider, 1981). De facto, foi encontrada uma correlação positiva entre o crescimento potencial do porta-enxerto e a taxa de transporte de IAA na seiva xilémica de pessegueiros (Sorce *et al*., 2002). Em macieira, Soumelidou *et al.* (1994b) referem que a taxa de transporte polar da auxina em segmentos de lançamentos é menor nos porta-enxertos ananicantes comparativamente aos mais vigorosos. Noda *et al.* (2000) encontraram uma correlação positiva

entre a concentração de IAA no ápice caulinar e o crescimento vegetativo de limoeiros enxertados em porta-enxertos de diferente gama de vigor. Adicionalmente, estudos em *Arabidopsis thaliana* (Heynh.) revelaram uma redução no transporte polar da auxina em fenótipos ananicantes (Ruegger *et al.*, 1997). Outros estudos correlacionaram positivamente o potencial de crescimento do porta-enxerto e a sua concentração de citocininas na seiva xilémica (Jones, 1986; Kamboj *et al.*, 1999; Sorce *et al.*, 2002). O crescimento potencial dos porta-enxertos apresentou uma correlação positiva com a taxa de citocininas exportadas das raízes em uvas (Skene e Antcliff, 1972), melancia (Yamasaki *et al.*, 1994), macieira (Kamboj *et al.*, 1999) e pessegueiro (Sorce *et al.*, 2002). Pilet e Saugy (1987) observaram uma correlação negativa entre o crescimento radicular e a concentração endógena de ABA. Relativamente ao crescimento caulinar de citrinos, Noda *et al.* (2000) encontraram também uma correlação negativa com a concentração de ABA e IAA nas raízes. O porta-enxerto ananicante induziu uma concentração baixa de IAA nos novos lançamentos e alta de IAA e ABA nas raízes fibrosas, comparativamente ao porta-enxerto vigoroso. Por último, Yadava e Dayton (1972), verificaram que os níveis de ABA eram mais altos em raízes, caules e folhas de macieiras ananicantes do que nas árvores mais vigorosas.

1.2.1.2. Efeito do Porta-enxerto na Fisiologia da Árvore

Diversos estudos em cerejeira (Schmitt *et al.*, 1989), pessegueiro (Bongi *et al.*, 1994; Basile *et al.*, 2003b) e macieira (Giulivo e Bergamini, 1982; Olien e Lakso, 1986), permitiram constatar a influência significativa dos porta-enxertos nas relações hídricas das respectivas plantas. Em condições de campo e em cerejeiras, Shackel *et al.* (1997) verificaram que o efeito do porta-enxerto no potencial hídrico foliar (Ψ) do meio-dia não se fez sentir, porém quando a irrigação foi reduzida, as árvores enxertadas em Colt reagiram com um declínio mais rápido do estado hídrico do que quando enxertadas em *Prunus mahaleb*. Neste estudo, as árvores em porta-enxertos vigorosos apresentavam sistematicamente maior Ψ do que as árvores com porta-enxertos ananicantes, devido a uma maior capacidade de absorção do sistema radicular ou à menor resistência hidráulica do sistema radicular e/ou do ponto de enxertia. Esse efeito dos porta-enxertos mais vigorosos tem depois consequências na actividade fisiológica das plantas. Com efeito, quer em cerejeira (Shackel *et al.*, 1997) quer em videira (Düring, 1994, Iacono *et al.*, 1998; Patakas *et al.*, 2003), os estudos referem maior actividade fotossintética e condutância estomática nas plantas enxertadas em porta-enxertos mais vigorosos. No entanto, Jiménez *et al.* (2004) constataram que o efeito do vigor do porta enxerto não influenciou a concentração de clorofila, uma vez que os maiores valores foram registados

indiscriminadamente em árvores enxertadas em *Prunus cerasus* (CAB 11E, CAB 6P e MM9), Maxma 14, Maxma 97 e SL 64, do que em Colt e Damil.

No que respeita à produtividade, Facteu *et al.* (1996) registaram maiores valores em cerejeiras enxertadas nas selecções Giessen (Gisela 6, 7, 12 e 154-7), que induzem efeito ananicante, do que no vigoroso *Prunus avium*. Contudo, o porta-enxerto ananicante Gisela 5, incluído também na selecção Giessen, levou a uma redução no crescimento do fruto relativamente ao vigoroso F 12.1 (Franken-Bembeck, 1998). Em macieira, Autio e Southwick (1993) encontraram diferenças na cor e peso do fruto de árvores enxertadas em porta-enxertos diferentes, enquanto que Ferree (1992) e Meheriuk *et al.* (1994) apenas assinalaram diferenças ligeiras nesses parâmetros e nos sólidos solúveis e firmeza.

De acordo com Schmid *et al.* (1988), a cultivar Sam (*Prunus avium*) enxertada em *Prunus cerasus*, com sintomas de incompatibilidade tardia, apresentou valores de Ψ menos negativos, menores taxas de transpiração, encerramento dos estomas em condições de elevada irradiância fotónica, alta concentração de hidratos de carbono e compostos fenólicos (catequinas e proantocianidinas) e menor concentração em clorofila do que as folhas das combinações sem sintomas de incompatibilidade. Outros autores também verificaram, em árvores de *Prunus avium* quando enxertada em *Prunus* spp. com sintomas de incompatibilidade, a acumulação de compostos fenólicos, nomeadamente de ácido clorogénico (Bauer *et al.*, 1989), de ácido *p*-cumárico e de genisteína (Usenik e Štampar, 2001). O artigo de revisão de Pina e Errea (2005) aprofunda as causas de incompatibilidade na enxertia.

1.2.2. Cultivares

A maioria das cultivares de cerejeira doce, hoje em dia disponíveis para os fruticultores, são auto-estéreis pelo que é necessário o recurso a cultivares polinizadoras compatíveis e com as florações coincidentes. Por outro lado, perante os acidentes climáticos a que os pomares estão tantas vezes sujeitos, será sempre de aconselhar a plantação com diferentes cultivares que permitam um período mais alargado de colheita, ou seja, cerca de dois meses; assim, também a gestão da mão-de-obra se torna mais eficiente. De facto, nos últimos anos foi notável a renovação varietal da cerejeira, com o aparecimento de cultivares de boa produtividade e frutos de grande calibre e excelente coloração. Segundo Edin *et al.* (1997), os objectivos do melhoramento genético para as cultivares desta espécie são: (i) quanto à árvore, a rapidez de entrada em produção, a auto-fertilidade, a floração tardia para resistir às geadas de Primavera e a resistência a doenças; (ii) quanto ao fruto, a qualidade gustativa, o calibre, em média 9 a 10 g (27 a 29 mm de diâmetro), a resistência ao rachamento, a resistência à

manipulação e transporte e a possibilidade de recolha mecânica. Simões (1998) aconselha para as nossas condições as cultivares auto-férteis Stella, Lapins, Vista, Sunburst, New Star, Celeste e Sweetheart; dentro das que não são auto-férteis, a Van, Summit, Canada Giant, Durona 3, Arcina, Noir de Meched, entre outras. Mais recentemente surgiram algumas cultivares de pouca exigência de frio invernal como a Garnet, Marvin e Brooks, que por isso poderão vir a ter maior expansão em certas regiões do Sul e do litoral de Portugal.

1.3. A CEREJA

O crescimento da cereja, como de outras prunóideas, é caracterizado por uma curva com dupla sigmóide onde se podem destacar três fases (Flore, 1994; Edin *et al.*, 1997):

Fase I: Com uma duração média aproximada de 20 a 25 dias após a floração, correspondente a um crescimento rápido em volume provocado pela multiplicação celular. Este processo vai determinar, em grande parte, o calibre do fruto;

Fase II: Varia entre 10 a 20 dias e corresponde ao endurecimento do caroço. Esta fase é tanto mais prolongada quanto mais tardio for o fruto. O crescimento é retardado;

Fase III: Marcada pelo engrossamento do fruto, geralmente 10 a 20 dias antes da maturação. Nesta fase a fotossíntese é essencial, caso as reservas da planta sejam escassas.

Nesta espécie, o desenvolvimento do fruto é simultâneo com o crescimento vegetativo, competindo ambos como "sinks" pelas mesmas fontes de carbono, minerais, água e hormonas (Flore e Layne, 1999). A força "sink" da cereja varia durante o desenvolvimento do fruto e é máxima na fase III. Nesta fase, a taxa fotossintética é máxima (64 dias após a floração) (Whiting e Lang, 2004).

Segundo Flore e Layne (1999), a produtividade da cerejeira está dependente do fornecimento de assimilados e de reservas, e na capacidade de translocação e distribuição de carbono para os órgãos reprodutivos de uma maneira eficiente sem comprometer o desenvolvimento vegetativo durante a época de crescimento em curso ou nas épocas futuras.

A cereja é muito menor do que a maioria dos frutos, tornando-se essencial uma produção numerosa de gomos florais, para atingir uma maior produtividade sem prejuízo do respectivo calibre. Sendo este parâmetro uma prioridade no acesso a mercados mais lucrativos, a gestão da fisiologia da árvore deve ser orientada com esse fim. De acordo com Flore e Layne (1999), um dos meios consiste em ajustar a razão folha:fruto através da poda de forma a favorecer o tamanho do fruto. Num outro estudo, Roper e colaboradores (1987) constataram

que a qualidade da cereja Bing foi correlacionada com a superfície foliar dos "ramalhetes de Maio" na madeira de dois anos e que a concentração em sólidos solúveis e o calibre foram sensíveis ao aumento da superfície foliar. Estes autores verificaram ainda que as folhas dos "ramalhetes de Maio", *per se*, não tinham capacidade fotossintética suficiente para suportar o crescimento dos frutos, sendo por isso necessária a translocação de hidratos de carbono das estruturas de reserva da árvore.

Serrano *et al.* (2004) estudaram diferentes parâmetros relacionados com a qualidade organoléptica e propriedades funcionais da cereja desde as fases iniciais de desenvolvimento até à sua maturação completa. Os resultados mostraram que a mudança de cor (diminuição da luminosidade – L^* e da coordenada cromática b^*), o aumento em sólidos solúveis e a diminuição da firmeza tiveram início quando o fruto apresentava 50% do seu tamanho final. Por outro lado, a actividade antioxidante total e a concentração em compostos fenólicos alcançaram os maiores níveis nos estados mais avançados de maturação.

Os reguladores de crescimento têm sido extensivamente testados pelos seus efeitos específicos na maturação e qualidade da cereja (Looney, 1996). Entre eles, a utilização de ácido giberélico (GA_3) nas cultivares Van, Sunburst e Elisa foi amplamente estudada por Usenik *et al.* (2005). Estes autores sublinham que a aplicação de GA_3 atrasou a maturação do fruto, mas aumentou a sua firmeza, peso e teor em sólidos solúveis e reduziu o índice de rachamento. Contrariamente ao GA_3, a cianamida cálcica ($CaCN_2$) parece ter capacidade para antecipar a floração e a maturação do fruto (Predieri *et al.*, 2003). Segundo Papa (2001), o efeito da cianamida hidrogenada (CH_2N_2) na cultivar Ferrovia foi semelhante à $CaCN_2$. A aplicação de CH_2N_2 provocou uma antecipação na floração de cerca de 13 dias e na maturação do fruto de 6–7 dias. Observou ainda um aumento consistente na homogeneidade da maturação do fruto e na redução do número de colheitas nas árvores tratadas com CH_2N_2, quando comparadas com as não tratadas.

A cereja é tradicionalmente considerada um fruto de grande importância nutritiva e dietética, pela enorme diversidade de constituintes nutritivos que contém (Quadro 1.2). Para além disso, a cereja possui um baixo índice glicémico e contém também pequenas quantidades de outros componentes não nutritivos, tais como:

- ácidos orgânicos, o málico e o cítrico, que actuam como estimulantes das glândulas digestivas e como depurativos do sangue;
- fibra vegetal de tipo solúvel, formada na sua maior parte por pectina, o que explica o seu suave efeito laxante e hipolipemiante (descida do colesterol);
- compostos fenólicos que lhe conferem propriedades diuréticas e antioxidantes;

– ácido salicílico, o precursor natural de actuais medicamentos de acção anti-inflamatória e anti-reumática.

Quadro 1.2
Valor nutricional da cereja (100 g de matéria edível). Baseado em Souci *et al.* (1994).

Valor calórico (kcal)	62,32	Aminoácidos (mg)	
Água (g)	82,80	Alanina	24,00
Proteínas (g)	0,90	Arginina	14,00
Lípidos (g)	0,31	Ácido aspártico	483,00
Hidratos de carbono (g)	13,27	Cistina	3,00
Fibra total (g)	1,31	Ácido glutâmico	31,00
Solúvel	0,50	Glicina	19,00
Insolúvel	0,81	Histidina	11,00
Ácidos orgânicos (g)	0,95	Isoleucina	16,00
Minerais		Leucina	23,00
Sódio (mg)	2,70	Lisina	31,00
Potássio (mg)	229,00	Metionina	4,00
Magnésio (mg)	11,00	Fenilalanina	16,00
Cálcio (mg)	17,00	Prolina	26,00
Fósforo (mg)	20,00	Serina	26,00
Ferro (µg)	350,00	Treonina	18,00
Zinco (µg)	72,50	Triptofano	8,00
Cobre (µg)	115,00	Tirosina	10,00
Boro (µg)	396,00	Valina	22,00
Vitaminas		Ácidos	
Retinol equivalentes (µg)	5,83	Ácido málico (mg)	940,00
Carotenóides totais (µg)	35,00	Ácido cítrico (mg)	13,00
β-Caroteno (µg)	35,00	Ácido oxálico total (mg)	7,20
Tocoferóis totais (µg)	130,00	Ácido oxálico solúvel (mg)	4,30
α-Tocoferol (µg)	130,00	Ácido clorogénico (mg)	6,10
Vitamina B1 (µg)	39,00	Ácido ferúlico (µg)	300,00
Vitamina B2 (µg)	42,00	Ácido cafeico (mg)	7,00
Nicotinamida (µg)	270,00	Ácido *p*-cumárico (mg)	9,00
Ácido pantoténico (µg)	190,00	Ácido salicílico (µg)	850,00
Vitamina B6 (µg)	45,00	Hidratos de carbono	
Biotina (ng)	400,00	Glucose (g)	6,93
Ácido fólico (µg)	52,00	Frutose (g)	6,14
Vitamina C (mg)	15,00	Sacarose (mg)	193,00
		Pectina (mg)	360,00

1.3.1. Aspectos Qualitativos e Conservação

O conceito de qualidade é subjectivo e varia consoante o tipo de consumidores, da sua nacionalidade, da idade, dos hábitos alimentares, etc. Na qualidade, para além dos factores

característicos dos próprios frutos, designadamente a aparência, textura, odor e sabor, valor nutritivo e segurança (Quadro 1.3), são decisivos também a apresentação e aspecto dos frutos no local de venda, assim como a embalagem e os rótulos utilizados. Concretamente para a cereja, de acordo com Edin *et al.* (1997), o calibre, a firmeza, a cor e o brilho da epiderme, e um bom equilíbrio açúcares/ácidos são os principais critérios qualitativos. No Quadro 1.4 apresentam-se alguns critérios de qualidade para a cereja. A qualidade exprime-se também pela ausência de defeitos tais como: fissuras, picadas de aves, engelhamento, podridão ou formas estranhas (ex. cerejas duplas). Os pedúnculos verdes e firmes estão também associados à qualidade do fruto. Os danos físicos durante a colheita e manuseamento, tais como quedas, excesso de pressão no fruto provocada por grande peso (em especial na fruta a granel) contribuem bastante para a posterior deterioração do fruto. Os danos físicos aceleram a perda de água, conduzem à contaminação por fungos nas superfícies danificadas e aumentam a taxa respiratória.

Os frutos quando colhidos são estruturas vivas, continuando com as suas reacções metabólicas e mantendo os seus processos fisiológicos durante um considerado período de tempo pós-colheita. A respiração é o processo natural de obtenção de energia a partir das reservas de nutrientes dos frutos. Os frutos frescos continuam a respirar após a colheita sendo este processo responsável por grandes perdas de qualidade, assim como pela senescência natural. A taxa de respiração varia de fruto para fruto e quanto mais alta for, mais rapidamente se degradará o fruto e menor será o seu tempo de vida útil. A cereja, numa escala de taxa de respiração muito baixa a alta, apresenta taxa moderada (Kader, 1992).

A transpiração é outro processo natural pelo qual o fruto fresco perde água, podendo isso ter efeitos negativos não só no peso mas também no aspecto do fruto, o que se reflecte inevitavelmente no seu valor económico. A temperatura de armazenamento tem uma influência muito marcada no período de vida útil das cerejas, influenciando as taxas respiratória e de transpiração (Edin *et al.*, 1997; Empis e Moldão, 2000). Na cereja é imperativo a utilização de sistemas de refrigeração logo após a colheita para a manutenção de uma boa qualidade do fruto. A temperatura é assim o factor mais importante para a conservação dos frutos e deve ser mantida em valores baixos. No entanto abaixo de um certo limite de temperatura (específico para cada produto), podem surgir lesões nos frutos. O tempo de vida das cerejas pode-se aumentar se forem armazenadas e/ou transportadas à temperatura óptima de –1 a –0,5 °C. A humidade relativa do ar que está em contacto com os frutos após a colheita tem também um papel fundamental na textura do fruto. Em geral, a humidade relativa deve ser mantida em valores elevados para reduzir a transpiração do fruto.

Quadro 1.3
Critérios de qualidade principais dos frutos (Adaptado de Kader, 1992).

Factor	Componentes
Aparência visual	Tamanho: dimensões, peso e volume
	Forma e aspecto: irregularidade e uniformidade
	Cor: intensidade e uniformidade
	Brilho: natural ou da cera
	Defeitos:
	Morfológicos
	Físicos ou mecânicos
	Fisiológicos
	Patológicos
	Entomológicos
Textura	Firmeza
	Estaladiço
	Fibroso
	Dureza
Sabor	Aromas
(aroma e paladar)	Maus sabores e maus odores
	Doçura
	Acidez
	Adstringência
	Amargo
Valor nutritivo	Vitaminas
	Compostos fenólicos
	Minerais
	Hidratos de carbono (incluindo as fibras dietéticas)
	Proteínas
	Lípidos
Segurança	Componentes tóxicos naturais
	Contaminantes: resíduos químicos de pesticidas e de metais pesados ou produtos de limpeza
	Micotoxinas
	Contaminação microbiana

A composição da atmosfera do ar tem influência na manutenção da qualidade, na medida em que altos teores de CO_2 e baixos teores de O_2 podem diminuir a respiração. Cada produto tem uma tolerância máxima ao CO_2 e mínima ao O_2 a partir da qual sofre lesões irreversíveis e alterações fisiológicas. Actualmente, o aparecimento de técnicas de embalamento sob atmosfera modificada e armazenamento/transporte sob atmosfera controlada tem já em conta esses pressupostos. A informação técnica sobre a composição gasosa óptima em cerejas é muito variada, sendo particularmente dependente das condições edafo-climáticas do local, da

cultivar e do porta-enxerto (Cavalheiro *et al.*, 2004). Para uma longa conservação da cereja Burlat, a melhor concentração gasosa estudada foi a de 2,6% de O_2 e 20% de CO_2, uma vez que os frutos apresentaram uma menor perda de massa, tinham maior luminosidade (L^*), uma cor mais distinta (C^* mais elevado) e a cor vermelha mais pronunciada (H^* mais baixo).

Quadro 1.4
Critérios de qualidade para a cereja (Adaptado de Edin *et al.*, 1997).

Critérios				
Firmeza	Muito firme	Firme	Média	Insuficiente
Durofel 25	> 70	63–70	56–62,9	< 56
Sólidos solúveis	Muito alta	Alta	Média	Insuficiente
Índice refractométrico (ºBrix)	> 17	14–17	11,5–13,9	< 11,5
Doçura	Muito doce	Doce	Ácida	Muito ácida
Acidez titulável (g l^{-1})	< 10	10–12,5	12,6–15	> 15

Entre os principais parâmetros de qualidade do fruto, destacamos no ponto seguinte os compostos fenólicos pela importância que têm na valorização nutritiva das cerejas.

1.3.2. Compostos Fenólicos. Ocorrência, Funções Biológicas e Aplicações

Historicamente, os frutos e outros vegetais, para além do seu valor alimentar, têm sido utilizados como agentes medicinais. Especialmente, nestas últimas duas décadas, a comunidade científica começou a reconhecer o valor destes alimentos para além da sua contribuição mineral e do seu papel na prevenção de deficiências vitamínicas. Um elevado número de fitoquímicos (compostos bioactivos não nutrientes das plantas) foram identificados nos frutos e outros vegetais e têm sido relacionados com a redução do risco de doenças crónicas, especialmente as do foro oncológico. Para além disso, são reconhecidos outros efeitos benéficos na saúde, ligados à prevenção de doenças coronárias, à manutenção de uma boa estrutura óssea e ao controlo saudável do peso corporal. Julga-se que estes benefícios estejam associados ao efeito sinérgico dos diversos constituintes presentes tanto nos frutos como nos vegetais em geral. Embora os tipos de fitoquímicos sejam muitos, como os compostos de enxofre, os carotenóides, as ditionas e os glucosinolatos, os compostos fenólicos têm sido ultimamente alvo de uma grande atenção.

O termo "composto fenólico" (relativo a fenol, do grego *phaineine*) abrange uma grande variedade de compostos químicos cujo aspecto estrutural comum é a presença, nas suas moléculas, de pelo menos um grupo hidroxilo ligado directamente a um anel aromático (Shahidi e Naczk, 1995; Croteau *et al.*, 2000).

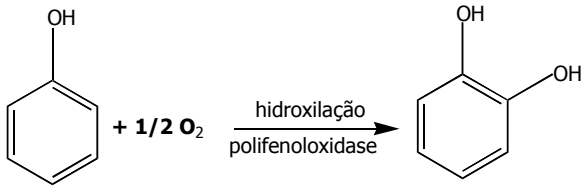
Os compostos fenólicos são metabolitos secundários presentes em frutos, hortaliças, sementes e flores, assim como na cerveja, vinho, chá verde e chá preto. Localizam-se preferencialmente nos tecidos mais externos das plantas, ou nas paredes celulares, onde a lenhina é depositada, ou nos vacúolos (Macheix *et al.*, 1990). Os fenóis naturais raramente se encontram livres, apresentando-se na sua grande maioria em combinações sob a forma de éster e glicosídica. Estes compostos são componentes substanciais da fracção não energética da dieta humana (Aherne e O'Brien, 2002) e também se podem utilizar sob a forma de suplementos nutricionais, juntamente com certas vitaminas e minerais.

A intervenção das substâncias fenólicas nos processos biológicos e tecnológicos é elevada, salientando-se os seguintes exemplos:

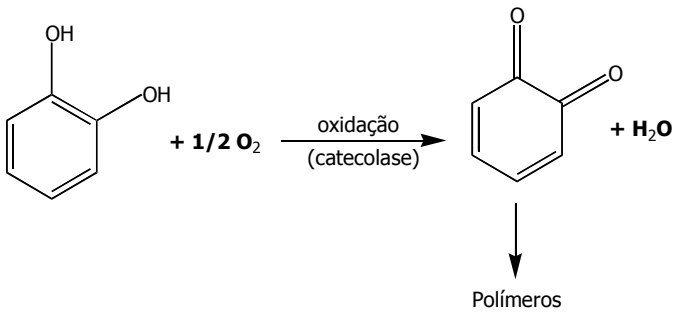
- As lenhinas são compostos poliméricos que fazem parte da estrutura da parede celular, funcionando como suporte mecânico e como barreira contra a invasão microbiana (Shahidi e Naczk, 1995; Strack, 1997; Croteau *et al.*, 2000);
- Alguns ácidos fenólicos simples, bem como taninos complexos e resinas, podem proteger as plantas dos ataques de herbívoros (Strack, 1997; Croteau *et al.*, 2000);
- Através da síntese de fitoalexinas (hidroxicumarinas e conjugados hidroxicinâmicos) em resultado de um ataque microbiano podem contribuir para o mecanismo de resistência a doenças das plantas (Macheix *et al.*, 1990; Shahidi e Naczk, 1995; Strack, 1997);
- Alguns fenólicos tóxicos solúveis em água (e.g., hidroquinona, hidroxibenzoatos e ácidos hidroxicinâmicos) podem influenciar a competição entre plantas, um fenómeno chamado alelopatia (Strack, 1997);
- Os flavonóides apigenina e luteolina podem actuar como moléculas sinal, que actuam selectivamente em *Rhizobia,* levando à indução da nodulação em algumas espécies da família *Fabaceae*, que permitem posteriormente a fixação do azoto atmosférico (Strack, 1997; Croteau *et al.*, 2000);
- São agentes quelantes de metais nocivos para as plantas (Formica e Regelson, 1995);
- Em resposta à luz, controlam os níveis de auxinas reguladoras do crescimento e diferenciação das plantas (Formica e Regelson, 1995);
- Os flavonóides absorvem radiação electromagnética na faixa do ultravioleta, sobretudo UV-B, e do visível e dessa maneira desempenham um papel fundamental de protecção das plantas contra agentes foto-oxidantes (Li *et al.*, 1993; Koes *et al.*, 1994; Dixon e Paiva, 1995; Strack, 1997; Croteau *et al.*, 2000);

– As antocianinas, flavonas e flavonóis contribuem para a cor das flores e frutos, que facilitam a atracção e orientação dos insectos até ao néctar, contribuindo muito para a polinização e para a dispersão de sementes (Strack, 1997; Croteau *et al.*, 2000);
– Os flavonóides estão envolvidos na regulação do crescimento do tubo polínico no estigma (Mo *et al.*, 1992; Vogt *et al.*, 1994);
– As antocianinas das epidermes das uvas ou de outros frutos após extracção e concentração são utilizados na indústria alimentar como corantes (Francis, 1993);
– O papel importante das antocianinas e dos taninos na cor do vinho e no seu envelhecimento (Liao *et al.*, 1992; Remy *et al.*, 2000);
– A intervenção de certos fenóis no escurecimento enzimático de frutos e outros vegetais (batata, banana, pêssego, chá, café, etc.) uma vez abertos e expostos ao ar (Strack, 1997; Tomás-Barberán e Espín, 2001). Por acção da enzima polifenoloxidase, os monofenóis são hidroxilados a *orto*-difenóis, que por sua vez são oxidados a *orto*-quinonas. Estas, por sua vez, polimerizam, dando origem a melaninas, compostos de cor castanha.

1) A inserção de um grupo hidroxilo na posição *orto* do anel aromático de um monofenol.



2) A oxidação de *orto*-difenóis (actividade catecolásica).



– A aplicação dos taninos no curtimento de peles (Strack, 1997);
– Podem ter um papel preponderante no sabor amargo, doce, picante (capsaicina, fenólico irritante em frutos de várias espécies de *Capsaicin*, Solanaceae) ou adstringente (taninos) dos alimentos (Croteau *et al.*, 2000). Singleton e Nobel (1976) relacionaram a

presença do ácido clorogénico e de outros ácidos hidroxicinâmicos e, em particular, as proantocianidinas com o sabor amargo e adstringente do vinho e cidra. Também na cerveja (Dadic e Belleau, 1973) e arando (Marwan e Nagel, 1982) foi relacionado com o ácido clorogénico;

- A 3,4-dihidroisocumarina é referenciado como sendo um fenólico 200 a 300 vezes mais doce do que a sacarose (Shahidi e Naczk, 1995);
- Os fenóis simples, especialmente os que são voláteis, e.g., vanilina, eugenol, isoeugenol e siringol contribuem para o aroma e sabor. Alguns deles estão presentes como precursores do aroma sob a forma de glicosídeos fenólicos, que após hidrólise, libertam os fenóis levando ao aroma (Crouzet *et al.*, 1997);
- Os flavonóides apresentam outras propriedades que incluem a estimulação das comunicações através das uniões de enxertia, o impacto sobre a regulação do crescimento celular e a indução de enzimas de desintoxicação, tais como as monooxigenases dependentes do citocromo P-450, entre outras (Stahl *et al.*, 2002);
- Desempenham um papel importante na saúde humana, uma vez que têm acção antioxidante, minimizando a peroxidação lipídica e o efeito dos radicais livres. Foi demonstrado que indivíduos que ingerem maiores quantidades de flavonóides, através de alimentos de origem vegetal (frutos, hortaliças, chá, mel, etc.), apresentam uma diminuição considerável do risco de morte por acidentes cardiovasculares (Kinsella *et al.*, 1993). Destacam-se ainda os seguintes efeitos dos flavonóides sobre os sistemas biológicos: actividade anti-inflamatória (Starvic, 1994) e do vasodilatador, acção antialérgica, antiviral e anticancerígena (Middleton, 1998), anti-hepatotóxica (Hikino *et al.*; 1984; Pathak *et al.*, 1991), antiulcerogénica (Pathak *et al.*, 1991; Harborne e Williams, 2000) e antiplaquetária (Amellal *et al.*, 1985). Recentemente, Lule e Xia (2005) num artigo de revisão aprofundam os atributos positivos e negativos dos compostos fenólicos dos alimentos.

Velioglu *et al.* (1998) determinaram a concentração de compostos fenólicos de diferentes produtos vegetais (Quadro 1.5). A cereja revelou uma concentração em fenóis totais cinco vezes inferior à da cebola, mas um coeficiente da actividade antioxidante (AAC; Mallet *et al.*, 1994) apenas 22% inferior.

Existe uma grande variabilidade na estrutura e ocorrência das substâncias de natureza fenólica, que inclui desde fenólicos muito simples, como os ácidos hidroxibenzóicos, até estruturas mais complexas, como os taninos condensados ou hidrolisáveis de elevado peso

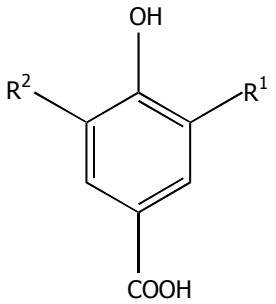
molecular (Tomás-Barberán e Espín, 2001). No entanto, as três classes principais de fenólicos na dieta são os ácidos fenólicos, os flavonóides e os polifenóis (taninos).

Quadro 1.5
Teor em compostos fenólicos (mg 100 g^{-1} peso fresco) e coeficiente da actividade antioxidante (AAC) de alguns frutos e vegetais (Velioglu *et al.*, 1998).

Fruto/Vegetal	Compostos fenólicos	AAC
Trigo	349	236
Batata	437	509
Linho	509	52
Girassol	1601	280
Cereja	2098	580
Mirtilo	4180	796
Cebola	10548	743

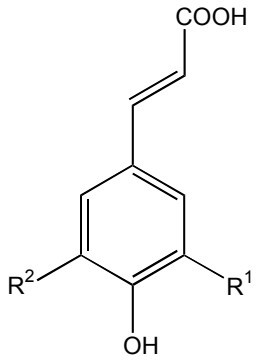
1.3.2.1. Ácidos Fenólicos

A denominação geral de ácidos fenólicos engloba os ácidos hidroxibenzóicos com uma estrutura base em C_6-C_1 (Figura 1.2) e os ácidos hidroxicinâmicos com uma estrutura base em C_6-C_3 (Figura 1.3) (Riberéau-Gayon, 1968; Shahidi e Naczk, 1995). O teor em ácidos hidroxibenzóicos nas plantas é geralmente baixo e integram estruturas complexas como as lenhinas e os taninos hidrolisáveis (Shahidi e Naczk, 1995). Contrariamente, os ácidos hidroxicinâmicos ocorrem frequentemente nos frutos e vegetais (Shahidi e Naczk, 1995). De acordo com Macheix *et al*. (1990), o ácido cafeico é o ácido hidroxicinâmico predominante em muitos frutos. Constitui mais de 75% dos ácidos hidroxicinâmicos totais em ameixa, maçã, damasco, mirtilo e tomate. Nos citrinos e ananás é o ácido *p*-cumárico que predomina.



R^1=R^2=H	ácido *p*-hidroxibenzóico
R^1=R^2=OH	ácido gálico
R^1=OCH_3, R^2=H	ácido vanílico
R^1=R^2=OCH_3	ácido siríngico

Figura 1.2
Estrutura base dos ácidos hidroxibenzóicos e alguns exemplos.



$R^1=R^2=H$	ácido *p*-cumárico
$R^1=$ OH, $R^2=H$	ácido cafeico
$R^1=OCH_3$, $R^2=H$	ácido ferúlico
$R^1=R^2=OCH_3$	ácido sináptico

Figura 1.3
Estrutura base dos ácidos hidroxicinâmicos e alguns exemplos.

Os ácidos hidroxicinâmicos são na sua maioria derivados dos ácidos *p*-cumárico, cafeico, ferúlico e mais raramente do ácido sináptico. Ocorrem nos alimentos frequentemente como derivados de ésteres e glicosídeos. O ácido 5-*O*-cafeoilquínico, conhecido como ácido clorogénico, éster do ácido cafeico e do ácido quínico, é a combinação mais frequente na natureza e um dos principais responsáveis pelo escurecimento enzimático que se observa quando se descascam alguns frutos como pêra, maçã e marmelo (Hulme, 1958). Por exemplo, o teor deste ácido é reduzido em 70% durante o acastanhamento da epiderme da pêra (Wang e Mellenthin, 1973).

Na cereja, os ácidos hidroxicinâmicos mais comuns são os ésteres dos ácidos cafeico e *p*-cumárico com o D-quínico e com a D-glucose: o ácido neoclorogénico (ácido 3-*O*-cafeoilquínico), o ácido *p*-cumaroilquínico e em menor quantidade o ácido clorogénico (Herrmann, 1989; Gao e Mazza, 1995). O primeiro autor refere para os três ácidos as seguintes concentrações, 73–628, 81–450 e 11–40 mg kg^{-1} de peso fresco, respectivamente. Gao e Mazza (1995) e Mozetič *et al*. (2002) avançaram os valores apresentados no Quadro 1.6 para os ácidos neoclorogénico e *p*-cumaroilquínico.

1.3.2.2. Flavonóides

Os flavonóides estão presentes na maior parte dos tecidos das plantas e constituem um grupo enorme com mais de 4500 flavonóides já identificados (Croteau *et al*., 2000). As funções biológicas deste grupo de compostos nos animais e seres humanos foram inicialmente sugeridas na década de 30 pelo prémio Nobel de fisiologia e medicina Albert Szent-Györgyi, quando isolou a citrina da casca do limão, possuindo, esta substância, a capacidade de

regulação da permeabilidade dos capilares. Assim, os flavonóides designaram-se inicialmente por vitamina P ("*preventive*", "*permeability*") e também vitamina C2, visto que algumas das substâncias pertencentes a esta classe apresentavam capacidade de fortificar as paredes dos vasos sanguíneos muito semelhante à da vitamina C (Singleton, 1981). Porém, cerca de duas décadas mais tarde e depois de numerosos ensaios clínicos, chegou-se à conclusão que, a diversidade química encontrada para os flavonóides associada ao não estabelecimento de uma relação directa entre os flavonóides e a permeabilidade capilar, não permitia a classificação destes compostos como vitamina (Varma e Kinoshita, 1993).

Quadro 1.6
Teor em ácidos hidroxicinâmicos (mg 100 g^{-1} peso fresco) em frutos de 9 cultivares de cerejeira ([a]Gao e Mazza, 1995; [b]Mozetič *et al.*, 2002).

Cultivar	ácido neoclorogénico	ácido *p*-cumaroilquínico	Total
Bing[a]	128	43	171
Bing[b]	27	8	35
Lambert[a]	119	47	166
Lambert[b]	36	9	45
Sam[a]	38	131	169
Stella[a]	93	33	126
Stella[b]	30	8	38
Summit[a]	29	84	113
Sylvia[a]	34	76	110
Van[a]	86	23	109
Napoleon[b]	20	51	71
Petrovka[b]	53	16	69

Os flavonóides são compostos de baixo peso molecular, com estrutura geral C_6-C_3-C_6 (Figura 1.4), caracterizados pela presença de dois anéis benzénicos (A e B) ligados através de um anel pirano (Shahidi e Naczk, 1995; Iwashina, 2000; Martínez-Flórez *et al.*, 2002). O esqueleto C_{15} dos flavonóides é biogeneticamente derivado do fenilpropano (C_6-C_3) e três unidades de acetato (C_6) (Middleton e Kandaswarni, 1993).

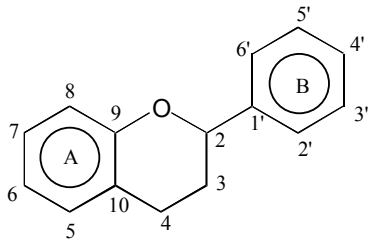


Figura 1.4
Estrutura base dos flavonóides.

O modo de ciclização, o grau de insaturação e o estado de oxidação do anel central dos flavonóides determinam os vários grupos, sendo os que se incluem na dieta humana essencialmente 6: flavonas **(1)**, e.g., apigenina e luteolina; flavonóis **(2)**, e.g., miricetina, quercetina, quempferol e rutina; antocianidinas **(3)**, e.g., cianidina, peonidina, pelargonidina; flavanóis **(4)**, e.g., catequina e epicatequina; isoflavonóides **(5)**, e.g., genisteína e coumestrol; e flavanonas **(6)**, e.g., hesperidina, naringina e naringenina (Figura 1.5) (Riberéau-Gayon, 1968; Mabry *et al.*, 1970; Markham *et al.*, 1982; Croteau *et al.*, 2000; Iwashina, 2000; Martínez-Flórez *et al.*, 2002; Yilmaz e Toledo, 2004).

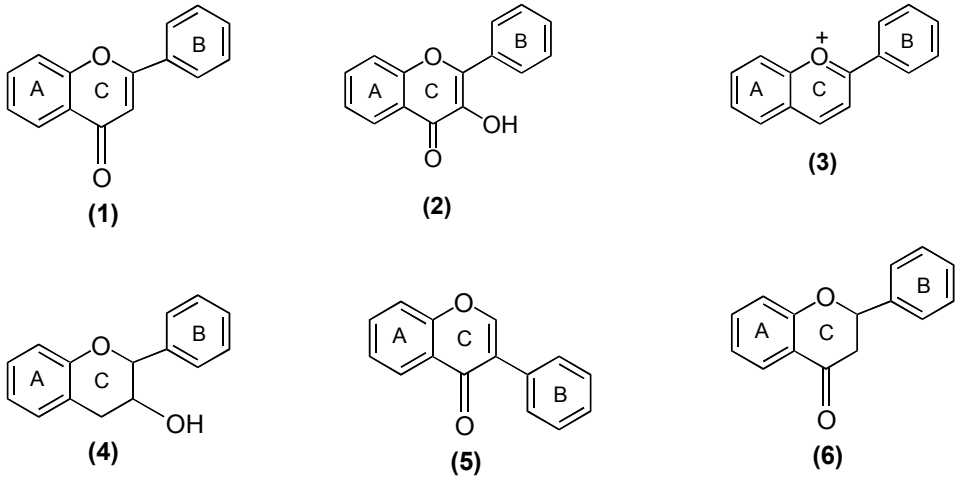


Figura 1.5
Estruturas base de alguns grupos característicos dos flavonóides: **(1)** flavonas, **(2)** flavonóis, **(3)** antocianidinas, **(4)** flavanóis, **(5)** isoflavonóides e **(6)** flavanonas.

Os flavonóides encontram-se na natureza sob a forma de glicosídeos. Estes formam-se através da união de resíduos de D-glucose à posição 3 do anel C ou à posição 5 ou 7 do anel A (os açúcares ligados ao anel B são raros) destes flavonóides, sendo a primeira substituição a mais frequente (Strack, 1997). Outros resíduos de açúcares que também se podem encontrar ligados a este tipo de compostos são a D-galactose, a L-ramnose, a L-arabinose, a D-xilose e o ácido D-glucurónico (Martínez-Flórez *et al.*, 2002).

Nos alimentos, esta classe de fenóis está largamente representada por uma vasto grupo de flavonóides corados e por outro grupo de flavonóides não corados de grande interesse. As antocianinas são pigmentos solúveis em água e responsáveis pela maioria da cor vermelha, azul e cores intermédias da maioria das espécies do reino vegetal (Mazza e Miniati, 1993; Shahidi e Naczk, 1995). Na natureza, todas as antocianinas são glicosídeos e a sua correspondente aglicona chama-se antocianidina. Cerca de 200 antocianinas diferentes foram identificadas nas plantas. Destas, aproximadamente 70 foram identificadas em frutos. Contudo as 6 antocianidinas mais comuns são a delfinidina, cianidina, malvidina, pelargonidina, peonidina e

petunidina (Shahidi e Naczk, 1995).

A cor das antocianinas varia em função do grau de hidroxilação e de metilação do anel B. A presença de um número elevado de grupos hidroxilo na molécula leva à predominância da cor azul, enquanto que os grupos metoxilo levam ao vermelho (Mazza e Miniati, 1993). No entanto, a intensidade da cor depende também do pH, presença de iões metálicos, mistura de pigmentos e co-pigmentos (Brouillard, 1983) e, ainda, das condições de processamento e de armazenamento (temperatura, teor em hidratos de carbono, presença de ácido ascórbico, etc.). Para a estabilidade da cor contribui a acilação do açúcar das antocianinas (Harborne, 1964). Esta está também associada com a capacidade das antocianinas em formar complexos com outros fenólicos, ácidos nucléicos, açúcares, aminoácidos e iões metálicos, tais como o cálcio, magnésio e potássio (Brouillard, 1983).

As cianidinas 3-rutinósido e 3-glucósido são as mais abundantes na cereja (Gao e Mazza, 1995; Esti *et al.*, 2002; Mozetič *et al.*, 2002; Belitz *et al.*, 2004). Em menor quantidade surgem as peonidinas 3-rutinósido e 3-glucósido e a pelargonidina-3-rutinósido (Gao e Mazza, 1995). Em frutos, o teor em antocianinas é muito variável, desde 3,4 mg na maçã vermelha e 232 mg em morango, até 1064 mg em groselha e 3090 mg em mirtilo, por 100 g de peso seco (Kähkönen *et al.*, 2001). Na cereja, o teor em antocianinas totais depende da cultivar (Quadro 1.7). Garcia-Viguera *et al.* (1997) investigaram a composição em antocianinas em compotas de cereja doce e também encontraram valores mais elevados de cianidina-3-rutinósido, que variaram entre 76 e 201 mg 100 g^{-1}.

Aproximadamente 12 flavanóis foram identificados em plantas, dos quais apenas 4 surgem em frutos: (+) catequina, (–) epicatequina, (+) galocatequina e (–) epigalocatequina (Figura 1.6, Macheix *et al.*, 1990). Os flavan-3-óis catequina e epicatequina são unidades monoméricas das proantocianidinas (Shahidi e Naczk, 1995), sendo os predominantes na cereja (Risch e Herrmann, 1988).

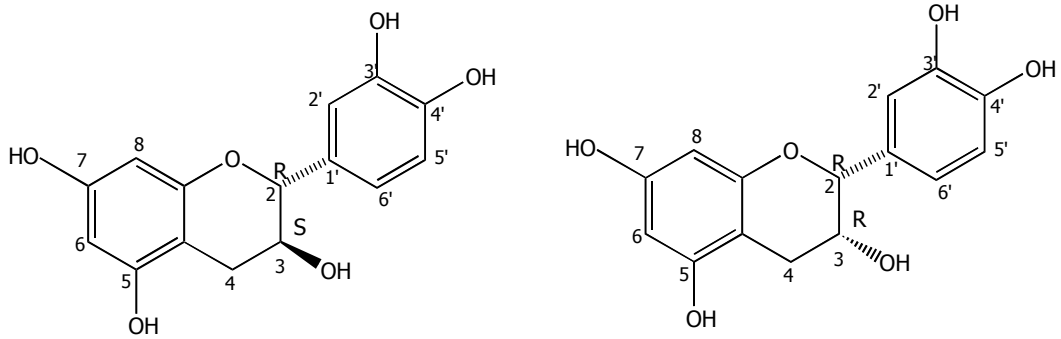


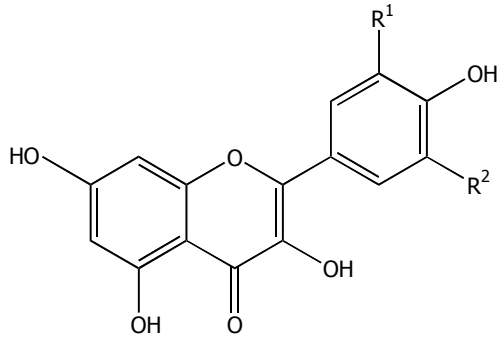
Figura 1.6
Série das catequinas e das epicatequinas.

Quadro 1.7
Teor em antocianinas (mg 100 g^{-1} peso fresco) em frutos de 9 cultivares de cerejeira ([a]Gao e Mazza, 1995; [b]Esti *et al.*, 2002).

Cultivar	cy-3-glu	cy-3-rut	pn-3-glu	plg-3-rut	pn-3-rut	Total
Bing[a]	31	181	1	3	9	225
Lambert[a]	44	151	1	1	2	199
Sam[a]	25	193	1	2	6	227
Stella[a]	18	129	qv	1	6	154
Summit[a]	6	72	qv	1	3	82
Sylvia[a]	15	211	1	2	16	230
Van[a]	12	130	1	1	7	151
Sciazza[b]	48	393	3	4	28	476
Ferrovia[b]	3	60	1	1	1	66

cy – cianidina; pn – peonidina; plg – pelargonidina; glu – glicósido; rut – rutinósido; qv – quantidades vestigiais.

Os flavonóis agliconas usualmente encontrados nos frutos são 4, nomeadamente o quempferol, a quercetina, a miricetina e a isoramnetina (Figura 1.7, Macheix *et al.*, 1990). A quercetina é um potente antioxidante, com concentrações elevadas nos seguintes frutos: marmelo, baga de sabugueiro, arando, framboesa e maçã (Belitz *et al.*, 2004). Na cereja, o flavonol rutina (quercetina-3-glucósido) é o mais abundante (Gao e Mazza, 1995; Belitz *et al.*, 2004).



R^1=R^2=H quempferol
R^1=R^2=OH miricetina
R^1=OH, R^2=H quercetina
R^1=OCH_3, R^2=H isoramnetina

Figura 1.7
Estrutura base dos flavonóis e alguns exemplos.

Pela análise do Quadro 1.8, observa-se que a cereja tem um valor elevado de quercetina, principalmente em cerejas sujeitas a processamento. Geralmente, os teores em flavonóides de alimentos processados são 50% mais baixos do que nos produtos frescos. No entanto, em relação à quercetina, as cerejas processadas têm aproximadamente o dobro deste flavonol do que as cerejas frescas (Velioglu *et al.*, 1998). O teor em quercetina em cerejas

processadas é similar ao da maçã, sendo esta uma das fontes principais de quercetina na dieta. Dos vegetais analisados, apenas a cebola (300–400 mg kg^{-1}), couve galega (110 mg kg^{-1}), feijão processado (39 mg kg^{-1}) apresentaram teores de quercetina mais altos do que as cerejas processadas (Hertog *et al.*, 1992).

Quadro 1.8
Teor em quercetina (mg kg^{-1} peso fresco) de diferentes frutos ([a]Velioglu *et al.*, 1998; [b]Belitz *et al.*, 2004).

Fruto	Quercetina[a]	Fruto	Quercetina[b]
Maçã	36	Baga de sabugueiro	170
Cereja processada	32	Marmelo	130
Cereja fresca	15	Arando	130
Damasco processado	25	Framboesa	70
Damasco fresco	<1	Maçã	49
Uvas brancas	12	Cereja	14
Uvas tintas	15	Groselha	13
Morango	9		
Ameixa	9		
Pêra	6		
Pêssego	<1		

1.3.2.3. Taninos

De acordo com Macheix *et al.* (1990), os taninos são polifenóis que têm a propriedade de precipitar proteínas em meio aquoso, podendo ser classificados em taninos hidrolisáveis (incluem galotaninos e elagitaninos, polímeros derivados do ácido gálico e elágico; também contêm uma molécula de glucose – Figura 1.8) e não-hidrolisáveis (ou taninos condensados ou proantocianidinas – Figura 1.9). Também formam complexos com certos tipos de polissacarídeos, ácidos nucléicos, alcalóides (Ozawa *et al.*, 1987) e minerais, em particular, iões metálicos bivalentes como o Fe^{2+} (Faithful, 1984). Os taninos hidrolisáveis são encontrados em plantas das famílias *Leguminosae* e *Rosaceae*. Em frutos como o morango e framboesa estão normalmente combinados com os taninos condensados (Foo, 1981; Foo e Porter, 1981). O peso molecular varia entre 500 e 2800 daltons (Haddock *et al.*, 1982).

Os taninos condensados são mais frequentes nos frutos que os taninos hidrolisáveis e estão distribuídos pela polpa do fruto, mas principalmente na epiderme. Os taninos condensados são dímeros, oligómeros ou polímeros de flavan-3-óis. O peso molecular varia entre 2000 e 4000 daltons (Macheix *et al.*, 1990). Como já foi referido, os taninos são responsáveis pela adstringência de muitos frutos, particularmente antes da maturação. Durante o amadurecimento há uma perda de adstringência nos frutos, usualmente concomitante com a

diminuição na concentração desses compostos (Goldstein e Swain, 1963; Joslyn e Goldstein, 1964).

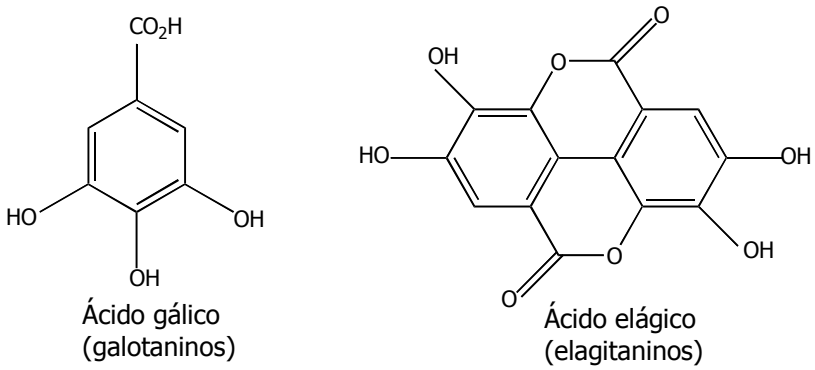


Figura 1.8
Estrutura do ácido gálico e do ácido elágico.

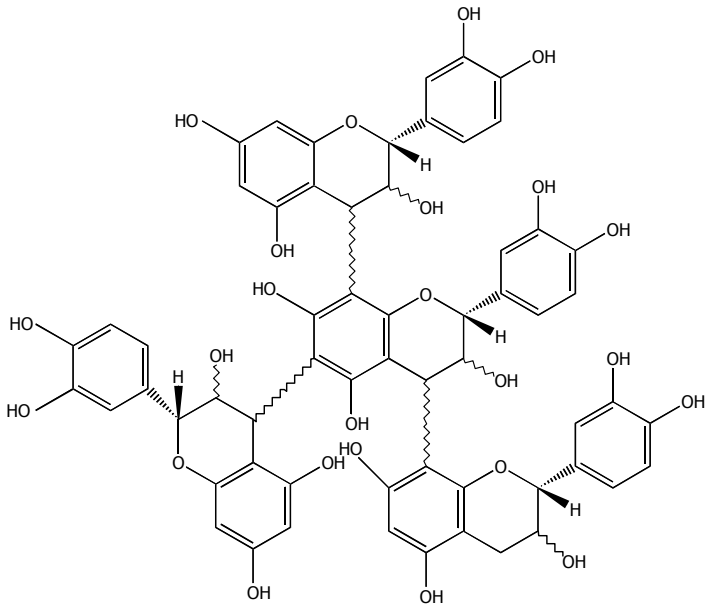


Figura 1.9
Estrutura base dos taninos condensados.

1.3.2.4. Biossíntese dos Compostos Fenólicos

Os aminoácidos aromáticos fenilalanina e tirosina são sintetizados através da Rota do Ácido Shiquímico [ver revisão de Dixon e Paiva (1995)]. Estes dois aminoácidos são os precursores chave da maioria das substâncias fenólicas produzidas pelas plantas (Figura 1.10), designadamente, os fenilpropanóides, os ácidos fenolcarboxílicos, os fenóis simples bem como o anel B e os carbonos 2, 3 e 4 do heterociclo central, dos flavonóides (Castro e Fernandes, 1995), que passamos resumidamente a descrever. Por desaminação não oxidativa, a

fenilalanina converte-se em ácido cinâmico e a tirosina em ácido *p*-cumárico (Croteau *et al.*, 2000). Estas reacções são promovidas por duas amoníaco-liases: a fenilalanina-amoníaco-liase (PAL) e a tirosina-amoníaco-liase. O ácido cinâmico pode, por sua vez, converter-se em ácido *p*-cumárico por hidroxilação (Strack, 1997). Os outros membros da família do ácido cinâmico formam-se por simples substituições nas posições 3, 4 e 5, conduzindo aos ácidos ferúlico e sináptico, que, juntamente com o ácido *p*-cumárico, são os precursores directos das unidades estruturais das lenhinas. Os grupos –OH e $–OCH_3$ resultam de reacções de hidroxilação e de *O*-metilação, que têm lugar no anel aromático, nas posições 3, 4 e 5 (Poulton, 1981; Strack, 1997).

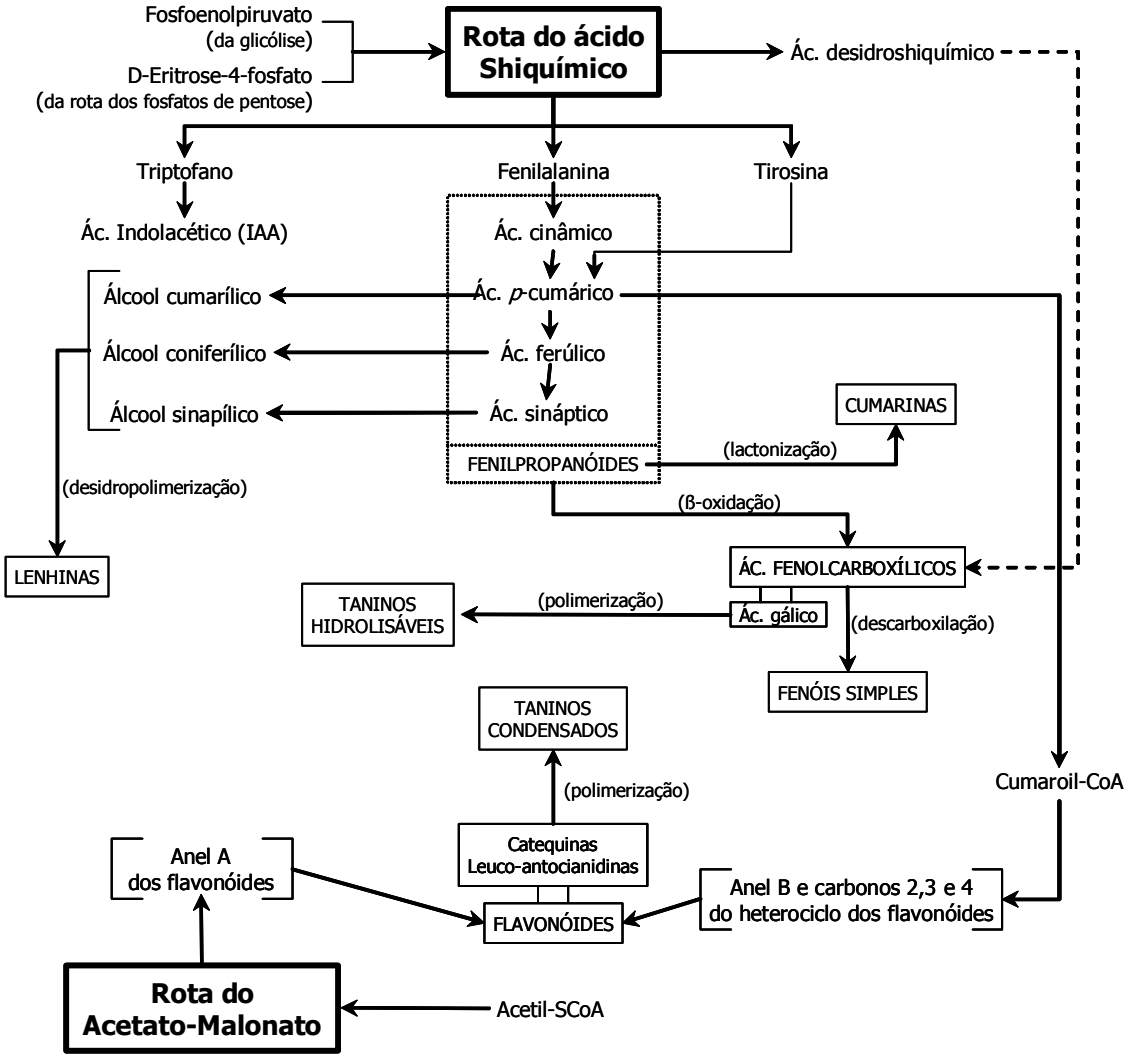


Figura 1.10
Biossíntese das substâncias fenólicas nas plantas superiores. Integração dos processos (Adaptado de Castro e Fernandes, 1995).

Nas plantas demonstrou-se a presença de *para*-hidroxilases, nomeadamente, a que converte o ácido cinâmico em *p*-cumárico. A formação de um grupo –OH, em posição *orto*, nos monofenóis, é, provavelmente, promovida por enzimas do tipo das fenolases (Macheix *et al*., 1990). As cumarinas são lactonas de derivados do ácido cinâmico. Se este ácido for oxidado de maneira a formar um grupo hidroxilo em posição *orto* e, em seguida, ocorrer a eliminação de uma molécula de água entre esse grupo hidroxilo e o grupo carboxilo do ácido, forma-se a lactona (Macheix *et al*., 1990; Strack, 1997). Por β-oxidação do ácido *p*-cumárico obtém-se o ácido *p*-hidroxibenzóico; por descarboxilação deste último forma-se a hidroquinona que poderá ser glicosilada a arbutina por intervenção da uridina-difosfato-glicose (UDPG). A UDPG (um nucleósido difosfatado) intervém na síntese da maioria dos glicosídeos fenólicos como dadora da molécula de açúcar. De forma análoga ao que acontece com o ácido *p*-cumárico, a β-oxidação do ácido cafeico conduz à formação do ácido protocatecóico e, a β-oxidação do ácido ferúlico, conduz à formação do ácido vanílico (Strack, 1997).

Os dois anéis aromáticos dos flavonóides formam-se por vias metabólicas diferentes (Halbrock e Grisebach, 1979; Halbrock, 1981; Grisebach, 1985). Com efeito, experiências com precursores radioactivos revelaram que o esqueleto de carbono de todos os flavonóides deriva de moléculas de acetato e da fenilalanina: o anel A é formado a partir de três moléculas de acetato pela Rota do Acetato-Malonato; por outro lado, o anel B e os átomos de carbono 2, 3 e 4 do heterociclo central, são formados a partir da fenilalanina sintetizada pela Rota do Ácido Shiquímico. Esta linha biossintética traz como consequência o esquema de hidroxilação que se encontra na maioria dos casos, ou seja, hidroxilação alternada no anel A (OH em posição 5 e 7) e *para* no anel B com um dos seguintes esquemas: 4'-OH, 3',4'-diOH, 3',4',5'-triOH. A variabilidade de estrutura encontrada nesta classe de compostos advém das posteriores modificações que poderão surgir, sendo as mais frequentes a glicosilação e a metilação dos hidroxilos presentes. Embora mais raramente, surgem na natureza outras modificações: metoxilação, hidroxilação adicional, glicosilação (formação de *C*-glicosilflavonóides), prenilação, acilação dos hidroxilos, do núcleo flavonóide ou dos açúcares que lhe estão ligados, metilenação de grupos *orto*-dihidroxilos e dimerização (Ferreira *et al*., 1997). O primeiro grupo de reacções compreende os passos comuns à síntese dos fenilpropanóides, em que o resultado global consiste na conversão da fenilalanina em ácido *p*-cumárico, com 9 átomos de carbono. No segundo grupo de reacções, o ácido *p*-cumárico, na forma activada de cumaroil-CoA, serve de ponto de partida para a formação de uma chalcona com 15 átomos de carbono. Ao cumaroil-CoA vão-se adicionar, por três vezes sucessivas unidades de acetato derivadas do malonil-CoA que sofre concomitante descarboxilação durante o processo. Formam-se assim

intermediários com 11, 13 e 15 átomos de carbono. Este mecanismo, promovido pela chalcona-sintetase, assemelha-se muito ao processo de biossíntese dos ácidos gordos, realizado também por acréscimos sucessivos de 2 carbonos, provenientes do malonil-CoA. A cadeia lateral do anel aromático B, inicialmente com 3 mas agora com 9 átomos de carbono, sofre ciclização espontânea originando uma chalcona que é a precursora geral dos outros flavonóides (Strack, 1997).

Tal como os flavonóides, as proantocianidinas são sintetizadas a partir da cumaroil-CoA e a estrutura C_6-C_3 deriva do ácido cinâmico. As proantocianidinas, por condensação com os flavan-3-óis originarão dímeros, trímeros e polímeros (Macheix *et al.*, 1990).

Para uma leitura mais aprofundada sobre a biossíntese de todas as classes de compostos fenólicos recomenda-se o trabalho de Strack (1997).

1.3.2.5. Factores que afectam a Biossíntese dos Compostos Fenólicos

A composição fenólica dos frutos e de outros vegetais está dependente de factores genéticos, ambientais, agronómicos, pós-colheita e de processamento (Tomás-Barberán e Espín, 2001; Anttonen e Karjalainen, 2005). A utilização de compostos fenólicos como marcadores químicos taxonómicos está bem documentada. Por exemplo, o composto fenólico naringina é utilizado para estabelecer afinidades ou diferenças taxonómicas entre espécies do género *Citrus* (Robards *et al.*, 1999). A síntese de compostos fenólicos é também dependente do clima (temperatura e intensidade de luz), do tipo de solo, das práticas culturais, da concentração de CO_2 na atmosfera e da época da colheita (Robards *et al.*, 1999; Wang, 2006). A água disponível e a composição mineral e orgânica do solo têm um efeito marcante na composição fenólica de frutos e de outros vegetais e na possibilidade de sofrerem oxidações expressas pelo acastanhamento ("browning") e outras alterações fisiológicas que podem surgir ainda no campo ou em pós-colheita (McClure, 1975). Assim, a deficiência em cálcio pode levar ao aparecimento de "bitter pit" em maçãs durante a conservação refrigerada (Watkins *et al.*, 2004). Uma aplicação excessiva de azoto reduz os níveis de antocianinas nos frutos porque os produtos da fotossíntese são desviados prioritariamente para a síntese de aminoácidos e proteínas (Macheix *et al.*, 1990). A presença de cálcio no solo induz a acumulação de antocianinas nas uvas das castas Merlot e Cabernet Sauvignon (Yokotsuka *et al.*, 1999). Também a temperatura pode ter uma forte influência na acumulação de antocianinas em diferentes tipos de frutos, por exemplo, em groselha (Rubinskiene *et al.*, 2005), ameixa (Tsuji *et al.*, 1983) e uva (Kataoka *et al.*, 1984). Geralmente, a exposição a baixas temperaturas promove a formação de antocianinas (Yamane *et al.*, 2006). Por exemplo, nas uvas Aki Queen

(*Vitis labrusca* x *V. vinifera*), no estado III de desenvolvimento do fruto, o teor em antocianinas era superior quando sujeitas a 20 ºC do que a 30 ºC. No entanto, Naumann e Wittemberg (1980) referem que groselhas produzidas a temperaturas mais baixas tinham menor concentração de antocianinas, comparativamente aos frutos produzidos em condições de temperaturas mais altas. A intensidade e a qualidade da luz também afectam o teor em antocianinas. Em maçãs das cultivares Gala e Royal Gala, a síntese de antocianinas foi reduzida pela exposição à radiação UV-B e do visível (Reay e Lancaster, 2001).

A manipulação durante a colheita, assim como o transporte e armazenamento pós-colheita podem ter um impacto negativo na composição fenólica e nas enzimas envolvidas no metabolismo fenólico (Tomás-Barberán e Espín, 2001). Contudo, se o armazenamento ocorrer a baixas temperaturas pode contribuir para aumentar o teor em antocianinas, designadamente em morango (Sanz *et al*., 1999), mirtilo (Skrede *et al.*, 2000) e uva (Cantos *et al.*, 2000). Contudo, Hansawasdi *et al.* (2006) registaram um decréscimo de compostos fenólicos e de antocianinas em morangos armazenados a 0 ºC.

As tecnologias de processamento alimentar, tais como o tratamento térmico, enzimático, fermentação, desidratação e radiação podem afectar a composição fenólica e a qualidade alimentar. Como regra, durante o processamento, a síntese de compostos fenólicos é interrompida pela destruição enzimática ou pela degradação da parede celular. No entanto, se as enzimas oxidativas não forem inactivadas a degradação destes compostos pode aumentar com o processamento (Cantos *et al*., 2001).

O trabalho de revisão de Tomás-Barberán e Espín (2001) aprofunda os efeitos destes factores na composição fenólica e enzimas relacionadas como determinantes da qualidade de frutos e outros vegetais.

1.3.2.6. Stresse Oxidativo e Actividade Antioxidante dos Compostos Fenólicos

Os organismos aeróbios necessitam de O_2 como aceitador de electrões para uma produção eficaz de energia. No entanto, o oxigénio é uma fonte oxidante, tornando-se impossível impedir oxidações secundárias promovidas por esta molécula, não envolvidas no metabolismo fisiológico, que podem ter consequências graves se os seus produtos não forem neutralizados por um sistema antioxidante eficiente (Sorg, 2004). Outras fontes possíveis de espécies oxidantes são poluentes do ar (e.g., ozono), a radiação UV, o tabaco e uma dieta rica em ácidos gordos saturados (Pietta, 2000; Sorg, 2004).

A redução completa do O_2 envolve quatro electrões, resultando em água como produto final da cadeia respiratória (Sorg, 2004). Porém, podem ocorrer situações em que o oxigénio é

parcialmente reduzido, originando como produtos destas reacções secundárias vários compostos intermédios com elevado poder oxidante. Os radicais livres formados são frequentemente englobados num grande grupo de compostos designados por espécies reactivas de oxigénio (*Reactive Oxygen Species*, ROS). Estas englobam espécies radicalares (superóxido, hidroxilo, peroxilo, alquilo, alcoxilo, hidroperoxilo) e não radicalares (peróxido de hidrogénio, ácido hipocloroso, ozono, oxigénio singleto, peróxidos lipídicos) (Halliwell e Gutteridge, 1999). A formação de ROS verifica-se numa grande variedade de componentes celulares, tais como mitocôndrias, lisossomas, peroxissomas, núcleo, retículo endoplasmático, membrana plasmática e mesmo no citoplasma (Machlin e Bendich, 1987).

Os alvos biológicos principais dos radicais livres e das ROS são: as proteínas, cuja oxidação conduz à perda de função ou à degradação prematura nos proteossomas; os lípidos, cuja oxidação altera as propriedades físicas das membranas celulares e, consequentemente, a sua função; e o DNA, cuja oxidação pode conduzir a mutações génicas, à síntese proteíca anormal, à alteração na expressão génica e à morte celular (Byers e Perry, 1992; Sorg, 2004).

Para contrariar o efeito nocivo das ROS, as células têm dois sistemas principais de defesa, sendo o primeiro um sistema de defesa enzimático, constituído pelas enzimas superóxido dismutase (SOD), catalase (CAT), glutationa peroxidase (GP), glutationa redutase (GR) e tioredoxina redutase e o segundo um sistema de defesa não enzimático, constituído por antioxidantes dietéticos, como o α-tocoferol (vitamina E), o ascorbato, as ubiquinonas (Coenzimas Q_9, Q_{10}, entre outras), a glutationa reduzida, os carotenóides e os compostos fenólicos (Radi *et al.*, 1997; Sies, 1997; Halliwell e Gutteridge, 1999; Yilmaz e Toledo, 2004). A quercetina e o quempferol, compostos fenólicos da classe dos flavonóis, são importantes no controlo das concentrações intracelulares de glutationa. Ao actuarem ao nível do gene de regulação são capazes de aumentar o seu nível a 50%, induzindo o sistema antioxidante celular, o que se relaciona com a sua potencial actividade anticancerígena (Martínez-Flórez *et al.*, 2002).

Embora o organismo humano tenha capacidade de prevenir reacções indesejáveis e de reparar moléculas e tecidos danificados, estes mecanismos de defesa não são suficientemente abrangentes para prevenir e reparar os danos causados por todas as reacções indesejáveis, ocorrendo a acumulação de produtos destas reacções, que se tornará prejudicial ao fim de um certo período de tempo. Esta situação de desequilíbrio entre a formação de espécies com poder oxidante e a sua destruição denomina-se por stresse oxidativo e pode conduzir a um metabolismo anormal, à perda de funções fisiológicas e, em casos extremos, à morte celular (Sorg, 2004). É do conhecimento geral que várias patologias, incluindo as doenças vasculares,

vários tipos de cancro, artrite e doença de Alzheimer estão associadas pelo menos em parte, aos danos causados pela produção descontrolada de radicais livres (Halliwell e Gutteridge, 1999).

Os compostos fenólicos são capazes de inibir a oxidação de vários substratos, desde moléculas simples a polímeros e biossistemas complexos, através de dois mecanismos: (i) abrange a inibição da formação de radicais livres que possibilitam a etapa de iniciação; (ii) abarca a eliminação de radicais importantes na etapa de propagação, como os radicais alcoxilo e peroxilo, através da doação de átomos de hidrogénio a estas moléculas, interrompendo a reacção em cadeia. Os produtos intermédios formados são relativamente estáveis devido à ressonância do sistema aromático, o que impede a propagação da cadeia (Figura 1.11) (Cotelle *et al.*, 1992; Rice-Evans *et al.*, 1996).

Muitas das acções biológicas dos flavonóides podem ser atribuídas às suas propriedades antioxidantes, quer através das suas capacidades redutoras, quer através da influência que exercem no estado redox do meio intracelular (Williams *et al.*, 2004). A capacidade antioxidante dos flavonóides aplica-se sobretudo na neutralização das ROS, embora também desempenhe uma função preventiva na oxidação das lipoproteinas de baixa densidade (LDL). Desta forma, anulam os efeitos negativos destas espécies sobre as células (Martínez-Flórez *et al.*, 2002). Por outro lado, os flavonóides inibem as enzimas responsáveis pela produção do anião superóxido, como a xantina oxidase e a proteina cinase C. Estes compostos inibem também a ciclooxigenase, lipooxigenase, monooxigenase microssomal, glutationa S-transferase, succinoxidase mitocondrial e NADH oxidase, estando todas estas enzimas envolvidas na produção de ROS (Pietta, 2000).

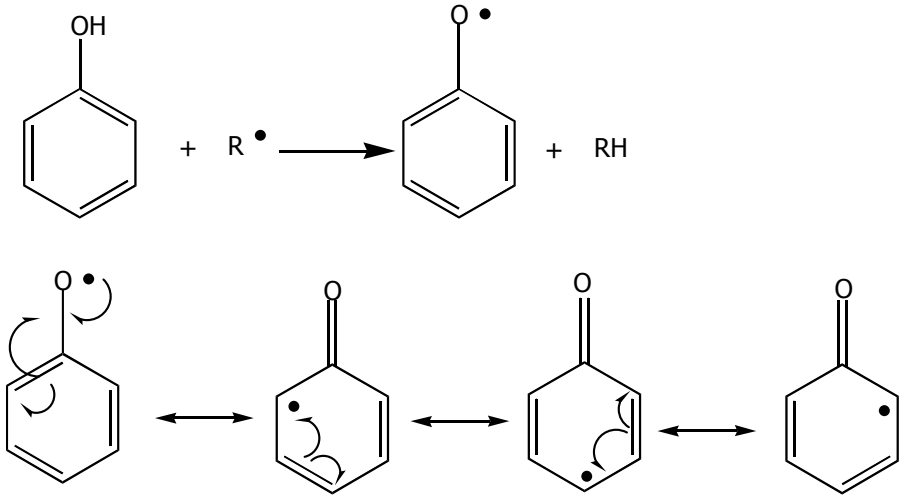


Figura 1.11
Eliminação de radicais livres pelos compostos fenólicos.

Nos alimentos, os antioxidantes têm sido definidos como substâncias que em pequenas quantidades são capazes de prevenir ou retardar a oxidação de materiais facilmente oxidáveis como as gorduras (Chipault, 1962). Nos sistemas biológicos, a definição de antioxidantes estendeu-se a qualquer substância que quando presente em baixas concentrações, comparando com a do extracto oxidável, atrasa ou previne significativamente a oxidação desse substrato (Halliwell, 1990). Esta definição cobre todos os substratos oxidáveis, i.e., lípidos, proteínas e DNA. Por seu lado, em biologia, todos os compostos que consigam retardar ou prevenir os efeitos da oxidação têm sido considerados antioxidantes, e.g., compostos que inibem enzimas específicas da oxidação ou que reagem com oxidados antes de provocarem danos em moléculas biológicas (Scott, 1997). Os antioxidantes biológicos assumiram correntemente uma definição mais ampla ao incluir sistemas de reparação como as proteínas que transportam o ferro (e.g., transferrina, albumina, ferritina, caeruplasmina), enzimas antioxidantes, factores que afectam a homeostase vascular, transdução do sinal e expressão génica (Frankel e Meyer, 2000).

As actividades antioxidantes relativas de extractos de frutos baseadas na inibição da oxidação das LDL do homem diminuíram na seguinte ordem: amora > groselha > cereja > mirtilo > morango (Heinonen *et al.*, 1998). No mesmo sentido, Velioglu *et al.* (1998) encontraram uma correlação positiva entre a concentração de fenóis totais e a actividade antioxidante de uma grande variedade de frutos e vegetais. Vinson *et al.* (1995) e Cao *et al.* (1997) referem que muitos flavonóides são antioxidantes mais potentes do que as vitaminas C e E. As antocianinas revelaram-se como inibidoras da oxidação das LDL (Tamura e Yamagami, 1994; Teissedre *et al.*, 1996; Wang e Cao, 1997; Satué-Gracia *et al.*, 1997). Particularmente, a cianidina-3-glucósido tem uma actividade antioxidante significativa (Tsuda *et al.*, 1994), contra os radicais hidroxilo e superóxido *in vitro* (Tsuda *et al.*, 1996). As antocianinas presentes no vinho tinto (Satué-Gracia *et al.*, 1997; Ghiselli *et al.*, 1998) ou no sumo de uva (Frankel *et al.*, 1998) evitam a oxidação das LDL *in vitro* e diminuem o aparecimento da aterosclerose (Steinberg *et al.*, 1989). A actividade antioxidante da catequina no plasma humano foi demonstrada através do retardamento da degradação endógena do α-tocoferol e do β-caroteno, bem como da inibição da oxidação dos lípidos plasmáticos (Yilmaz e Toledo, 2004). Os ácidos fenólicos são também excelentes antioxidantes (Radtke *et al.*, 1998). Os compostos fenólicos purificados tiveram actividades antioxidantes que diminuíram na seguinte ordem: catequina > miricetina = epicatequina = rutina > ácido gálico > quercetina (Rice-Evans *et al.*, 1996; Teissedre *et al.*, 1996; Cao *et al.*, 1997; Huang *et al.*, 1997; Satué-Gracia *et al.*, 1997; Soleas *et al.*, 1997; Wang e Cao, 1997; Meyer *et al.*, 1998). No entanto, é de salientar que a

actividade antioxidante relativa de muitos compostos fenólicos varia fortemente com o método utilizado. Por exemplo, não há concordância da actividade antioxidante de vários fenólicos determinada pelo método da inibição da oxidação das LDL do homem induzida pelo cobre *in vitro* comparado com a determinação pelos métodos da actividade antioxidante total (TAS), da actividade antioxidante em equivalentes trolox (TEAC) e da capacidade de absorção de radicais de oxigénio (ORAC) (Quadro 1.9).

Quadro 1.9
Actividade antioxidante relativa de diversos compostos fenólicos por diferentes métodos (Frankel e Meyer, 2000).

	Inibição da oxidação das LDL (%)	TEAC (mM Trolox)	TAS (Randox) (mM)	ORAC (µM Trolox)
Compostos fenólicos (5 µM GAE)	Teissedre *et al.* (1996)	Rice-Evans *et al.* (1996)	Soleas *et al.* (1997)	Cao *et al.* (1997)
Catequina	74,9	2,40	3,50	2,49
Miricetina	68,1	3,12	4,04	----
Epicatequina	67,6	2,50	4,96	2,36
Rutina	67,6	2,42	----	0,56
Ácido gálico	63,3	3,01	3,00	1,74
Quercetina	61,4	4,72	4,24	3,29
Ácido elágico	36,6	----	----	----
Ácido sináptico	35,1	----	----	----
α-Tocoferol	32,6	0,97	0,90	----
Antocianinas	Satué-Gracia *et al.* (1997)			Wang e Cao (1997)
Cianidina	79,4	4,42	----	2,2
Delfinidina	71,8	4,44	----	1,8
Malvidina	59,3	2,06	----	2,0
Pelargonidina	39,0	1,30	----	1,1
Ácidos hidroxicinâmicos	Meyer *et al.* (1998)			Cao *et al.* (1997)
Cafeico	96,7	1,26	3,64	2,23
Clorogénico	90,7	1,20	----	----
Ferúlico	24,3	1,90	1,84	1,33
p-cumárico	24,5	2,22	1,56	1,09
Catequinas do chá	Lipossomas (Huang e Frankel, 1997)			
Galato de epigalocatequina	82,0	4,75	----	
Epicatequina	80,2	2,50	----	
Galato de epicatequina	59,6	4,93	----	
Epigalocatequina	22,2	3,82	----	

1.3.2.7. Absorção, Metabolismo e Excreção dos Compostos Fenólicos

Conclusões sobre a absorção, metabolismo e excreção dos compostos fenólicos no homem são contraditórias e escassas (Hollman *et al.*, 1996, Hollman e Katan, 1997; Hollman *et al.*, 1997ab; Manach *et al.*, 1997; Hollman e Katan, 1999). Segundo Yilmaz e Toledo (2004), o local onde os compostos fenólicos desempenham inicialmente o seu principal papel como antioxidantes é no tracto intestinal limitando a formação de ROS e capturando as ROS formadas. No entanto, pensava-se que a absorção de flavonóides era relativamente baixa porque, com a excepção das catequinas, os flavonóides ocorrem como glicosídeos e apenas as agliconas seriam capazes de passar através da parede intestinal, uma vez que não existiam aqui enzimas capazes de quebrar as ligações glicosídicas (Hollman e Katan, 1997). Contudo, investigação posterior concluiu que os flavonóides são absorvidos com uma eficiência relativamente alta (Hollman *et al.*, 1997ab; Gee *et al.*, 1998; Yilmaz e Toledo, 2004). No entanto, o composto glicosilado apresenta menor reactividade na neutralização de radicais livres do que a aglicona correspondente, bem como uma maior hidrossolubilidade (Martínez-Flórez *et al.*, 2002). A ingestão de flavonóides está associada à redução do risco de cancro (Knekt *et al.*, 1997), diminuição de doenças coronárias (Hertog *et al.*, 1993; Knekt *et al.*, 1996; Keli *et al.*, 1997) e até da SIDA, uma vez que a provável redução do stresse oxidativo tem um papel preponderante na redução da infecção pelo vírus da imunodeficiência adquirida (Zhang *et al.*, 1997).

Embora os hábitos alimentares sejam muito diversos no mundo, o valor médio de ingestão de flavonóides estima-se em 23 mg dia^{-1} (Hertog e Hollman, 1996), com predominância de flavonóis, especialmente quercetina. Este valor excede assim a de outros antioxidantes na dieta, tais como o β-caroteno (2 a 3 mg dia^{-1}) e da vitamina E (7 a 10 mg dia^{-1}) e é aproximadamente um terço da vitamina C (70 a 100 mg dia^{-1}) (Rice-Evans e Packer, 1998). Segundo Martínez-Flórez *et al.* (2002), a quercetina inibe a peroxidação lipídica produzida pelo ferro e inibe a produção de óxido nítrico no fígado, alterando vias de expressão de proteínas celulares. Alguns flavonóides podem reduzir a oxidação dos ácidos gordos poli-insaturados presentes nos microssomas do fígado de ratos (Yilmaz e Toledo, 2004). A catequina e a hesperidina apresentaram efeitos quimioprotectores no cancro do cólon induzido por aminas heterocíclicas em ratos (Yilmaz e Toledo, 2004). Cao e Prior (1999) estudaram a absorção de outro grupo de flavonóides, especificamente de cianidinas em extracto de sabugueiro. Trinta minutos após consumir 25 g do extracto, o nível antocianinas no plasma de homens de 35 anos era de 100 μg l^{-1}. Uma vez que as cianidinas são as antocianinas predominantes da cereja (Gao e Mazza, 1995), o estudo de Cao e Prior (1999) sugere que as antocianinas da cereja sejam

facilmente absorvidas. E ainda, como se pode constatar no Quadro 1.6, as cerejas são fontes importantes de ácidos fenólicos e Bourne e Rice-Evans (1999) constataram que os ácidos hidroxicinâmicos e os flavonóides de uma grande diversidade de frutos, incluindo a cereja, são facilmente absorvidos.

Cao *et al.* (1998) mediram a actividade antioxidante do soro e urina utilizando diferentes métodos, em oito mulheres adultas após a ingestão de 240 g de morangos, 294 g de espinafres, 300 ml de vinho tinto, ou 1250 g de vitamina C. Estes autores observaram que a actividade antioxidante da urina aumentou 8, 10, 28 e 45% após o consumo de vinho, espinafres, morangos e vitamina C, respectivamente. Em contraste, a actividade antioxidante do soro aumentou mais após o consumo de espinafres em comparação com os outros tratamentos. Outro estudo *in vivo* conduzido por Renaud e Lorgeril (1992) demonstrou que o consumo moderado de vinho tinto (5–10 g dia^{-1}) está correlacionado com a baixa incidência de doenças coronárias em França, apesar de uma dieta rica em gorduras, a que se designou por "Paradoxo Francês". A presença de antocianinas e outros fenólicos que actuam como antioxidantes, reduzindo a oxidação das LDL (Renaud e Lorgeril, 1992; Frankel *et al.*, 1993; Frankel *et al.*, 1995; Abu-Amsha *et al.*, 1996), pode ser a explicação para a inibição da aterosclerose (Yilmaz e Toledo, 2004).

Sintetizando, de acordo com vários estudos epidemiológicos efectuados, verificou-se uma relação inversa entre uma dieta constituída por antioxidantes e a incidência de cancro, trombose, aterosclerose podendo também ter um papel preponderante na redução de doenças neurodegenerativas, como a doença de Alzheimer e a doença de Parkinson (Sorg, 2004). Para uma revisão mais aprofundada sobre a biodisponibilidade dos compostos fenólicos recomenda-se a leitura do trabalho de Scalbert e Williamson (2000).

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CAPÍTULO 2

EFFECT OF PRUNING AND PLANT SPACING ON THE GROWTH OF CHERRY ROOTSTOCKS AND THEIR INFLUENCE ON STEM WATER POTENTIAL OF SWEET CHERRY TREES

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2. EFFECT OF PRUNING AND PLANT SPACING ON THE GROWTH OF CHERRY ROOTSTOCKS AND THEIR INFLUENCE ON STEM WATER POTENTIAL OF SWEET CHERRY TREES

2.1. Abstract

The aims of this work are to describe the effects of pruning and planting density on growth and water relations of ungrafted and grafted sweet cherry trees. A trial with cherry rootstocks: *Prunus avium*, CAB 11E, Maxma 14, Gisela 5 and Edabriz was begun in 1997. Pruning severities were applied to the rootstocks (0, 30, 60 and 90% of the vegetative growth was removed corresponding to P1, P2, P3 and P4 treatments, respectively) after planting to two plant spacings (S1 = 0.25 x 1.0 m and S2 = 0.45 x 1.5 m). Canopy, root growth and leaf water potential (Ψ_{leaf}) were quantified throughout the growing season. Pruning significantly affected root length and root weight of the rootstocks. Uncut plants (P1) showed a heavier and expanded root biomass (231 g and 108 m) than the intensively pruned plants (P4) (187 g and 75 m). The greater root biomass was obtained with the spacing/pruning combination, S1/P1 (285 g), and the smaller with S1/P4 (180 g) and S2/P4 (176 g). Ψ_{leaf} varied significantly between the rootstocks and plant spacing but not with pruning. Maxma 14 and *Prunus avium* attained the lowest values of midday Ψ_{leaf}, –2.28 and –2.04 MPa, but the highest values of predawn Ψ_{leaf}, –0.29 and –0.25 MPa, respectively. Generally, with high density (S1), the rootstocks exhibited lower predawn and midday Ψ_{leaf}. In 1998, cultivars Burlat, Summit and Van were grafted onto rootstocks and a trial was installed in 1999. Predawn and midday stem water potential (Ψ_{stem}) on cherry trees, measured in 2002, were affected significantly by the rootstock/genotype combination. Cultivars grafted on *Prunus avium* and Maxma 14 showed the less negative midday Ψ_{stem}, –1.36 and –1.42 MPa, respectively, so these rootstock genotypes perhaps induced a higher drought resistance to the scion. Recorded data show that the scion-rootstock interaction with regard to production performance under water deficits may be an important consideration in cherry tree planting strategies.

Key words: *Prunus avium* L., scion-rootstock interaction, stem water potential, vegetative growth.

2.2. Introduction

Tree size plays a central role in orchard management and production of quality fruit. Some authors have reported that scion vigour is controlled by various means: pruning, nutrition and/or rootstocks (Faust, 1989; Webster, 2001). Chalmers *et al.* (1983) stated that plant spacing and competition for water can interact synergistically to reduce vegetative growth.

The growth of woody perennial species is affected by rootstocks, scions and their resulting interactions (Tubbs, 1976, 1977, 1980; Iacono *et al*., 1998). Growth and physiological characteristics were evaluated in autografted and reciprocally grafted plants of *Prunus avium* x *Prunus pseudocerasus* cv. Colt and *P. cerasus* cv. Meteor. Rootstock influenced growth, morphology (leaf area:root surface area) and specific leaf area, and physiological (net assimilation rate) characteristics of grafted plants (Ranney *et al*., 1991a). Düring (1994) and Iacono *et al.* (1998), concluded that rootstock genotype induced drought resistance in the scion in grafted grapevines.

In a study by Schmitt *et al.* (1989), leaf water potential (Ψ_{leaf}) was determined on cherry trees and was most negative in *Prunus cerasus* seedlings, followed by Sam on F 12.1, Sam on *Prunus cerasus* clones and Sam on *Prunus acida*. Measurements of stomatal conductance (g_s), Ψ_{leaf} and Ψ_{stem} on apple, grapevine and nectarine trees under several irrigation treatments from early morning to mid-afternoon showed that Ψ_{stem} was more closely correlated with g_s than Ψ_{leaf} was correlated with g_s (Naor, 1998). Centritto *et al*. (1999) observed that g_s of cherry seedlings was highly correlated with soil water status. In a combined rootstock-irrigation trial on cherry Bing, Ψ_{stem} was correlated with g_s and rates of shoot growth, with shoot growth essentially stopping once Ψ_{stem} fell to between –1.5 and –1.7 MPa (Shackel *et al*., 1997).

Information on the effects of rootstock on growth, drought resistance and water relations of cherry trees are incomplete, so the aims of this study were to determine the influence of pruning and plant spacing on Ψ_{leaf}, canopy and root growth in cherry rootstocks throughout the growing season and to determine the effect of rootstock genotype on Ψ_{stem} of four year old cherry cultivars, after grafting.

2.3. Materials and Methods

2.3.1. Experimental Trials

The trials were set up at Vila Real, in the northeast of Portugal, at 470 m a.s.l., 41º 19' N and 7º 44' W. According to the Thornthwaite classification, the regional climate is humid, mesotermic, with high deficit of water in the summer, and with moderate thermic

efficiency in summer ($C_2B'_2s_2b'_4$) (Thornthwaite, 1948). The average annual rainfall is about 1100 mm, mainly from October to April. Warmest months are July/August and coldest are December/January, with average daily temperatures of 21–22ºC and 6–7°C, respectively. Mean annual sunshine values over a thirty-year period are 2392 h, the lowest monthly values (100 h) occurring in December and the highest (342 h) in July (Figure 2.1).

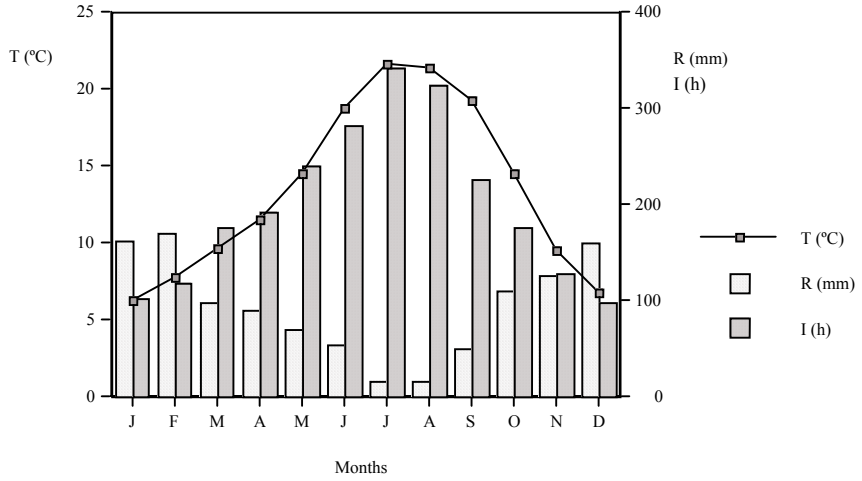

Figure 2.1
Average monthly temperature (T), rainfall (R) and sunshine hours (I) for Vila Real (period 1961–90).

Trial 1. In February an experimental plot of five cherry rootstocks with different vigour: *Prunus avium*, CAB 11E, Maxma 14, Gisela 5 and Edabriz was planted. Different pruning severities were applied to 2000 stools (400 plants per rootstock): 0, 30, 60 and 90% of the vegetative growth was removed, corresponding to P1, P2, P3 and P4 treatments, respectively, after planting to two plant spacings: S1 = 0.25 x 1.0 m and S2 = 0.45 x 1.5 m. The soil is a dystrochrept silt loamy, with pH 5.4 and an organic matter content of 1.45%; P_2O_5 and K_2O contents are 63 and 348 mg kg^{-1}, respectively.

Trial 2. Sweet cherry (*Prunus avium*) Burlat, Summit and Van were grafted by chip-budding in September onto those five rootstocks. The scions were transplanted to the orchard in February 1999, in a randomized complete block design in a trial where the trees have 5 m between rows and in-row spacings vary according to the relative vigour of the rootstock; with a minimum of 3.0 m and a maximum of 5.5 m for Edabriz and *Prunus avium*, respectively. In this phase of the orchard life, these spacings do not affect tree behaviour. The soil is a deep (> 100 cm) sandy loam dystric arid antherosol, pH 4.7 with an organic matter content of 1.5%, high content of fine sand (0.2–0.02 mm), high content of K_2O (150–200 mg kg^{-1}) and medium on P_2O_5 (50–100 mg kg^{-1}).

2.3.2. Canopy and Root Growth

From each combination (5 rootstocks x 4 pruning severities x 2 spacings), three plants were recorded to evaluate canopy and root growth. The trees were carefully removed from the soil using water and an iron bar, were enclosed in a plastic bag and immediately analysed. Leaf area was estimated using a portable area meter (CI-201 Portable Leaf Area Meter–CID, USA) and fine root (root diameter $<$ 2 mm) extension was measured with a Comair Root Length Scanner (Commonwealth Aircraft Corporation Limited, Australia). When this value was higher than 50 m, to obtain a more rigorous determination according to the instruction manual, the following equation was used: $A = -0.2246 + 0.9655E + 0.00123E^2$, where E was the root length scan. All the shoots and roots were measured and weighed.

2.3.3. Leaf and Stem Water Potential

Predawn and midday leaf water potentials of the cherry rootstocks were measured on 25 July and 19 August 1997, using a pressure chamber (ELE International, UK), according to the method described by Scholander *et al.* (1965). The Ψ_{leaf} was measured on three fully expanded healthy leaves on sun-exposed shoots per combination. To avoid evaporative loss, leaves were enclosed in a plastic bag just prior to cutting the petiole.

Predawn and midday stem water potentials of grafted cherry trees were determined on 21 June 2002. Several field studies (Garnier and Berger, 1985; McCutchan and Shackel, 1992; Naor and Wample, 1994; Naor *et al.*, 1995) have showed that midday Ψ_{stem} was more closely correlated with soil water availability than midday Ψ_{leaf}. So, in our study, Ψ_{stem} was measured on shaded leaves taken from inside the canopy; leaves were placed in a plastic bag covered with aluminium foil for at least 90 min before measurements were taken, to allow Ψ_{leaf} to equilibrate with Ψ_{stem}. Each measurement period included four measurements of Ψ_{stem} by scion-rootstock interaction.

2.3.4. Statistics

The data were analysed using analysis of variance by Super ANOVA (1.11 Abacus Concepts Inc, 1991) program. Mean separations were made using Fisher's Protected LSD Test ($P < 0.05$), designed to allow all possible linear combinations of group means to be tested.

The Discriminant Cannonical Analysis (DCA) was obtained using the STATISTICA program (STATSOFT, 1995). DCA is used to determine which variables discriminate between two or more naturally occurring groups (Hair *et al.*, 1995). The method used was Stepwise Discriminant Function Analysis, which "builds" a model of discrimination step-by-step,

reviewed all variables and evaluate which one will contribute most to the discrimination between groups (SPSS, 1997). This method use the *Wilks's lambda* statistic for the overall discrimination that is computed as the ratio of the determinant (det) of the within-groups variance/covariance matrix over the determinant of the total variance covariance matrix.

$$\textit{Wilks's lambda} = \det(W)/\det(T)$$

The *F* value for a variable indicates its statistical significance in the discrimination between groups, that is, it is a measure of the extent to which a variable makes a unique contribution to the prediction of group membership.

2.4. Results and Discussion

2.4.1. Canopy and Root Growth

All the vegetative parameters measured differed significantly ($P < 0.001$) between the five rootstocks (Table 2.1). CAB 11E displayed the longest and heaviest root system compared with the other rootstocks, essentially composed of fine roots (root diameter < 2 mm). The root system of Edabriz was mainly composed of roots thinner than 2 mm, but was nevertheless well fixed. Edin (1993) and Kappel (1993) also verified that under adverse climatic conditions, such as wind or heavy rain, such roots were brittle and that they cannot tolerate drought, because these roots include apical regions where cellular growth is rapid and, for this reason, are sensitive.

Pruning significantly affected total root length and fine root length ($P < 0.01$) and root weight ($P < 0.05$) of cherry rootstocks. Uncut plants (P1) showed a higher root growth (108 m) than the intensively cut plants (P4) (75 m), where there was a drastic reduction both in length and weight, i.e. the more the rootstocks were cut at planting the less their root system developed (Figure 2.2). Ranney *et al.* (1989), observed that the pruning (dormant shoots pruned to 20 cm in length) of Colt trees had no effect on the leaf area:root area ratio. Asamoah and Atkinson (1985) also verified that root pruning reduced root, leaf and stem weight in Colt cherry rootstocks. The growth of the rootstocks is very important, because the volume of soil explored by the roots defines the amount of water available from a given soil volume. Available soil moisture, i.e., the water that can be extracted by roots, held at a water potentials ranging between –0.1 and –1.5 MPa, which approximate field water-holding capacity and the permanent wilting percentage, respectively (Kramer, 1983). However, it should be stressed that the rate of root growth of the majority of plant species declines as the soil moisture increases above the soil water-holding capacity, due to a reduction in soil aeration (hypoxia). In this rhizosphere condition, the respiratory quotient

(RQ = mol CO_2/mol O_2) becomes greater than 1, root growth stops and root tips entering the low-oxygen zones die off (Larcher, 1995).

The greatest growth of the root system was obtained with the spacing/pruning combination S1/P1 (113 m) – and the less favourable ones were obtained with S1/P4 (76 m) and S2/P4 (74 m).

The Discriminant Canonical Analysis (DCA) done with all vegetative characteristics measured on rootstocks showed that canopy fresh weight (CFW) had a large discriminating effect, demonstrated by the higher *F* value (*F* = 110.05), contrasting with the other lower *F* values corresponding to the other vegetative variables (Table 2.2). Figure 2.3 shows that the two *Prunus cerasus* clones, CAB 11E and Edabriz, had more vegetative affinity and Maxma 14, an interspecific hybrid between *Prunus mahaleb* x *Prunus avium* was closer to *Prunus avium*. Gisela 5, an interspecific hybrid between *Prunus cerasus* x *Prunus canescens*, seemed to have more affinity with Edabriz than CAB 11E. So, phylogenetic affinity leads to a more vegetative proximity between the species.

Table 2.1
Vegetative parameters measured on cherry rootstocks in the growing season. Values are the mean ± *SD* (*n* = 24). Means flanked by the same letter are not significantly different at *P* < 0.05 (Fisher's test).

Vegetative characteristics	*Prunus avium*	CAB 11E	Maxma 14	Gisela 5	Edabriz
Fine roots[a] (m)	69.87 ±66.28 a	204.74 ±80.89 b	33.33 ±15.56 a	52.68 ±21.16 a	42.24 ±18.99 a
Root length (m)	73.00 ±68.25 a	214.01 ±82.70 b	34.21 ±16.27 a	54.51 ±21.39 a	44.59 ±19.66 a
Root weight (g)	160.45 ±140.25 b	583.76 ±181.66 c	41.93 ±31.41 a	113.69 ±37.08 ab	81.90 ±42.40 a
Stem length (cm)	123.04 ±50.40 b	140.17 ±24.67 bc	122.83 ±30.86 ab	155.96 ±22.66 c	102.62 ±31.60 a
Stem diameter (mm)	13.13 ±5.12 b	19.20 ±3.25 c	8.04 ±2.95 a	13.52 ±3.46 b	11.73 ±3.49 b
Branch length (cm)	91.10 ±65.44 a	654.04 ±300.85 c	139.25 ±103.52 ab	317.75 ±174.93 b	200.75 ±111.68 ab
Canopy fresh weight (g)	184.06 ±181.71 a	490.30 ±187.87 b	92.70 ±78.35 a	209.02 ±89.62 a	125.38 ±78.27 a
No. Leaves	55.79 ±29.53 a	228.58 ±79.25 c	71.67 ±41.36 ab	109.33 ±37.40 b	122.54 ±76.53 b
Leaf area (m^2)	0.45 ±0.24 a	2.15 ±0.75 b	0.33 ±0.19 a	0.48 ±0.16 a	0.35 ±0.22 a

[a]Fine roots (Ø < 2 mm diameter).

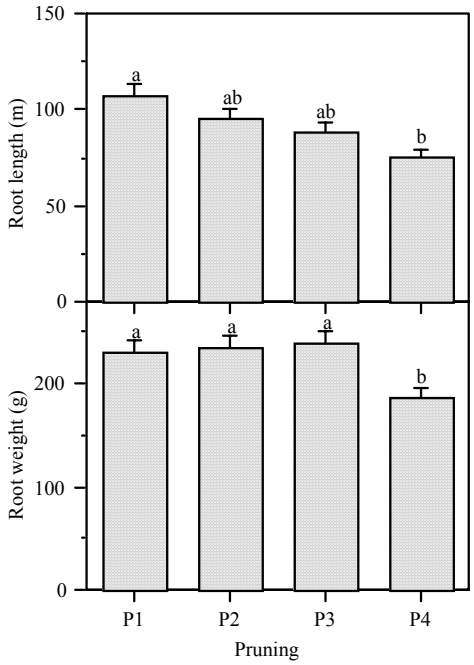

Figure 2.2
Root system length and weight of cherry rootstocks, affected by pruning severities (P1 to P4). The columns are the means (n = 24) and vertical bars represent standard errors. Columns with the same letter are not significantly different at $P < 0.05$ (Fisher's test).

2.4.2. Leaf and Stem Water Potential

Generally, Ψ_{leaf} of the rootstocks was close to zero during the first hours of the morning, but became gradually more negative through the day and until early afternoon, recovering thereafter during the night until sunrise, when it reached similar values as before (data not show). Ψ_{leaf} varied significantly ($P < 0.001$) between the rootstocks and plant spacing but not with pruning treatments. Maxma 14 attained the lowest midday Ψ_{leaf} (–2.28 MPa) due to a higher canopy:root weight ratio (2.21), but it also had the higher value of predawn Ψ_{leaf}, meaning that this rootstock had a more favourable water status recovery during the night. A different behaviour was observed for CAB 11E (–1.82 MPa) (Figure 2.4), may be due to its lowest canopy:root weight ratio (0.84). The less negative midday Ψ_{leaf} values observed on CAB 11E and Edabriz (–1.82 MPa and –1.93 MPa), although they had different vigour, being semi-vigorous and dwarfing respectively, could be related to some phylogenetic affinity (Figure 2.3).

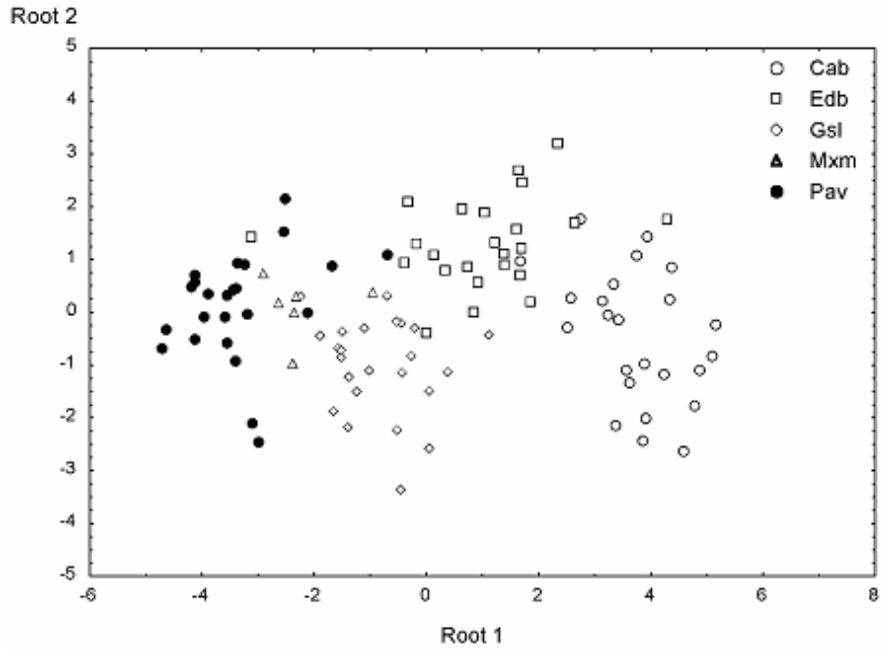

Figure 2.3
Discriminant Cannonical Analysis (DCA) of vegetative characteristics of cherry rootstocks (Cab – CAB 11E, Edb – edabriz, Gsl – gisela 5, Mxm – maxma 14, Pav – *Prunus avium*), by forward stepwise analysis.

Table 2.2
Discriminant Cannonical Analysis (DCA)[a] between vegetative characteristics of cherry rootstocks, by forward stepwise analysis.

Vegetative characteristics	Step	*Wilks' Lambda*	df 1	df 2	*F*[b]	df 1	df 2	*P* - level
Canopy fresh weight	1	0.181	4	97	110.05	4	97	0
Branch length	2	0.114	4	96	14.07	8	192	4.507E-09
Stem length	3	0.096	4	95	4.52	12	251.64	0.0022
Stem diameter	4	0.081	4	94	4.23	16	287.81	0.0034
Root weight	5	0.072	4	93	2.97	20	309.40	0.0236
No. leaves	6	0.066	4	92	2.15	24	322.16	0.0803
Root length	7	0.063	4	91	1.04	28	329.53	0.3889

[a]DCA done by STATISTICA program (STATSOFT, 1995).
[b]The *F* value for a variable indicates its statistical significance in the discrimination between groups.

In general, at 0.25 x 1.0 m the rootstocks exhibited lower predawn and midday Ψ_{leaf} (Figure 2.5). The likely reason for this is that the available soil water is reduced, causing lowered Ψ_{leaf}. Edabriz followed a different trend in the predawn period.

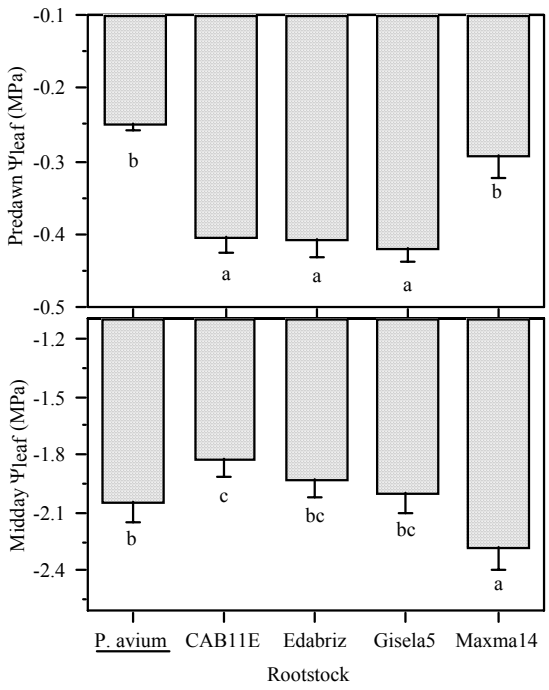

Figure 2.4
Leaf water potential of ungrafted cherry rootstocks at predawn and midday. The columns are the means (n = 3) and vertical bars represent standard errors. Columns with the same letter are not significantly different at $P < 0.05$ (Fisher's test).

Rootstock genotype significantly affected ($P < 0.01$) the predawn and midday stem water potential of the cherry cultivars. Cultivars grafted on *Prunus avium* and Maxma 14 showed the higher midday Ψ_{stem}, –1.36 and –1.42 MPa, respectively (Figure 2.6), so these rootstock genotypes maybe induced drought resistance in the scion. Düring (1994) and Iacono *et al*. (1998) reported similar observations in grafted grapevines. Cherry cultivars had a better performance (higher Ψ_{stem}) in these two rootstocks probably due to a deeper root system than the more dwarfing, Edabriz and Gisela 5. Shackel *et al.* (1997) observed no rootstock effect on midday Ψ_{stem} under fully irrigated conditions, but when irrigation was reduced, trees on Colt rootstock exhibited a more rapid decline in water status than those on *Prunus mahaleb*. Under these water stress conditions, sorbitol was the soluble carbohydrate present at the highest concentration in Colt and Meteor (Ranney *et al*., 1991b). Under the same conditions, abscisic acid (ABA) in the xylem sap can increase substantially as a function of reduced soil water availability (Loveys, 1984; Zhang and Davies, 1989). So, several authors and more recently Wilkinson and Davies (2002), concluded there is now strong evidence that the plant hormone ABA is important in the regulation of stomatal behaviour and gas exchange of droughted plants. Probably, *Prunus avium* and Maxma 14 have hormonal regulation mechanisms, namely stronger ABA signals that determine the observed results.

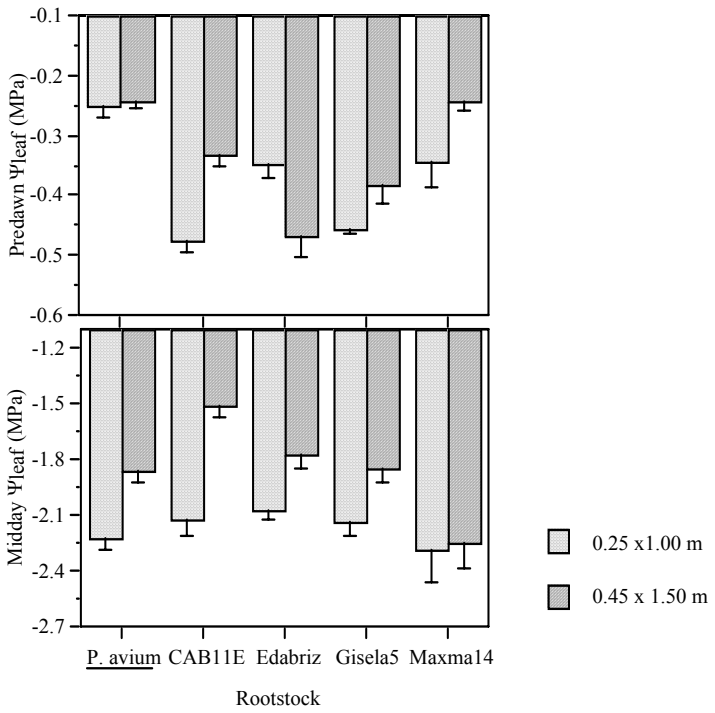

Figure 2.5
Leaf water potential of ungrafted cherry rootstocks at predawn and midday affected by plant spacing (S1 = 0.25 x 1.00 m and S2 = 0.45 x 1.50 m). The columns are the means (n = 3) and vertical bars represent standard errors.

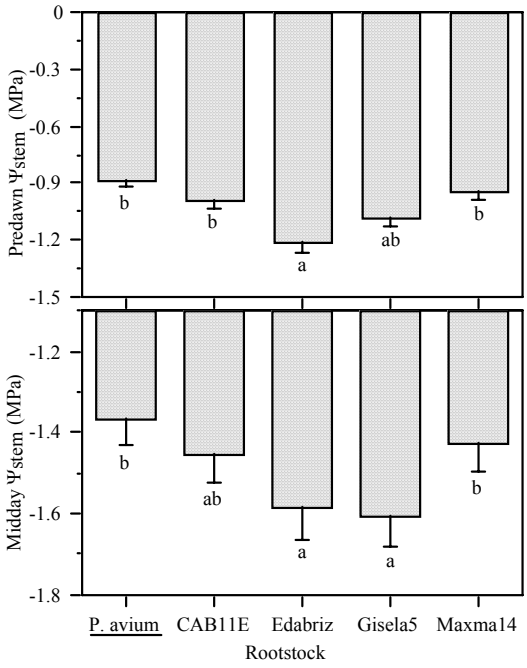

Figure 2.6
Stem water potential of grafted cherry rootstocks at predawn and midday. The columns are the means (n = 4) and vertical bars represent standard errors. Columns with the same letter are not significantly different at $P < 0.05$ (Fisher's test).

Another aspect that could affect Ψ_{leaf} and Ψ_{stem} is the incompatibility observed in the scion-rootstock combination, although we did not detect these symptoms. Schmid *et al.* (1988), observed that leaves of grafting combinations of *Prunus avium* cv. Sam on *Prunus cerasus* with symptoms of delayed incompatibility showed less negative Ψ_{leaf} during the daytime, lower transpirations rates, closure of stomata at times of high photosynthetically active radiation and higher content of carbohydrates, catechins and proanthocyanidins, in spite of their chlorophyll content being lower than that of the leaves of combinations without the symptoms.

2.5. Conclusions

In some cases, sweet cherry cultivars, grafting to different rootstock genotype increased Ψ_{stem}. These results are of interest to horticulture especially in dry areas as they offer the opportunity to increase water-use efficiency and drought resistance by selecting appropriate rootstock varieties.

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CAPÍTULO 3

VARIATION IN XYLEM STRUCTURE AND FUNCTION IN ROOTS AND STEMS OF SCION-ROOTSTOCK COMBINATIONS OF SWEET CHERRY TREE (*Prunus avium* L.)

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3. VARIATION IN XYLEM STRUCTURE AND FUNCTION IN ROOTS AND STEMS OF SCION-ROOTSTOCK COMBINATIONS OF SWEET CHERRY TREE (*Prunus avium* L.)

3.1. Abstract

To assess hydraulic architecture and limitations to water transport across rootstock-scion combinations [*Prunus avium* L. cultivar Van grafted on five differing size-controlling rootstocks: *Prunus avium* (vigorous) > CAB 11E > Maxma 14 > Gisela 5 > Edabriz (dwarfing)], we compared xylem anatomy, and calculated relative hydraulic conductivity (RC) and vulnerability index (VI) of roots (small, medium and large diameter) and stems. Water relations, leaf gas exchange and variations in growth were also determined. Roots exhibited larger-diameter xylem conduits (VD), greater RC and VI than stems in all Van-rootstock combinations. Moreover, there was a significantly higher vessel frequency (VF), lower VD, RC and VI in dwarfed trees, especially grafted on Gisela 5 than trees on the invigorating rootstocks, *Prunus avium* L., CAB 11E and Maxma 14. Anatomical constraints on water status imposed by the smaller VD (and/or in lower xylem thickness and root system length) of dwarfed trees imply a series of negative feedbacks, like a decrease in RC, stem water potential, leaf gas exchange and growth. On the other hand, Van grafted on CAB 11E and Maxma 14, with wide vessels and high VI, could be more susceptible to embolism, especially during periods of severe water stress, than trees on dwarfing rootstocks, which had small vessels and low VI.

Key words: plant water transport, relative hydraulic conductivity, rootstock effects, vulnerability to cavitation, whole-tree hydraulic architecture, xylem anatomy.

3.2. Introduction

Variations in xylem anatomy and hydraulic properties occur at several levels: interspecific, intraspecific and intraplant (Zimmermann, 1983; Ewers, 1985; Tyree and Ewers 1991; Sperry and Saliendra, 1994; Jackson *et al.*, 2000). Moreover, variations in xylem conduit diameter can radically affect the conducting system according to the fourth-power relationship between radius and flow through a capillary tube, as described by the Hagen-Poiseuille law (Zimmermann, 1983; Tyree and Ewers, 1991). Thus, even a small increase in mean conduit diameter has exponential effects on specific hydraulic conductivity (K_s). Consistent with this theory, numerous studies have shown that K_s is higher in shallow roots

than in stems (Alder *et al.*, 1996; Kavanaugh *et al.*, 1999; Martínez-Vilalta *et al.*, 2002; McElrone *et al.*, 2004). Other studies reported a decline in whole-plant hydraulic conductance with height (Mencuccini and Grace, 1996; Ryan *et al.*, 2000; McDowell *et al.*, 2002). According to Lovisolo and Schubert (1998), K_s can change as a result of (i) modifications of the size of the xylem vessels or (ii) interruption of the water column in the vessels by embolism.

Clonal rootstocks of cherry tree are widely used to control the vegetative vigour of the trees and improve fruit quality. The mechanisms for these commercially useful rootstock effects are complex and poorly understood, but a common hypothesis is that rootstocks that reduce scion vigour have low hydraulic conductance (Syvertsen and Graham, 1985; Atkinson and Else, 2001). According to Baas *et al.* (1984), in various dwarf trees there is a strong correlation between tree height and vessel element size. However, this correlation must be treated with caution since water movement and balance are not only regulated by anatomical characteristics. They may be also influenced by water potential gradients within the plant, as well as stomatal features and their sensitivity, especially when water availability is limited in the soil (Reyes-Santamaría *et al.*, 2002).

A restriction of water flow is entirely consistent with the anatomical changes associated with graft tissues and the different degrees of shoot dwarfism showed in grafted plants (Simons, 1986; Soumelidou *et al.*, 1994; Sekse, 1998). Several authors have reported morphological alterations, namely small vessels and swirling of vascular tissue (Soumelidou *et al.*, 1994), and presence of necrotic areas and large amounts of non-conducting phloem (Simons and Chu, 1984) in the graft union of apple trees grafted onto dwarfing rootstocks. These anatomical changes may be due to limitations in polar auxin (IAA) transport across the graft and its accumulation at the graft (Simons, 1986; Soumelidou *et al.*, 1994). IAA is a key leaf-derived regulator of xylem cell differentiation and division within the cambial zone and an initiator of vascular redifferentiation across the graft union (Hess and Sachs, 1972; Parkinson and Yeoman, 1982; Aloni, 1987; Savidge, 1988). A reduced flow of IAA to roots could provide an explanation to the lower xylem/phloem ratio presented by the trees grafted on dwarfing rootstocks, compared with trees on invigorating rootstocks (Simons, 1986; Kurian and Iyer, 1992).

Xylem water transport in plants is the subject of intensive research because of its agronomic and ecological implications (Lovisolo and Schubert, 1998). On the one hand, the aim of investigations focused on crop water management is to improve water-use efficiency (Jones, 1990) whilst on the other hand, factors affecting water transport parameters are important determinants of drought tolerance and relative habitat preferences of native and

cultivated species (Sperry and Tyree, 1990; Cochard *et al*., 1994; Pockman *et al*., 1995), and predictors of carbon and water balance in environmental models (Williams *et al*., 1996).

Development of the concept of hydraulic architecture by Zimmermann (1978, 1983) has led to numerous studies of the structure and properties of the water transport system of trees that govern the balance between efficiency of water supply and total transpiring leaf area (e.g., Ewers and Zimmermann, 1984; Tyree *et al*., 1991). In particular, water uptake and transport through the xylem are essential for replacing water lost during transpiration, preventing desiccation, and allowing continued photosynthesis (Kramer and Boyer, 1995). In a previous study, we demonstrated that water relations and leaf gas exchange of sweet cherry tree were mainly influenced by the rootstock genotype (Gonçalves *et al*., 2006). However, we know of no previous data related to xylem structure and function of different diameter roots, rootstock stems and scion stems in sweet cherry, which can help us to explain that behaviour. Therefore, in this study, the purpose was to test the hypothesis that reduced hydraulic conductance can provide an explanation for reductions in plant vigour caused by rootstocks. Specifically, the experiment was designed to determine secondary xylem anatomical variation, relative hydraulic conductivity (RC) and vulnerability index (VI), water potential, leaf gas exchange and growth in cultivar Van grafted on five differing size-controlling rootstocks: *Prunus avium*, CAB 11E, Maxma 14, Gisela 5 and Edabriz, growing in natural field conditions.

3.3. Materials and Methods

3.3.1. Plant Material and Growth Conditions

The study was carried out on adult plants of *Prunus avium* in an experimental plot near Vila Real, northeast Portugal (41º 19' N and 7º 44' W; altitude 470 m above sea level), between 1997 and 2002, as described in Gonçalves *et al.* (2003, 2006). Briefly, sweet cherry trees used in the field experiment were a mid to late maturing cultivar Van grafted on five different rootstocks: *Prunus avium* (vigorous), CAB 11E (clone of *Prunus cerasus* L.; semi-vigorous) Maxma 14 (*Prunus avium* x *Prunus mahaleb* L. hybrid; semi-dwarfing), Gisela 5 (*Prunus cerasus* x *Prunus canescens* Bois hybrid; dwarfing) and Edabriz (clone of *Prunus cerasus*; very dwarfing). Two representative trees of each combination were randomly selected to make all the measurements.

In 1997, rootstocks were planted in the nursery. After grafting in 1999, trees were transplanted in a north-south orientation with rows 5.5 m apart. Within rows, trees were spaced according to the vigour of the rootstock: 5.5, 5.0, 4.5, 4.0 and 3.0 for *Prunus avium*, CAB 11E, Maxma 14, Gisela 5 and Edabriz, respectively.

Routine disease and pest control treatments were provided according to a commercial protocol for fruit production. The cherry orchard was fertilized and daily drip-irrigated (3 h, between May and September; drippers were in line, 1 m apart, and 4 l h^{-1} flow rate). The trees were not pruned during the experiment.

3.3.2. Xylem Anatomical Analyses and Electron Micrographs

From each of two replicate plants used, per rootstock, 15 stems and 15 fine (Ø < 2 mm), medium (Ø, 2–5 mm) and large (Ø > 5 mm) roots were removed in 1997 for microscopic investigation of xylem anatomy. In 2001 and 2002, 15 stems were collected from each of two plants per scion-rootstock combination for light microscopic investigation of xylem anatomy. One year old stem transverse sections, approximately 50 µm thick, were dissected at the same distance from the apex (10 cm) with a hand microtome, roots were also cut, stained in a combination of alum carmine and iodine green (Deysson, 1965) and then mounted with synthetic resin. This double staining brought out the lignified elements in green and the cellulose in pink. Measurements of xylem vessel frequency and xylem vessel diameter were made on each cross section. Vessel frequency (vessels mm^{-2}) represents the mean of 30 randomly fields of view per treatment and each vessel diameter (µm) was determined from the mean of two orthogonal measurements across the widest part of the vessel lumen. Randomly selected vessels within the vascular pathway (i.e., area of maximum water transport) were chosen for measurement (n = 30). Efficiency and susceptibility to damage during water conduction was evaluated through relative hydraulic conductivity (Zimmermann, 1983) and vulnerability index (Carlquist, 1977). The relative hydraulic conductivity was estimated using a modified Hagen-Poiseuille equation (Fahn *et al.*, 1986): $RC = r^4 VF$, where RC is the relative hydraulic conductivity, r the individual vessel radius and VF the vessel frequency. The vulnerability index (VI) was calculated as proposed by Carlquist (1977): VI = VD/VF, where VD is the vessel diameter and VF the vessel frequency.

For scanning electron microscopy (SEM) observations, transverse sections of roots and stems were performed, as described above for xylem analysis. Micrographs of the different organs were obtained by SEM Philips/FEI Quanta 400 (Brno, Czech Republic).

3.3.3. Water Relations and Leaf Gas Exchange

Morning (Ψ_{PD}) and midday (Ψ_{MD}) stem water potentials of sweet cherry trees were determined with a pressure chamber (ELE International, Bedfordshire, U.K.) according to McCutchen and Shackel (1992). Stem water potential was measured on fully-expanded healthy leaves on June 21 and July 8, 2002. Previously, they were placed in a black

polyethylene bag wrapped in aluminium foil for at least 90 min before measurements, to allow leaf water potential to equilibrate with stem water potential. In all cases, leaves were placed in the chamber within a few seconds after excision. Eight measurements of Ψ were done by Van-rootstock combination, in each diurnal period and date.

Leaf gas exchange parameters were measured with a portable gas exchange system (ADC-LCA-3, Analytical Development, Hoddesdon, U.K.) and a leaf chamber clip (ADC-PLC, surface: 6.25 cm^2, volume: 16 cm^3) with quantum, temperature and humidity sensors. The gas exchange unit was operated in the open mode at 300 ml min^{-1} flow rate and at ambient CO_2 partial pressure of 35–37 Pa. Eight measurements were done on 'sun' fully-expanded healthy leaves in the morning (09.00–11.00 h) and afternoon (14.00–16.00 h). Net CO_2 assimilation rate (A) and stomatal conductance (g_s) were estimated from gas exchange measurements using the equations of von Caemmerer and Farquhar (1981). Measurements were conducted in cloudless days of summer, where the photosynthetic photon flux density ranged between 1850 and 1950 µmol m^{-2} s^{-1}, air temperature ranged between 25 and 30 ºC and air vapour pressure deficits were around 2.6 and 3.1 kPa in the morning and afternoon, respectively.

3.3.4. Plant Growth Parameters

Plant height and trunk diameters below (in the rootstock) and above (in the cultivar) the grafting points were measured in all plants between 1998 and 2002, in each dormancy period.

3.3.5. Statistics

One-way analysis of variance (ANOVA) was used to examine organ or rootstock effect on xylem hydraulic properties and rootstock effect on xylem hydraulic properties, water relations, leaf gas exchange and growth of cherry trees, and least significant difference tests were used to compare individual treatment means ($P < 0.05$). All data sets satisfied the assumptions of ANOVA based on homogeneity of variances, normality of errors, and independence of errors. Associations among characters were examined by a Fisher correlation analysis. The relationships between A and g_s, plant height and rootstock stem diameter, and plant height and scion stem diameter were analysed by simple linear regression. The slopes and the equations of the linear regression of each rootstock were compared by t-test and Fisher's test, respectively.

3.4. Results

3.4.1. Xylem Hydraulic Properties

Vessel diameter (VD), vessel frequency (VF), and the calculated values of relative hydraulic conductivity (RC) and vulnerability index (VI) were significantly ($P < 0.001$) related to organ (Table 3.1). The values of VD were significantly smaller in the scion stems, intermediate in rootstock stems, and larger in roots, mainly in those with medium diameter. Medium size roots had mean VD that was 2.7 times larger than vessels from terminal shoots. In relation to vessel frequency, the lowest VF was observed in large size roots and the highest in fine roots (+120%).

In roots, a significant negative correlation was found between VD and VF, in all rootstocks, and the values of the coefficient of correlation (r) varied between –0.35 and –0.72 ($P < 0.001$; data not shown). However, there was no correlation between these two parameters in stems.

Table 3.1
Means of xylem vessel diameter (VD), vessel frequency (VF), relative hydraulic conductivity (RC) and vulnerability index (VI) of the different organs. Means ($n = 150$) followed by the same letter are not significantly different at $P < 0.05$ (Duncan's test).

Organ	VD (μm)	VF (vessels mm^{-2})	RC (μm^4 10^6)	VI
Fine roots	23.9 c	576.4 d	15.44 c	55.9 b
Medium roots	34.2 e	348.8 b	35.08 e	147.8 d
Large roots	32.9 d	261.6 a	22.5 d	155.8 e
Rootstock stem	24.3 c	349.5 b	8.76 b	75.9 c
Cultivar stem (2001)	14.0 b	533.3 c	1.53 a	26.8 a
Cultivar stem (2002)	12.8 a	524.7 c	1.05 a	25.4 a
P - value	0.0001	0.0001	0.0001	0.0001

Medium size roots had higher RC (2620%) than terminal shoots of Van, on average of the years 2001-02 (Table 3.1). As sampling depth increased, the greater proportion of large conduits in each distribution and the concurrent increased mean conduit diameter (Figure 3.1) resulted in greater RC (Table 3.1). The VI of the cherry vessels ranged from 25 to 156, where large roots had comparatively high values.

Statistical analysis showed significant differences ($P < 0.05$) in VD, VF, RC and VI among rootstocks (Tables 3.2–3.4). VD values were significant largest in invigorating plants, especially in CAB 11E, which it had a mean vessel diameter of 25 μm, 45% larger than vessels of trees on Gisela 5 (Tables 3.2–3.4, Figure 3.2). In general, the dwarfing Edabriz and Gisela 5 induced significantly higher VF than trees on invigorating rootstocks. On the other hand, the rootstock Gisela 5 induced the lowest RC and CAB 11E the highest. Notably,

Table 3.2
Xylem vessel diameter (VD), vessel frequency (VF), relative hydraulic conductivity (RC) and vulnerability index (VI) of the fine (Ø < 2 mm), medium (Ø, 2-5 mm) and large (Ø > 5 mm) roots of the five rootstocks. Means (n = 30) followed by the same letter are not significantly different at $P < 0.05$ (Duncan's test).

Rootstock	VD (µm)			VF (vessels mm^{-2})			RC (μm^4 10^6)			VI		
	Ø < 2	2-5	Ø > 5	Ø < 2	2-5	Ø > 5	Ø < 2	2-5	Ø > 5	Ø < 2	2-5	Ø > 5
Prunus avium	29.9 c	34.1 b	29.1 a	292.5 a	198.2 a	347.3 bc	21.7 b	20.7 a	21.3 ab	102.1 e	172.0 c	83.7 a
CAB 11E	32.1 c	46.2 d	40.0 b	394.4 b	192.5 a	173.6 a	31.8 c	62.9 c	30.7 b	81.4 d	239.8 d	230.5 c
Maxma 14	20.8 b	38.2 c	36.8 b	400.1 b	164.2 a	130.2 a	6.4 a	26.2 b	17.6 a	52.0 c	232.6 d	282.2 d
Gisela 5	16.1 a	19.9 a	30.8 a	1098.4 d	690.7 c	292.5 b	5.6 a	8.9 a	19.8 a	14.6 a	28.8 a	105.3 b
Edabriz	20.5 b	32.9 b	28.1 a	696.4 c	498.2 b	364.2 c	11.7 a	56.7 b	23.1 ab	29.5 b	66.0 b	77.1 a
P - value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0482	0.0001	0.0001	0.0001

stems of Van on the dwarfing Edabriz had higher RC than those observed on invigorating Maxma 14 and *Prunus avium*. Among the five rootstocks studied (data not shown), Van on the dwarfing Gisela 5 and Edabriz had the lowest VI (< 40), intermediate values in *Prunus avium* (66) and highest in Maxma 14 and CAB 11E (> 95). In addition, dwarfed trees had significantly (P < 0.001) lower xylem/phloem thickness ratio than invigorating trees (Figure 3.3), due to a low xylem thickness (data not shown).

Table 3.3
Xylem vessel diameter (VD), vessel frequency (VF), relative hydraulic conductivity (RC) and vulnerability index (VI) of the stems of the five rootstocks in 1997. Means (n = 30) followed by the same letter are not significantly different at P < 0.05 (Duncan's test).

Rootstock	VD (μm)	VF (vessels mm^{-2})	RC (μm^4 10^6)	VI
Prunus avium	25.1 b	369.9 b	10.3 bc	67.8 b
CAB 11E	29.6 c	218.9 a	11.4 c	135.4 d
Maxma 14	24.3 b	326.5 b	7.8 b	74.3 c
Gisela 5	18.6 a	371.8 b	3.1 a	50.1 a
Edabriz	23.9 b	460.5 c	11.2 c	51.9 a
P - value	0.0001	0.0001	0.0001	0.0001

Table 3.4
Xylem vessel diameter (VD), vessel frequency (VF), relative hydraulic conductivity (RC) and vulnerability index (VI) of the stems of Van grafted onto five rootstocks in 2001–2002. Means (n = 30) followed by the same letter are not significantly different at P < 0.05 (Duncan's test).

Rootstock	VD (μm)		VF (vessels mm^{-2})		RC (μm^4 10^6)		VI	
	2001	2002	2001	2002	2001	2002	2001	2002
Prunus avium	14.8 b	13.0 ab	564.3 b	423.7 a	2.2 b	0.83 a	26.3 b	31.2 d
CAB 11E	13.4 a	12.3 a	605.8 b	562.4 c	1.4 a	0.97 a	23.1 a	22.4 ab
Maxma 14	14.3 ab	13.2 b	457.7 a	486.0 b	1.4 a	1.05 a	31.5 d	28.1 c
Gisela 5	13.7 a	12.2 a	488.8 a	502.9 b	1.3 a	0.82 a	28.3 c	24.4 b
Edabriz	13.7 a	13.4 b	550.1 b	648.3 d	1.4 a	1.58 b	24.8 ab	20.8 a
P - value	0.0073	0.0127	0.0001	0.0001	0.0004	0.0001	0.0001	0.0001

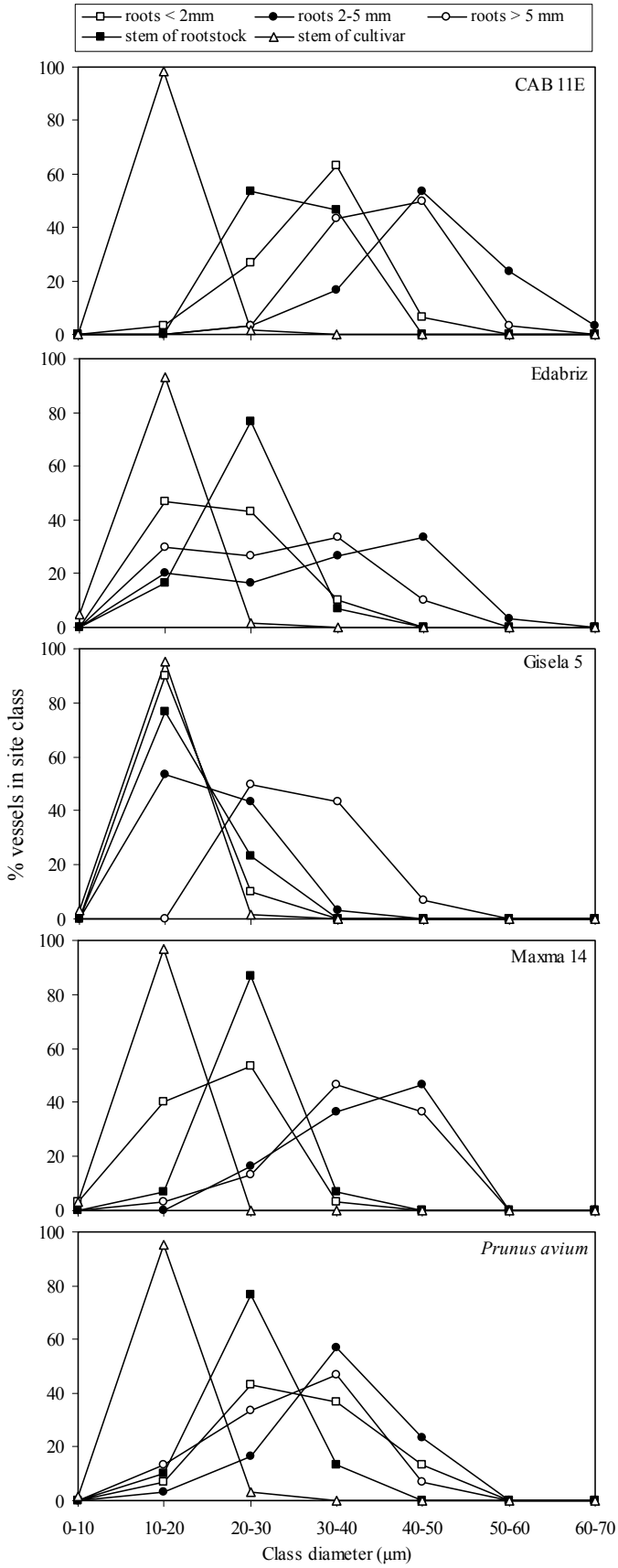

Figure 3.1
Percentage of vessels per class diameter in organs of Van grafted onto five rootstocks.

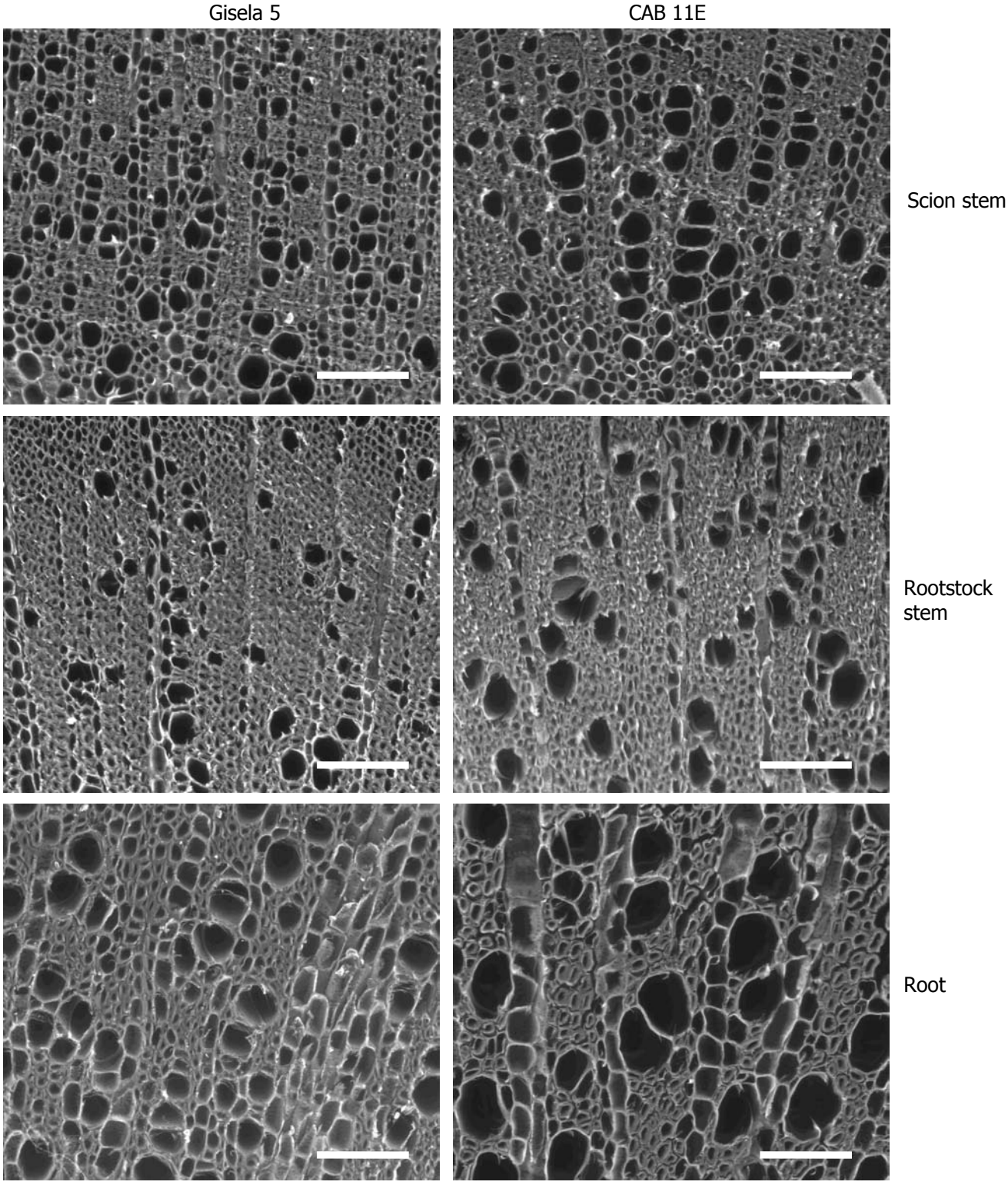

Figure 3.2
Scanning electron micrographs of roots and stems of Gisela 5 (dwarfing rootstock) and CAB 11E (invigorating rootstock). Scale bar = 50 µm.

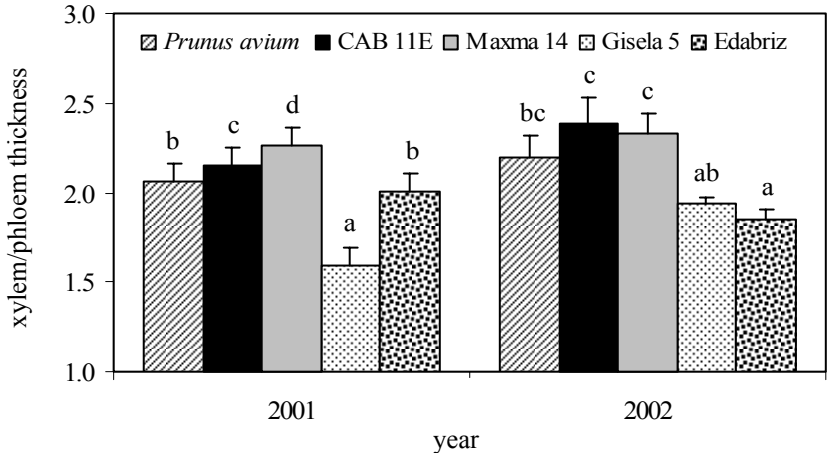

Figure 3.3
Xylem/phloem thickness ratio of stems of Van grafted onto five rootstocks in years 2001–2002. Means (n = 10) followed by the same letter are not significantly different at $P < 0.05$ (Duncan's test).

3.4.2. Water Relations and Leaf Gas Exchange

There were significant ($P < 0.05$) differences in midday stem water potential (Ψ_{MD}) among the rootstocks in the two studied dates. In fact, trees on dwarfing rootstocks, Edabriz and Gisela 5, had lower Ψ_{MD}, especially when grafted on Gisela 5 (Figure 3.4). However, no significant ($P > 0.05$) differences were observed in morning stem water potential (Ψ_M) among rootstocks.

Regarding leaf gas exchange, as expected, A correlated positively with g_s (Figure 3.5). However, the coefficients of determination (r^2, indicating the proportion of the variability in A that is explained by g_s) and the slopes of the linear regression were higher in dwarfing rootstocks than in invigorating ones. A comparing of all the slopes and the equations of the linear regression showed significant differences between the dwarfing and the invigorating rootstocks ($P < 0.001$), which suggest a strong regulation of photosynthesis by stomatal aperture on cherry trees grafted on Edabriz and Gisela 5.

3.4.3. Plant Growth Parameters

Trees on dwarfing rootstocks had significantly ($P < 0.001$) lower plant height and lower trunk diameters above and below the grafting point than trees on invigorating rootstocks (data not shown). In fact, for the same increase in plant height, the diameter of the trunk (above the grafting point) increased more in trees on invigorating rootstocks than in dwarfing rootstocks, as expressed by the slopes of the linear regression (Figure 3.6). The coefficients of correlations between the trunk diameters below and above the grafting point

were always high (r > 0.96; P < 0.001); as a result we found a similar relationship between plant height and the trunk diameter below the grafting point, so only the first relationship is presented.

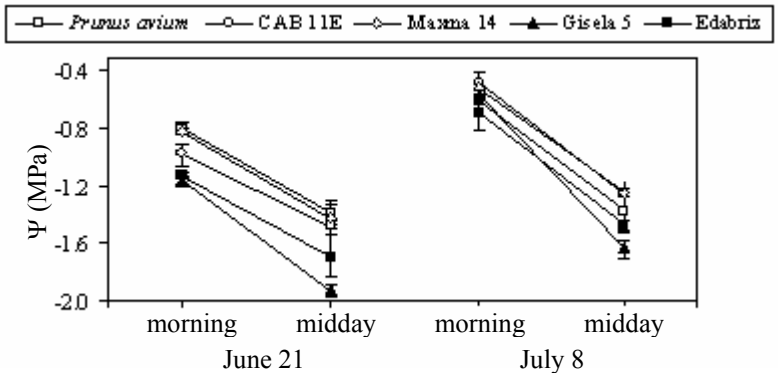

Figure 3.4
Diurnal changes in stem water potential (MPa), measured at cherry ripeness period (two dates), for leaves of Van grafted onto five rootstocks. Within each diurnal period (morning: 09.00–10.00 h; midday: 14.00–15.00 h) mean ± S.E. (n = 8).

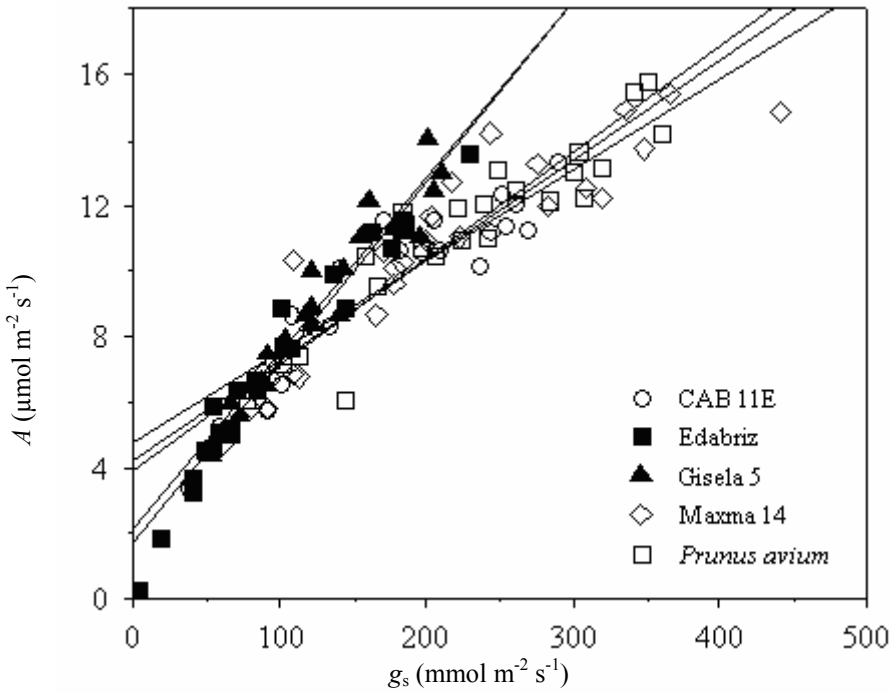

Figure 3.5
Relationship between photosynthesis (A) and stomatal conductance (g_s) in Edabriz (A = 1.627 + 0.055g_s, r^2 = 0.954, P < 0.001), Gisela 5 (A = 2.111 + 0.054g_s, r^2 = 0.928, P < 0.001), Maxma 14 (A = 4.763 + 0.028g_s, r^2 = 0.818, P < 0.001), CAB 11E (A = 3.854 + 0.032g_s, r^2 = 0.867, P < 0.001) and *Prunus avium* (A = 4.239 + 0.030g_s, r^2 = 0.867, P < 0.001). Measurements were taken throughout the diurnal period on June 21 and July 8, 2002.

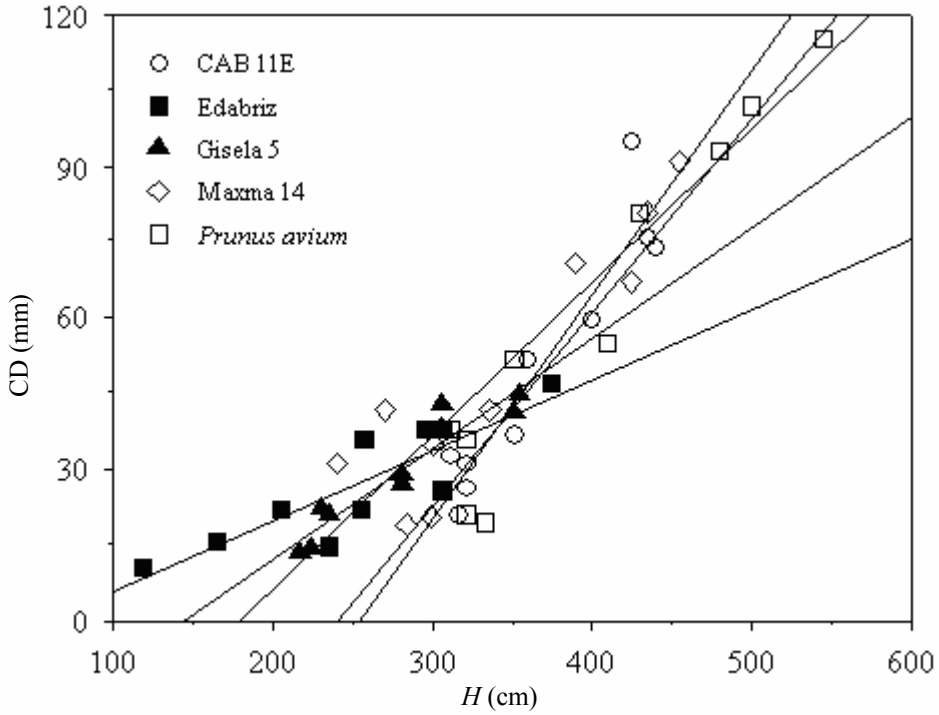

Figure 3.6
Relationship between plant height (*H*) and trunk diameter above the grafting point (CD) in Edabriz (CD = –8.158 + 0.140*H*, r^2 = 0.756, *P* < 0.001), Gisela 5 (CD = –31.476 + 0.219*H*, r^2 = 0.904, *P* < 0.001), Maxma 14 (CD = –54.645 + 0.305*H*, r^2 = 0.834, *P* < 0.001), CAB 11E (CD = –112.434 + 0.443*H*, r^2 = 0.884, *P* < 0.001) and *Prunus avium* (CD = –92.351 + 0.384*H*, r^2 = 0.928, *P* < 0.001). Measurements were taken between 1998 and 2002, in each dormancy period.

3.5. Discussion

3.5.1. Xylem Structure and Resistance to Water Transport among Roots and Stems

Plant anatomists have known for some time that xylem conduits (vessels and tracheids) within a plant tend to decrease in diameter in acropetal direction, from roots to the terminal branches (Tyree and Zimmermann, 2002). In accordance, our study showed that vessel lumen diameter decreased sharply from the roots to the terminal branches (Table 3.1) and in all Van-rootstock combinations (Tables 3.2–3.4). Differences in conduit diameter for root and stem xylem have been reported for a wide range of species. For trees in the *Proteaceae*, Pate *et al.* (1995) reported increases in VD between shallow roots and so-called sinker roots and along sinker roots with increasing depth. In the conifer *Juniperus ashei* Buchh., tracheids in shallow roots and deep roots were about three and four times wider, respectively, than tracheids in stems. Moreover, for the three dicotyledonous trees investigated, vessels in roots were an average of 1.5 (shallow) and 2.3 (deep) times wider

than vessels in stems (McElrone *et al*., 2004). The large conduits of the roots, observed mainly in medium size roots (Tables 3.1 and 3.3), are thought to be necessary to minimize the hydraulic resistance associated with the great path length from deep roots to the canopy (McElrone *et al*., 2004). Other reasons why xylem conduits are wider in deep roots than elsewhere within trees involve constraints that are related to the soil environment (North, 2004). As discussed by McElrone *et al*. (2004), deep roots are supported by the soil and unlike shallow roots are relatively unaffected by mechanical forces acting on the shoot. The reduced need for the xylem to provide structural support allows deep roots to be specialised for transport, with fewer xylem fibres, fewer rays (Pate *et al*., 1995), more vessels or tracheids per transverse area, and conduits with larger lumens than in shallow roots and stems. Such specialization results not only in more efficient water uptake but also in reduced carbon allocation per unit length of root (North, 2004).

Extensive research on species from a wide range of habitats generally supports the theory that xylem structure and function is optimised to balance the conflicting demands of xylem safety *versus* efficiency (Tyree *et al*., 1994; Pockman and Sperry, 2000; Hacke and Sperry, 2001). Despite the RC and VI being mathematically deduced, our results showed a gain in hydraulic safety associated with a loss in hydraulic conductivity (Table 3.1). These results are consistent with the findings of McElrone *et al*. (2004) in stems, shallow roots and deep roots of four woody species. In a recent study, Pittermann *et al.* (2005) refer that the vulnerability to cavitation is dependent on the pit membrane, specifically on size of pores in the membrane, not on conduite diameters, *per si*. However, there is often a correlation, perhaps because the size of the vessel increases the area of the membrane, thereby increasing the probability that there will be higher pore sizes.

3.5.2. Xylem Structure and Resistance to Water Transport among Rootstocks

Across rootstocks, VD was significantly larger in invigorating plants, i.e., Van grafted on *Prunus avium*, CAB 11E and Maxma 14, than vessels of trees on dwarfing Edabriz and Gisela 5 (Tables 3.2–3.4), which may be due to genetic differences, as it has been suggested that dwarfing rootstocks inherently produce smaller vessels (Beakbane and Thompson, 1947). However, VD is not the only factor to influence xylem water transport. The dwarfed trees (grafted on Gisela 5 and Edabriz) had significantly lower xylem/phloem thickness ratio than trees on invigorating rootstocks (Figure 3.3), mainly due to a low xylem thickness and not to a high phloem thickness (data not shown). Moreover, dwarfed trees had smaller root systems than invigorating ones, as demonstrated in a previous study (Gonçalves *et al*., 2003). These observations are consistent with the view that clonally produced dwarfing

rootstocks possess innate factors, such as lower xylem/phloem ratios and changes in xylem vessel anatomy (Simons, 1986; Kurian and Iyer, 1992), which might explain how they influence shoot behaviour when used in grafted plants. According to Atkinson *et al.* (2003), the development of vascular abnormalities (e.g., the production of excess callus cells in the graft union) may reduce xylem water transport and contribute to the dwarf stature. An alternative hypothesis for the apparent reduction in water transport through the graft union is that there is a reduction in transpiration of dwarfed trees due to smaller evaporative surface area or, perhaps, root-scion signalling molecules such as ABA (Olmstead *et al*., 2006).

We concluded that dwarfed plants, mainly grafted on Gisela 5, had adjustments in xylem structure and function that reduce hydraulic conductance and enhance hydraulic safety. Therefore, those trees had substantially lower VD and, consequently, lower RC. These findings are further supported by the observation that, as predicted by Poisseuille's law, although relative differences in VD between dwarfed and invigorating trees were similar at all organs tested, relative differences in RC (dependent on the fourth power of the vessel radius) were larger. Other factors, however, may contribute to conductivity changes: vessel transectional areas and length (Zimmermann and Jeje, 1981), and so reduced vessel length may have co-operate with reduced vessel diameter in decreasing xylem conductivity in dwarfed plants.

In all rootstock genotype, VD and VF of the roots were inversely related (Table 3.2), similar to other plants (Carlquist, 1988; Reyes-Santamaría *et al*., 2002). In spite of the differences in xylem hydraulic properties in roots, these features were similar among the stems of Van-rootstock combinations suggesting an adjustment of these trees in adult phase.

It is common that genotypes with narrow and abundant vessels show low vulnerability values (Carlquist, 1977). This indicates that among combinations, Van grafted on Gisela 5 and Edabriz probably have a safer water flow system because of their low VI (Tables 3.2–3.4). This supposition is supported by Hargrave *et al*. (1994) observations, who found that wider vessels are more susceptible to show dysfunction compared to vessels with narrower diameter. In addition, this interpretation supports the idea that Van on CAB 11E and Maxma 14 with wide vessels could be more susceptible to have embolism especially during periods of severe water stress (Hargrave *et al*., 1994). Although vulnerability to cavitation in *Prunus avium* increased with depth, deeper roots may not operate any closer to critically low xylem water tensions because xylem water potential in deep roots is typically higher than in stems (McElrone *et al*., 2004).

In conclusion, xylem features of different rootstocks affected axial relative hydraulic conductance. However, rootstocks may also influence the radial conductance, according to several recent researchers (Atkinson *et al.*, 2003; Basile *et al.*, 2003a). Therefore, in future we intend to measure axial and radial conductance and correlate them with xylem properties and plant size.

3.5.3. Relationships between Xylem Structure and Water Relations, Leaf Gas Exchange and Growth

There is ample evidence that the structure of the plant hydraulic system – the hydraulic architecture – has the potential to limit water flow through plants, thus restricting their water balance, leaf gas exchange and growth (Tyree and Ewers, 1991). Therefore, studying the differences in the hydraulic architecture of sweet cherry trees may help to select the best scion-rootstock combinations with regard to water availability in soils and specifically, in this study, how scion differs in growth when grafted on five differing-size controlling rootstocks.

The pattern in Ψ of the different Van-rootstock combinations was the same in the two dates (Figure 3.4), and these findings were also observed in the same cultivar-rootstock combinations in year 2003 by Gonçalves *et al.* (2006). The higher the RC in invigorating plants, the less negative is the stem water potential (Tables 3.2–3.4, Figure 3.4). The same results were obtained by Lemoine *et al.* (2001) in three woody species.

Davis *et al.* (1999) and Schubert *et al.* (1999) reported that water movement from the roots to the atmosphere is controlled by the conductivity of the water pathway components. Conductivities affecting water flow in the plant are hydraulic (root conductivity and shoot conductivity), and diffusive (stomatal conductance). In accordance, our study showed that smaller conduits of trees on Gisela 5 had lower relative hydraulic conductivity (Tables 3.2–3.4), and led to lower stomatal conductance and photosynthetic rate (Figure 3.5). In fact, Franks (2004) referred that stomatal regulation of leaf gas exchange is directly influenced by the water status and hydraulic structure of the whole plant. Any change in transpiration rate will be translated almost immediately into a change in the water status of every leaf cell, including stomatal guard cells. Similarly, a change in the hydraulic properties will alter the water status of guard cells, and therefore stomatal conductance. The positive correlation between hydraulic and stomatal conductance has been also observed in several studies (Meinzer and Grantz, 1990; Meinzer *et al.*, 1995; Saliendra *et al.*, 1995; Comstock, 2000). Therefore, when xylem hydraulic conductance is reduced, stomatal conductance also decreases (Sperry *et al.*, 1993; Hubbard *et al.*, 2001; Cochard *et al.*, 2002), indicating that

hydraulic conductance to transpirational flux is an integral and dynamic component of the stomatal control mechanism (Franks, 2004). Therefore, the lower *A* in trees grafted on the dwarfing Edabriz and Gisela 5 was due to high stomatal limitations, represented by the low g_s in these two rootstocks (Figure 3.5). The same results were observed in fifteen scion-rootstock combinations of sweet cherry in field-grown conditions (Gonçalves *et al*., 2006).

The lower Ψ, g_s and *A* presented by the dwarfed trees led to lower vegetative growth (Figures 3.4–3.6). Basile *et al.* (2003b) reported a positive correlation between integrated diurnal patterns of stem water potential and daily stem extension growth of peach trees on rootstocks with different size control potentials. They documented the effect of decreased water potential on shoot growth in a manner similar to previous works that linked water stress effects with plant growth in annual species (Hsiao, 1973; Boyer, 1985).

In conclusion, it is possible to say that much of this research has addressed the hydraulic limitation hypothesis, which proposes that reduced growth in trees grafted on dwarfing rootstocks may be linked to reductions in vessel diameters (and/or in xylem thickness and root system length) and consequently lower hydraulic conductivity, which in turn lead to reductions in stem water potential, ultimately decreasing stomatal conductance and photosynthesis. Nevertheless, measurements of the root and stem hydraulic conductivity and vulnerability are necessary to corroborate the theoretical calculations based on xylem anatomy.

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CAPÍTULO 4

CANOPY ARCHITECTURE EFFECTS ON LEAF STRUCTURE AND FUNCTION OF SWEET CHERRY TREE (*Prunus avium* L.)

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4. CANOPY ARCHITECTURE EFFECTS ON LEAF STRUCTURE AND FUNCTION OF SWEET CHERRY TREE (*Prunus avium* L.)

4.1. Abstract

Canopy architecture effects on morpho-anatomy (leaf mass per unit area – LMA, leaf density – LD, leaf tissue thickness and stomatal density), chemical composition (photosynthetic pigments, total soluble sugars – SS, starch – St and total phenols – TP concentrations), gas exchange rates and stem water potential (Ψ_{stem}) were studied in leaves of *Prunus avium* L. cultivars Burlat, Summit (upright, high dense canopies) and Van (spreading, low dense canopy). Van presented the highest value of canopy light transmittance. Therefore, Van allowed an easier penetration of the visible radiation through the canopy, which improves photosynthetic rates, especially at harvest, compared to Summit and Burlat. However, no differences in Ψ_{stem} were found among cultivars. Van showed the highest LMA and variations in this parameter were mainly associated with alterations in leaf thickness than in leaf density. Leaves of Summit and Van were thicker than those of Burlat, mainly due to increased palisade and spongy parenchyma thickness. However, Burlat leaves had the highest palisade/spongy ratio. Total chlorophyll concentration per area (Chl_{area}) and the chlorophyll a/b ratio was always higher in Van leaves than in the other two cultivars. As expressed on dry mass (Chl_{mass}), no significant differences were found among cultivars. On the other hand, Van leaves had the lowest concentrations of soluble carbohydrates and starch. In dense canopies of Summit and Burlat, light reduction and lower growth habit caused a notable increase in the relative concentration of total phenols, since surplus carbon is probably diverted to phenylpropanoid synthesis.

Key words: canopy architecture, gas exchange, leaf anatomy, carbohydrates, phenolic compounds, photosynthetic pigments.

4.2. Introduction

Under natural conditions, leaf structure and function characteristics exhibit a large spatial variability at canopy scale (Frak *et al.*, 2002). Several microclimatic (e.g., vapour pressure deficit, light quality) and physiological (leaf transpiration and carbon gain) parameters change concurrently with light intensity within plant canopies (Combes *et al.*, 2000). The plant ability to acclimate to different light environments include alterations both

at leaf level, associated with morphological, anatomical and physiological characteristics and at the whole-plant level mainly related to shoot architecture and biomass allocation patterns (Givnish, 1988; Muraoka *et al*., 1997; Niinemets, 1997a; Valladares and Pearcy, 1998; Mendes *et al*., 2001). Leaves at low light usually present an increased assimilate investment in leaf size to improve light interception (Pearcy and Sims, 1994; Niinemets *et al*., 1998), whereas at high light leaves are comparatively thicker, with a high leaf dry mass per area, reflected in a more efficient quantum utilization and, thus, a high photosynthetic capacity per unit leaf area (Wayne and Bazzaz, 1993; Niinemets and Tenhunen, 1997; Génard *et al*., 2000; Frak *et al*., 2002). Adaptation to irradiance also involves changes in foliar chemistry (Mendes *et al*., 2001). In fact, the biogenesis of photosynthetic machinery involves the light-dependent conversion of non-green plastids to green, photosynthetically competent plastids (Thomson and Whatley, 1980) and the primary reactions of photosynthesis require chloroplasts with fully differentiated thylakoid membrane system (Vothknecht and Westhoff, 2001). Several studies (Björkman, 1981; Meletiou-Christou *et al*., 1994; Niinemets and Tenhunen, 1997; Frak *et al*., 2002) reported positive correlations between leaf nitrogen content and irradiance, since a significant fraction of foliar nitrogen is invested in the form of photosynthetic proteins. Consistently, greater leaf chlorophyll concentrations per dry biomass are reported for low light leaves as compared to high light leaves, resulting in enhanced foliar nitrogen investment in light harvesting at low irradiance (Naidu and DeLucia, 1998; Niinemets, 1999a). In addition, the content of foliar non-structural carbohydrates tends to relate positively with irradiance (Niinemets, 1995; Johnson *et al*., 1997; Niinemets, 1997b), reflecting increased daily photosynthetic production, due in part to the high stomatal conductance that enables greater carbon acquisition. On the other hand, when growth is restricted due to irradiance limitation, more carbon can be diverted to defensive structures and to the production of carbon-based secondary metabolites such as phenolics (Herms and Mattson, 1992).

Cherry has heterobaric leaves (Nikolopoulos *et al*., 2002), characterized by many mesophyll transparent regions separated by bundle sheaths extensions (BSE) compartments (Terashima, 1992). Vogelmann (1989) pointed out that BSEs could modify the light microenvironment of the mesophyll layers because these transparent regions may create heterogeneous light gradients within leaves. Nikolopoulos *et al.* (2002) found that variations in anatomical-morphological factors of heterobaric leaves contribute to differences in photosynthetic capacity. However, the leaf mass per unit area (LMA) and internal leaf anatomy often affect net gas exchange because of their effects on internal CO_2 conductance to the site of carboxylation (Niinemets, 1999b), internal shading, competition for CO_2 among

carboxylation sites, nitrogen concentration and its partitioning (Meadiavilla *et al.*, 2001). In particular, several authors have considered the effects of the internal cell surface available for CO_2 diffusion (Romero-Aranda *et al.*, 1997) and the internal air volume (Parkhurst, 1986; Evans and von Caemmerer, 1996; Roderick *et al.*, 1999a).

This study is part of our ongoing research about the relationships between morpho-anatomical, chemical and physiological traits of scion-rootstock interactions in sweet cherry tree. The first observations indicate that rootstock genotype affects essentially gas exchange, chlorophyll a fluorescence and water relations responses whilst cultivar genotype affects more the morphology and chemistry of leaves (Gonçalves *et al.*, 2006).

In the current study, we investigate the leaf anatomical and chemical characteristics, as well as leaf gas exchange and water relations of three *Prunus avium* cultivars with different canopy architecture which affect light microenvironment in natural field conditions in order to test the following hypotheses: (i) foliage acclimation to light conditions integrates adjustments in leaf anatomy, physiology and chemistry; (ii) phenolic compounds represent an alternative to growth sink for organic carbon at low light irradiance.

4.3. Materials and Methods

4.3.1. Plant Material and Growth Conditions

The study was carried out on adult plants of *Prunus avium* L. in an experimental plot near Vila Real, northeast Portugal (latitude 41º 19' N and longitude 7º 44' W; altitude 470 m above sea level), during 2003, as described in Gonçalves *et al.* (2006). Briefly, four-year old sweet cherry trees used in the field experiment were an early-maturing cultivar Burlat and two mid-to-late-maturing cultivars Summit and Van grafted on Edabriz (clone of *Prunus cerasus* L.; dwarfing rootstock). The three cultivars have different canopy architecture; Van is rather spreading, whereas Burlat and Summit are upright (Edin *et al.*, 1997; Santos *et al.*, 2006). Trees were trained under a freely growing system in a North-South orientation rows, and planted at 5.5 m between rows and spaced 3.0 m. Routine disease and pest control treatments were provided according to a commercial protocol for fruit production. The cherry orchard was fertilized and daily drip-irrigated (3 h during night, between May and September; drippers were in line, 1 m apart, and 4 l h^{-1} flow rate). The trees were not summer-pruned during the experiment.

4.3.2. Light Microclimate, Leaf Gas Exchange and Water Relations

Canopy light transmittance (LC_t) values were used as an indirect indicator of the canopy size, in accordance to Dufrêne and Bréda (1995). Midday LC_t was calculated as the

ratio of photosynthetic photon flux density (PPFD) measured horizontally below and above the canopy as described by Campbell (1986), using a Sunfleck Ceptometer (Model SF-80, Decagon Devices, Cambridge, U.K.). Eight averages of LC_t were determined per cultivar on two dates (11 June, at harvest; and 5 July, at postharvest, in 2003), as well as in all physiological parameters. Each average consisted in ten readings taken over the ground area shaded by canopy.

Leaf gas exchange parameters were measured with a portable gas exchange system (LCA-3, Analytical Development Corp., Hoddesdon, U.K.) and a leaf chamber clip (ADC-PLC, surface: 6.25 cm^2, volume: 16 cm^3) equipped with a quantum, temperature and humidity sensors. The gas exchange unit was operated in the open mode at 300 ml min^{-1} flow rate and at ambient CO_2 partial pressure of 35–37 Pa. Eight measurements were done on sunny and fully-expanded healthy leaves (as in all leaf determinations), at morning (09.00–11.00 h) and at afternoon (14.00–16.00 h). Net CO_2 assimilation rate (A), stomatal conductance (g_s), transpiration rate (E) and intercellular CO_2 concentration (C_i) were estimated from gas exchange measurements using the equations of von Caemmerer and Farquhar (1981). Values for liquid phase diffusive conductance to CO_2 (g_m) and intrinsic water-use efficiency (A/g_s) were calculated in accordance with Izuta *et al.* (1996) and Düring (1994), respectively. Air temperature, PPFD and water vapour pressure deficit (VPD) were recorded simultaneously during all measurements, in the two diurnal periods and in both dates (Table 4.1).

Table 4.1
Environmental parameters determined during each measurement period. Means (n = 8) followed by the same letter are not significantly different at $P < 0.05$ (Duncan's test).

		June 11	July 5
PPFD	Morning	1960 ± 46 a	1696 ± 57 a
(µmol m^{-2} s^{-1})	Afternoon	2039 ± 51 a	2000 ± 25 b
T_{air}	Morning	28.6 ± 0.6 a	29.0 ± 0.6 a
(ºC)	Afternoon	33.8 ± 0.3 b	34.1 ± 0.3 b
VPD	Morning	2.09 ± 0.05 a	2.37 ± 0.11 a
(kPa)	Afternoon	3.26 ± 0.13 b	3.16 ± 0.16 b

Predawn (Ψ_{PD}) and midday (Ψ_{MD}) stem water potentials of sweet cherry trees were determined with a pressure chamber (ELE International, Bedfordshire, U.K.) according to McCutchen and Shackel (1992). Stem water potential was measured in leaves previously placed in a black polyethylene bag wrapped in aluminium foil for at least 90 min before measurements, to allow leaf water potential to equilibrate with stem water potential. In all

cases, leaves were placed in the chamber within a few seconds after excision. Eight measurements of Ψ were done by cultivar, in each diurnal period.

4.3.3. Leaf Morpho-Anatomical Traits

Thirty leaves were gathered in July 5 from two trees of each cultivar. Sampling was done at morning, and only mature leaves were considered. Measurements of leaf morphology included leaf area (LA, cm^2) (LICOR 3100, Lincoln, NE, USA), fresh mass (FM) and dry mass (DM), by oven-dried at 70 ºC to constant mass. The leaf moisture index, water content (WC; %) = [1 – (DM / FM)] 100, was expressed as percentage of FM. Further, the leaf mass per unit area (LMA, g m^{-2}) and leaf tissue density (LD, g kg^{-1}) were calculated according to Dijkstra (1989).

Tissue samples for anatomical measurements were taken from twenty randomly chosen healthy, sun-exposed, fully expanded leaves from two trees of each cultivar. The lamina thickness (LTh) was found to be quite regular throughout the lamina width except its borders and the protruding veins areas. Therefore, the sampling points avoid both regions, where leaf transverse sections (8 µm) midway between the leaf edge and the mid-vein were cut, dehydrated in alcohol and stained in a combination of alum-carmine and iodine-green (Deysson, 1965) and then mounted with synthetic resin. Sections were placed on a slide to measure whole leaf blade and adaxial plus abaxial epidermis, palisade and spongy parenchyma. Tissues thickness was measured on cross-sections under light microscope equipped with a calibrated micrometric grid. Stomatal density (stomata per unit leaf area) was measured in artificial replicas of nail varnish in the central region around the midrib of abaxial epidermis, since sweet cherry leaves are hypostomatic. For each leaf impression, ten fields of view were selected for analysis.

For scanning electron microscopy (SEM), transverse sections or direct observations of the abaxial leaf surface were performed. Observations and micrographs of the abaxial epidermis and the lamina were studied by SEM Philips/FEI Quanta 400.

4.3.4. Photosynthetic Pigments and Metabolites Assays

Leaf discs (1.57 cm^2) were taken in the field experiment at morning July 5, frozen in liquid N_2 and stored at –80 ºC prior to analysis. Total chlorophyll (Chl) and carotenoid (Car) were quantified spectrophotometrically from leaf extracts with 80% acetone (v/v) using the methods of Sesták *et al.* (1971) and Lichtenthaler (1987), respectively.

Total soluble sugars (SS) were extracted by heating leaf discs in 80% ethanol and quantified according to Irigoyen *et al.* (1992). Starch (St) was extracted with 30% perchloric acid and quantified according to Osaki *et al.* (1991).

The concentration of total phenols (TP) in leaf extracts was determined on the same extract used for pigment analysis, according to the Folin-Ciocalteu's procedure (Singleton and Rossi, 1965).

4.3.5. Statistical Analysis

Data were analysed using analysis of variance and means were separated by Duncan's significant difference test, when ANOVA showed significant variable effect ($P < 0.05$). Canopy light transmittance expressed as percentage was arcsine square-rooted transformed. A Fisher correlation analysis including all morphological and anatomical parameters was also performed.

4.4. Results

4.4.1. Light Microclimate, Leaf Gas Exchange and Water Relations

The highest midday canopy light transmittance (LC_t) was recorded in spreading Van (~ 52%), whereas the lowest LC_t was observed in upright Summit (~ 13%), and the intermediate value in Burlat (~ 20%) (Figure 4.1). Therefore, cultivars Summit and Burlat were characterized by high dense canopies, and, in opposition, Van had a less dense canopy that allowed the penetration of more visible radiation that reaches the soil surface. Moreover, canopies of the three sweet cherry cultivars tended to close during the growing season leading to a higher leaf area density, which was revealed by the decrease in LC_t values from June to July.

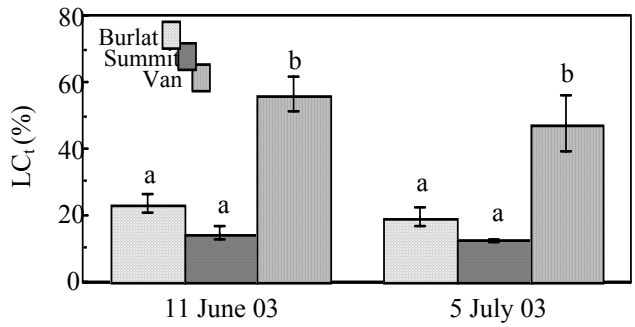

Figure 4.1
Canopy light transmittance, LC_t, at midday, of the three cherry cultivars. Means ± S.E ($n = 8$) followed by the same letter are not significantly different at $P < 0.05$ (Duncan's test).

No differences were observed between the two sampling dates (i.e., at harvest and postharvest periods) both in morning and afternoon measurements of gas exchange parameters (A, g_s, C_i, g_m and E) and A/g_s of the three cultivars (Figure 4.2). However, the low dense canopy of Van presented higher photosynthetic rates, especially at harvest, compared to the other two cultivars that had high dense canopies. As expected, values of A, E, g_s, C_i and g_m determined in the morning were consistently higher than in the afternoon period, except in E value of Van, which presented an increase from morning to afternoon (4.8 to 6 mmol m^{-2} s^{-1}), in July 5. Higher decreases of A were measured in Burlat, with a maximum reduction of 53% in June 11, where this cultivar achieved the minimum value, 6 µmol m^{-2} s^{-1}. The values of A/g_s showed an increase from the morning to the afternoon, mainly in Summit cherry trees, which ranged from 35 to 55 µmol mol^{-1}, more 57% in June 11.

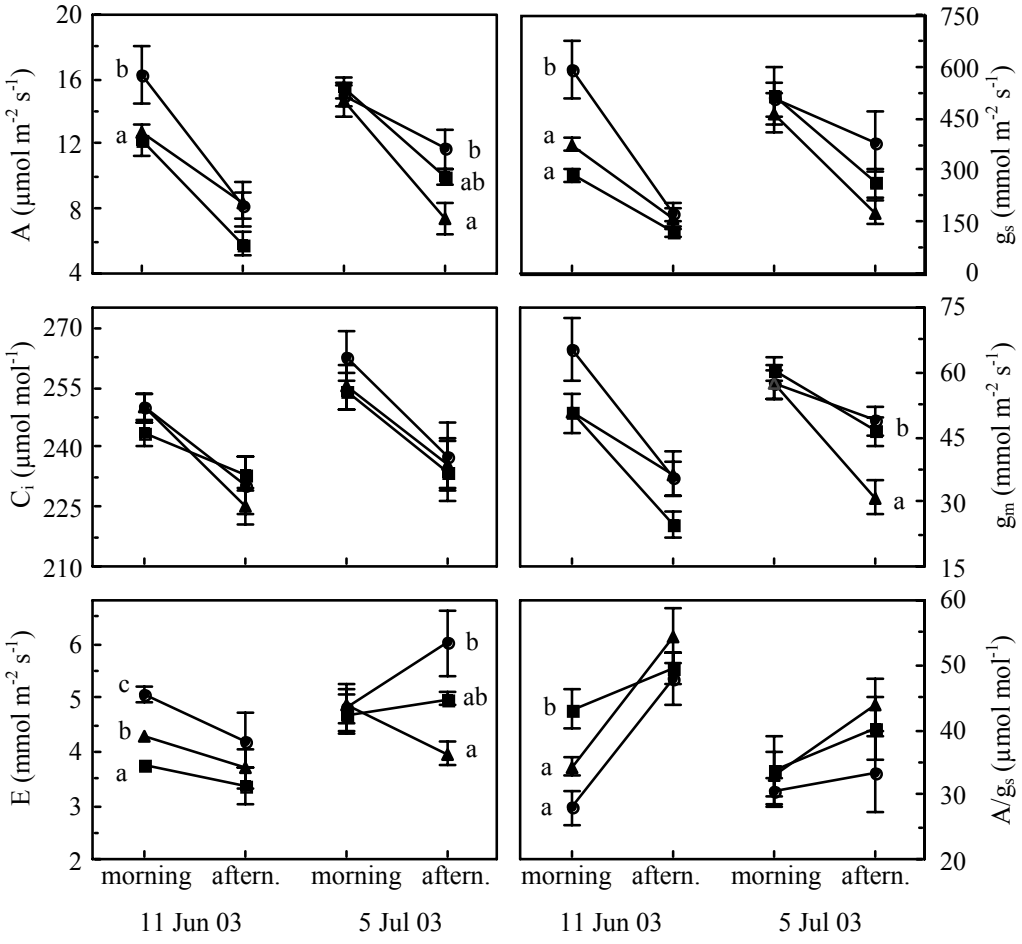

Figure 4.2
Diurnal changes in net CO_2 assimilation rate (A), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), liquid phase diffusive conductance to CO_2 (g_m), transpiration rate (E), and intrinsic water-use efficiency (A/g_s), for leaves of Burlat (*squares*), Summit (*triangles*), and Van (*circles*) cherry cultivars. Within each diurnal period (morning and afternoon) mean ± S.E. (n = 8) followed by different letter are significantly different ($P < 0.05$) according to Duncan's test.

Regarding the diurnal periods, cultivars did not present significant differences in values of stem water potential determined at predawn (Ψ_{PD}) or at midday (Ψ_{MD}) (Figure 4.3). Stem water potential values presented similar decreases from predawn to midday in all cultivars.

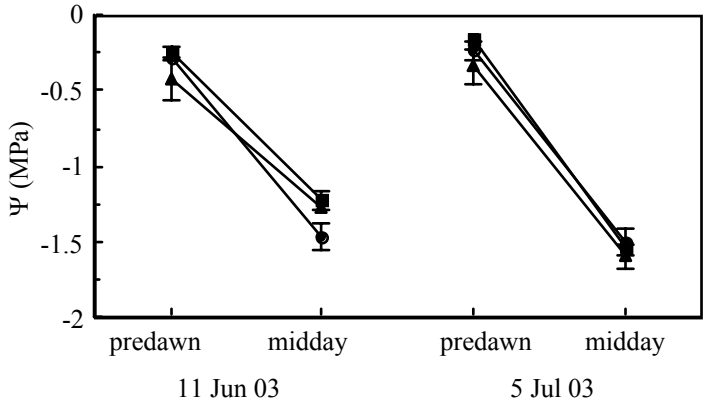

Figure 4.3
Diurnal changes in stem water potential (Ψ) of Burlat (*squares*), Summit (*triangles*) and Van (*circles*) cherry cultivars. Within each diurnal period (predawn and midday) mean ± S.E. (n = 8).

4.4.2. Leaf Morpho-Anatomical Traits

Foliar morphological characteristics of the three cultivars are presented in Table 4.2. Cultivar genotype significantly influenced leaf area (LA) and leaf mass per unit area (LMA). Fully developed Van leaves had higher LMA (+20%), but smaller surface area per leaf, compared to Summit (–31%) and Burlat (–24%). Leaf density (LD) and leaf water content (WC) were unaffected by genotype, with an average of 412 g kg^{-1} and 59 %, respectively.

Table 4.2
Leaf area (LA), leaf mass per unit area (LMA), leaf density (LD) and leaf water content (WC) of cherry cultivars grafted on Edabriz. Means (n = 60) followed by the same letter are not significantly different at $P < 0.05$ (Duncan's test).

Cultivar	LA (cm^2)	LMA (g m^{-2})	LD (g kg^{-1})	WC (%)
Burlat	69.4 b	64.8 a	411.8	58.8
Summit	75.7 b	64.8 a	412.1	58.8
Van	52.6 a	79.8 b	411.0	58.9
P	***	***	n.s.	n.s.

P = statistical significance of differences: n.s. indicates not significant; *** indicates $P < 0.001$.

Anatomical differences among the cultivars were investigated by means of scanning electron microscopy (SEM) and light microscopy. In leaf cross sections of all cultivars, the lamina was bifacial and hypostomatous (Figure 4.4). The glabrous adaxial epidermis was

composed by large irregular cells. The abaxial epidermis was characterized by one layer of small irregular cells surrounding numerous, irregularly scattered with differing size-stomata. Palisade mesophyll was constituted by three layers of cells in cultivars Summit and Van. The upper two formed by elongated, regularly compacted cells, whereas the lower one was formed by irregular, loosely packed cells. Meanwhile, Burlat palisade parenchyma had only two layers of elongated cells. In the three cultivars, spongy parenchyma was characterized by irregularly elongated cells surrounding small lacunae and numerous lateral veins.

According to the results from the anatomical study, leaf tissues thickness varied significantly among cultivars (Table 4.3). Total leaf lamina thickness (LTh) ranged from an average of 136 µm in Burlat to 184 µm in Summit. In Summit and Van, higher LTh was mainly due to a thicker palisade and spongy parenchyma and, in a less extent, due to a thicker adaxial epidermis. In addition, Burlat leaves had the highest palisade/spongy tissue ratio (> 30%), while laminae from Summit and Van had similar ratios. The mesophyll thickness was approximately 75% of the LTh in the three cultivars. Relatively to the protection tissues, adaxial epidermis was thicker than abaxial epidermis in all cultivars. In our study, stomatal density varied significantly between 436 in Van and 507 stomata mm^{-2} in Burlat.

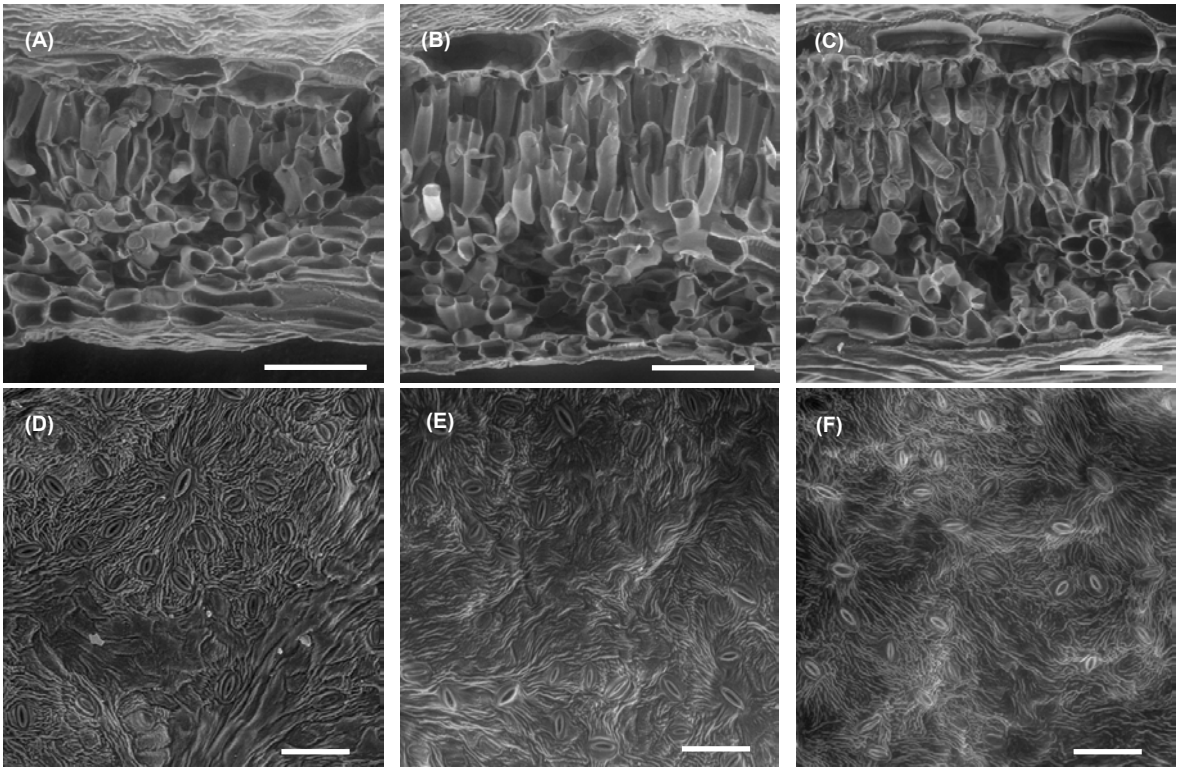

Figure 4.4
SEM micrographs of lamina transverse sections (A – Burlat; B – Summit; C – Van) and tangential of the abaxial page sections (D – Burlat; E – Summit; F – Van) of the three cherry cultivars. Scale bar = 50 µm.

Table 4.3
Leaf tissue thickness and stomatal density of cherry cultivars grafted on Edabriz. Means (n = 40) followed by the same letter are not significantly different at $P < 0.05$ (Duncan's test).

Cultivar	Thickness (µm)						Stomatal density (stomata mm^{-2})
	LTh	Upper Epidermis*	Palisade parenchyma	Spongy parenchyma	Palisade/ Spongy	Lower Epidermis*	
Burlat	135.8 a	23.0 a	53.1 a	49.1 a	1.147 b	12.7 a	507 b
Summit	184.4 b	28.0 b	65.0 b	77.9 c	0.853 a	13.9 a	465 ab
Van	178.3 b	29.9 b	62.8 b	72.4 b	0.884 a	15.5 b	436 a
P	***	***	***	***	***	***	*

Abbreviation: LTh – total lamina thickness. *Values also include the cuticle layer.
P = statistical significance of differences: * indicates $P < 0.05$; *** indicates $P < 0.001$.

4.4.3. Leaf Chemical Traits

Total chlorophyll concentrations per unit leaf area (Chl_{area}) of Van leaves was significantly higher than in Burlat (+35 %) and Summit (+23 %) (Table 4.4). On the contrary, as expressed on leaf dry mass (Chl_{mass}), no significant differences were apparent for the three cultivars, although the tendency was the same as for Chl_{area} concentrations. Less dense canopy of Van also showed significantly higher concentrations of total carotenoids expressed per leaf area (Car_{area}), with 31% more than Burlat and 27% more than Summit. However, when carotenoids concentrations where expressed per dry mass (Car_{mass}), no significant differences where detected among cultivars. Moreover, Van had the highest $Chl_{a/b}$ ratio, whereas no significant differences were detected in Chl/Car ratio.

Table 4.4
Photosynthetic pigments concentration per dry mass (mg g^{-1} DM) and per unit leaf area (mg dm^{-2}) of cherry cultivars grafted on Edabriz. Means (n = 8) followed by the same letter are not significantly different at $P < 0.05$ (Duncan's test).

Cultivar	Chl_{mass} (mg g^{-1} DM)	Chl_{area} (mg dm^{-2})	$Chl_{a/b}$	Car_{mass} (mg g^{-1} DM)	Car_{area} (mg dm^{-2})	Chl/ Car
Burlat	6.374	4.130 a	3.057 a	1.247	0.808 a	5.162
Summit	7.400	4.873 ab	3.063 a	1.305	0.860 a	5.711
Van	7.987	6.326 b	3.890 b	1.483	1.175 b	5.302
P	n.s.	*	*	n.s.	**	n.s.

Abbreviations: Chl – chlorophyll; Car – carotenoids.
P = statistical significance of differences: n.s. indicates not significant; * indicates $P < 0.05$; ** indicates $P < 0.01$.

Total phenols, total soluble sugars and starch concentrations expressed per area or dry mass varied significantly among cultivars and the variations were higher when concentrations were expressed by mass (Table 4.5). Van leaves had lower TP, SS and St concentrations, when expressed per dry mass, but presented the highest SS/St ratio.

Table 4.5
Leaf metabolites concentration per dry mass (mg g^{-1} DM) and per unit leaf area (mg dm^{-2}) of cherry cultivars grafted on Edabriz. Means (n = 8) followed by the same letter are not significantly different at $P < 0.05$ (Duncan's test).

Cultivar	TP_{mass} (mg g^{-1} DM)	TP_{area} (mg dm^{-2})	SS_{mass} (mg g^{-1} DM)	SS_{area} (mg dm^{-2})	St_{mass} (mg g^{-1} DM)	St_{area} (mg dm^{-2})	SS/St
Burlat	88.9 b	57.6 b	128.0 b	82.9 b	105.1 b	68.1 ab	1.303 ab
Summit	74.0 b	48.8 ab	105.1 b	69.3 ab	116.3 b	76.6 b	0.899 a
Van	39.3 a	31.1 a	57.9 a	45.8 a	47.5 a	37.6 a	1.767 b
P	**	*	*	*	**	*	*

Abbreviations: TP – total phenols; SS – total soluble sugars; St – starch.
P = statistical significance of differences: * indicates $P < 0.05$; ** indicates $P < 0.01$.

4.5. Discussion

In a previous study dealing with the same plants, Santos *et al.* (2006) reported that the three cultivars exhibited different canopy architecture, i.e., Van exhibited greatest growth with an abundant and open shooting habit than the other two cultivars. These canopy architectures affected light microenvironment of the sweet cherry cultivars that induced marked alterations in leaf morphology, anatomy and chemical composition (Tables 4.2–4.5). Moreover, sweet cherry tree canopies tended to close during the growing season leading to a higher leaf area density, which was revealed by the decrease in LC_t values from June to July (Figure 4.1). Therefore, according to Baraldi *et al.* (1994) and Baldini *et al.* (1997), is expected a substantial modification of the light quality inside the canopy that reduces the red/far-red (R/FR) ratio and induces consequent morphogenetic responses. Rossi *et al.* (1995) reported similar results in peach trees. In our study, Summit and Burlat with high dense canopies [i.e., with low shoot angle and low light penetration (low LC_t – Figure 4.1)] displayed a lower LMA, but higher leaf area than high light penetration trees of Van (Table 4.2). Changes in LMA among cultivars were produced by the development of thicker leaves under high light and not by differences in leaf density. This result is in accordance to those observed in many other species (Abrams and Kubiske, 1990; Niinemets and Kull, 1998; Mendes *et al.*, 2001).

The assumption that LMA and LTh are closely related was confirmed for this set of cherry cultivars, but the relation varied with the genotype. Accordingly, Aranda *et al.* (2004) found that LMA increased as LTh increased in eight temperate tree species, including *Prunus avium*, following a linear pattern. However, different leaves may differ in tissue density, causing a lack of correlation between LMA and LTh (Witkowski and Lamont, 1991), but it was not observed in this study, since there were no significant differences in leaf density among cultivars (Table 4.2).

In our study, the highest LTh values of Van and Summit leaves were mainly due to the greater spongy parenchyma (+ ~35%), but also due to the thicker palisade parenchyma (+ ~17%), and adaxial epidermis (+ ~20%) (Table 4.3). Therefore, cross-cultivars variations in LTh were mainly attributable to differing proportions of mesophyll components in leaves. These findings are in agreement with Castro-Díez *et al.* (2000), who studied 52 European woody species, including two belonging to genus *Prunus*. The increase in LTh involved a higher development of tissues per leaf area. So, adaxial and abaxial epidermis, as well as both spongy and palisade parenchyma, were positively correlated with LTh. In fact, a strong positive correlation was found between LTh and the thickness of palisade parenchyma, specifically in Summit (r = 0.965, P < 0.001), in Burlat (r = 0.933, P < 0.001) and in Van (r = 0.915, P < 0.001). A similar correlation between LTh and the thickness of palisade parenchyma was also shown by Rhizopoulou *et al.* (1991) in studies with four Mediterranean evergreen sclerophylls. According to Evans (1999), the differences in mesophyll thickness among cultivars can play a role in altering the profile of light capture through the leaf. Palisade tissue enables a better light penetration to the chloroplasts, while spongy tissue enhances the light capture by scattering light. So, it is presumed that leaves of Van and Summit, having a thicker palisade parenchyma, present an efficient structure in terms of photosynthesis.

Niinemets and Tenhunen (1997) analysing canopy carbon acquisition in *Acer saccharum* concluded that the acclimation to high light was dominated by adjustments in leaf anatomy rather than in foliar chemistry. In our experiment, photosynthetic pigments and leaf metabolites were also dependent on the canopy architecture effect (Tables 4.4 and 4.5). Among cultivars, Chl_{area} and Car_{area} concentrations were generally significantly greater in high light leaves of Van than in low light leaves of Burlat, with intermediate values in Summit. However, no significant differences were found for Chl_{mass} and Car_{mass}, which may be due to a higher LTh and a greater proportion of sclerified tissues (i.e., xylem and sclerenchyma) in the midrib and in secondary veins (data not shown) of Van leaves than in leaves of Burlat. According to Karabourniotis (1998), the greater abundance of these tissues in Van may contribute to the enhancement of the light microenvironment within internal mesophyll layers with advantages in the photosynthetic performance.

Since adaptation to low irradiance can involve an increase in light-harvesting complexes of photosystem II (Anderson and Osmond, 1987; Demmig-Adams, 1998), a decrease of $Chl_{a/b}$ was expected in low light leaves comparatively to high light leaves. Indeed, the ratio was affected by canopy architecture in the present study, where Burlat and Summit with high dense canopy showed low $Chl_{a/b}$.

It is well known that the intrinsic photosynthetic capacity of deciduous orchard trees depend on the leaf structural characteristics, such as LTh, size and arrangement of mesophyll cells that determine the amount of photosynthetic tissue per unit leaf area. In addition, greater photosynthetic capacity is often related to higher LMA, which is enhanced by ambient irradiance (Ellsworth and Reich, 1992; Reich *et al.*, 2000; Le Roux *et al.*, 2001). In fact, high light leaves of Van were comparatively thicker with high LMA, high leaf Chl_{area} and low SS and St concentrations than low light leaves of Burlat and Summit, reflected in a more efficient quantum utilization and a greater active sink capacity so slightly high photosynthetic capacity per unit area (Azcon-Bieto, 1983; Wayne and Bazzaz, 1993; Niinemets and Tenhunen, 1997). Accordingly, Centritto *et al.* (2000) reported a thinner mesophyll, a large specific leaf area and a reduced photosynthetic rate in cherry saplings adapted to shaded conditions throughout the growing season. An increase in mesophyll thickness represents a great cell wall area for CO_2 diffusion and so should to decrease liquid-phase resistance. In contrast, Mediavilla *et al.* (2001), in intraspecific comparisons, found that photosynthetic rates were significantly lower in high LMA leaves than in low LMA leaves; and may be due to a lower proportion of leaf nitrogen in the photosynthetic machinery (Evans, 1989; Niinemets, 1999b; Roderick *et al.*, 1999b). On the other hand, an increase in thickness should tend to increase the path length from the stomata to cell wall surfaces increasing gaseous diffusion resistance (Mediavilla *et al.*, 2001).

The high light leaves of Van showed lower concentration of TP, as compared to low light leaves of Burlat and Summit (Table 4.5). According to the growth/differentiation balance hypothesis (Herms and Mattson, 1992) and the predictions of Koricheva *et al.* (1998), the pathways leading either in phenylpropanoid or in protein synthesis, both compete for phenylalanine as the common precursor. Under water, light and nutrient sufficiency, growth as the priority and the bulk of phenylalanine is used for protein synthesis. However, under water, light or nutrient limitations, growth and protein synthesis are restricted and surplus carbon is diverted to phenylpropanoid synthesis. The above predictions are followed by the results of the present study for the concentrations of phenolic compounds. In fact, light is considered one of the most important factors in the control of phenolic synthesis acting upon a complex system involving several photoreceptors: R/FR systems (phytochromes), blue light systems (cryptochromes), UV systems and the photosynthetic pigments system (Arakawa, 1988). Specifically, phytochrome activity is controlled by the R/FR ratio and is believed to regulate phenolic gene expression in many plant systems (Mohr and Herrel, 1983; Tobin and Silverthorne, 1985).

The light environment influenced gas exchange parameters (Figure 4.2) and high light plants of Van exhibited higher A and g_s. Increases in maximum foliar conductance with irradiance or canopy height has been well documented in other species (Abrams and Mostoller, 1995; Muraoka *et al*., 1997; Niinenets *et al*., 1998; Mendes *et al*., 2001). The high light leaves of Van displayed during the day higher conductance and transpiration rates that low light ones, also presenting a greater change of g_s from morning to afternoon, at harvest. This stomatal closing response appears to be directly related to the increase of the leaf-to-air water vapour deficit, enabling water conservation and to maximize water-use efficiency (Pereira and Chaves, 1993; Tenhunen *et al*., 1994; Niinemets *et al*., 1999a; Mendes *et al*., 2001).

From morning to midday a drop in Ψ_{stem} occurred in response to soil water depletion (Figure 4.3). However no significant differences in Ψ_{stem} were found among genotypes, which indicate a similar plant water status. This result associated with similar soil nutrient availability suggests that differences in leaf morpho-anatomical and chemical composition among cultivars were mainly due to irradiance regimes influenced by canopy architecture.

The variation in the internal leaf structure of sweet cherry tree suggests that it may play a role in the profile of light capture through the leaf. So, it is presumed that Summit and Van leaves, having a thicker palisade parenchyma (Table 4.3; Figure 4.4), present a more efficient photosynthetic structure in terms of photosynthesis than thin leaves of Burlat. However, Burlat leaves had the highest palisade/spongy tissue ratio (1.2), suggesting a compact arrangement of cells and high mesophyll surface area per unit leaf area and higher stomatal density that could facilitate CO_2 uptake and thus maintain a high photosynthetic activity (Chartzoulakis *et al*., 2000), similar to the other two cultivars. On the other hand, as Burlat had the highest stomatal density of the studied cultivars, it probably avoids low Ψ, since it presented similar values to the other cultivars (Figure 4.3), by possessing flexible stomata regulation (Bolhar-Nordenkampf, 1987).

In conclusion, the results of this study indicate that *Prunus avium* cultivars with different canopy architecture, which affects light microenvironment, exhibited plastic responds to light in leaf morpho-anatomy, chemical composition and physiology. All the adjustments observed will enable better efficient carbon assimilation, specifically high LMA and Chl_{area} in high light plants, and high surface area and stomatal density in low light ones. In response to high evaporative demand, both high and low light trees exhibited water saving mechanisms, through an efficient stomatal regulation, more marked in high light plants.

In order to increase tree efficiency, it is important to ensure optimal conditions (light interception, water and nutrient availability, protection from diseases, etc.) when the demand for assimilates is high. In particular, it is important to manipulate factors such as orchard exposure, row orientation, plant spacing, training system, pruning, etc. which are able to reduce shading in the crown.

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CAPÍTULO 5

SCION-ROOTSTOCK INTERACTION AFFECTS THE PHYSIOLOGY AND FRUIT QUALITY OF SWEET CHERRY TREE

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5. SCION-ROOTSTOCK INTERACTION AFFECTS THE PHYSIOLOGY AND FRUIT QUALITY OF SWEET CHERRY TREE

5.1. Abstract

Water relations, leaf gas exchange, chlorophyll a fluorescence, canopy light transmittance, leaf photosynthetic pigments and metabolites and fruit quality indices of cherry cultivars Burlat, Summit and Van growing on five rootstocks with differing size–controlling potentials: *Prunus avium* L. (vigorous) > CAB 11E > Maxma 14 > Gisela 5 > Edabriz (dwarfing), were studied during 2002 and 2003. Rootstock genotype affected all physiological parameters. Cherry cultivars grafted on invigorating rootstocks had higher values of midday stem water potential (Ψ_{MD}), net CO_2 assimilation rate (A), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and maximum photochemical efficiency of photosystem II (PSII) (F_v/F_m) than cultivars grafted on dwarfing rootstocks. The Ψ_{MD} was positively correlated with A, g_s and C_i. Moreover, A was positively correlated with g_s, and the slopes of the linear regression increased from invigorating to dwarfing rootstocks, indicating a stronger regulation of photosynthesis by stomatal aperture in trees on dwarfing Edabriz and Gisela 5. The effect of rootstock genotype was also statistically significant for leaf photosynthetic pigments, whereas metabolite concentrations and fruit physicochemical characteristics were more dependent on cultivar genotype. Among cultivars, Burlat leaves had the lowest concentrations of photosynthetic pigments, but were richest in total soluble sugars, starch and total phenols. Compared with the other cultivars, Summit had heavier fruits, independent of the rootstock. Burlat cherries were less firm and had lower concentrations of soluble sugars and a lower titratable acidity than Van cherries. Nevertheless, Van cherries had lower lightness, chroma and hue angle, representing redder and darker cherries, compared with Summit fruits. In general, Ψ_{MD} was positively correlated with fruit mass and A was negatively correlated with lightness and chroma. These results demonstrate that: (1) water relations and photosynthesis of sweet cherry tree are mainly influenced by the rootstock genotype; (2) different physicochemical characteristics observed in cherries of the three cultivars suggest that regulation of fruit quality was mainly dependent on the cultivar genotype, although the different size–controlling rootstocks also had a significant effect.

Key words: chlorophyll a fluorescence, fruit physicochemical characteristics, gas exchange, leaf metabolites, canopy light transmittance, photosynthetic pigments, *Prunus avium*, size–controlling rootstocks, water relations.

5.2. Introduction

Several reports have documented the relationships between various physiological parameters of grafted trees and fruit quality (Naor *et al.*, 1995; Shackel *et al.*, 1997; Naor, 1998). These relationships are important from a horticultural point of view, because they provide a basis for selecting the best graft combination for particular environmental conditions and high fruit quality. Selection of an appropriate graft combination is crucial for the production of deciduous orchard species, because the scion–rootstock interaction influences water relations, leaf gas exchange, mineral uptake, plant size, blossoming, timing of fruit set, fruit quality and yield efficiency (Schmitt *et al.*, 1989; Nielsen and Kappel, 1996; Gonçalves *et al.*, 2003).

In grafted trees, the control of plant size is mainly exerted through the rootstock. The mechanism by which the rootstock regulates scion vigor has not been identified (Sorce *et al.*, 2002; Basile *et al.*, 2003), although several potential dwarfing mechanisms have been studied, including the presence of small vessels that influence hydraulic conductivity (Simons and Chu, 1984; Ussahatanonta and Simons, 1988; Soumelidou *et al.*, 1994; Salvatierra *et al.*, 1998), lower total sap solute content (Jones, 1984), low nutrient uptake capacity (Simons and Swiader, 1985; Tagliavini *et al.*, 1992; Nielsen and Kappel, 1996; Rosati *et al.*, 1997; Ebel *et al.*, 2000) and reduced production of growth hormones (Chen *et al.*, 1985; Sorce *et al.*, 2002).

Rootstocks generally affect tree water relations. For cherry (Schmitt *et al.*, 1989; Gonçalves *et al.*, 2003), peach (Bongi *et al.*, 1994; Basile *et al.*, 2003) and apple (Giulivo and Bergamini, 1982; Olien and Lakso, 1986), trees grafted on invigorating rootstocks have consistently higher stem water potentials than trees grafted on dwarfing rootstocks. Moreover, in cherry (Shackel *et al.*, 1997) and grapevine (Düring, 1994; Iacono *et al.*, 1998; Patakas *et al.*, 2003), trees grafted on invigorating rootstocks have higher A and g_s. In grapevines, the graft combination also affects leaf photosynthetic pigments and metabolites (Bavaresco and Lovisolo, 2000). Furthermore, fruit quality and maturity are reported to be affected by rootstock (Ezzahouani and Williams, 1995). Autio and Southwick (1993) found differences in fruit color and fruit mass in apple grafted on different rootstocks, whereas Ferree (1992) and Meheriuk *et al.* (1994) found small or no difference in fruit soluble solids, skin color, firmness and mass in apple grafted on different rootstocks.

The northeast region of Portugal produces almost 40% of the Portuguese cherry crop. Although the cultivars most frequently grown in the region are considered to be well adapted to the local environment, there have been few field studies on the newer dwarfing rootstocks, which vary greatly in the physiological and morphological traits that determine

their horticultural performance. We tested the following hypotheses: (1) size–controlling rootstocks affect the physiology of grafted sweet cherry trees; and (2) physicochemical characteristics of fruits are affected by the physiology of the scion–rootstock interaction. Specifically, the experiment was designed to evaluate stem water potential, leaf gas exchange, chlorophyll a fluorescence, canopy light transmittance, photosynthetic pigments, leaf metabolites and fruit quality of cherry cultivars Burlat, Summit and Van grafted onto rootstocks with contrasting size–controlling potentials: *Prunus avium*, CAB 11E, Maxma 14, Gisela 5 and Edabriz.

5.3. Materials and Methods

5.3.1. Experimental Trial

The study was carried out in Vila Real, in the northeast Portugal (41º 19' N and 7º 44' W), at 470 m above sea level, during 2002 and 2003.

The climate is typical Mediterranean with mild rainy winters and long, hot and dry summers. Mean annual rainfall is about 1100 mm, mainly from October to April. The warmest months are July and August and the coldest are December and January, with mean daily temperatures of 21–22 °C and 6–7 °C, respectively. Mean annual sunshine values over a 30-year period are 2392 h, the lowest monthly values (100 h) occurring in December and the highest (342 h) in July. The soil is a deep (> 100 cm) sandy loam, dystric arid antherosol, pH 4.7, with an organic matter content of 1.5%, a high content of fine sand (0.2–0.02 mm), a high content of K_2O (150–200 mg kg^{-1}) and a medium content of P_2O_5 (50–100 mg kg^{-1}).

Three cultivars of sweet cherry trees (*Prunus avium*) were each grafted on five different rootstocks. The cultivars were an early-maturing Burlat, and the mid- to late-maturing Summit and Van, and the rootstocks were *Prunus avium* (vigorous), CAB 11E (clone of *Prunus cerasus* L.; semi-vigorous) Maxma 14 (*Prunus avium* × *Prunus mahaleb* L. hybrid; semi-dwarfing), Gisela 5 (*Prunus cerasus* × *Prunus canescens* Bois. hybrid; dwarfing) and Edabriz (clone of *Prunus cerasus*; very dwarfing). In 1999, trees were planted in a north–south orientation with rows 5.5 m apart. Within rows, trees were spaced according to the vigor of the rootstock: 5.5, 5.0, 4.5, 4 and 3.0 m for *Prunus avium*, CAB 11E, Maxma 14, Gisela 5 and Edabriz, respectively. Routine disease and pest control treatments were provided according to a commercial protocol for fruit production. The cherry orchard was drip-irrigated daily between May and September and periodically fertilized. The trees were not pruned in the summer during the experiment.

5.3.2. Water Relations

Predawn (Ψ_{PD}) and midday stem water potentials (Ψ_{MD}) of grafted cherry trees were determined on four dates (June 21 and July 8, 2002 and June 11 and July 9, 2003). Stem water potential (Ψ_{stem}) was measured on fully expanded healthy leaves. Branches were placed in black polyethylene bags wrapped in aluminium foil for at least 90 min before measurements, to allow leaf water potential to equilibrate with Ψ_{stem}. We measured Ψ_{stem} with two pressure chambers (ELE International, Bedfordshire, England) as described by Scholander *et al.* (1965). In all cases, leaves were placed in the chamber within a few seconds after excision. Eight measurements of Ψ_{stem} by the scion–rootstock combination were made during each diurnal period.

5.3.3. Gas Exchange and Chlorophyll a Fluorescence

On July 9, 2003, leaf gas exchange rates were measured with a portable gas exchange system (ADC-LCA-3, Analytical Development, Hoddesdon, U.K.) and a leaf chamber clip (ADC-PLC, surface = 6.25 cm^2, volume = 16 cm^3) with a quantum sensor, air temperature and humidity sensors. The gas exchange unit was operated in the open mode at a flow rate of 300 ml min^{-1} and an ambient CO_2 partial pressure of 35–37 Pa. Twelve measurements were made on fully expanded healthy leaves in the morning (09.00–11.00 h) and afternoon (14.00–16.00 h). Net CO_2 assimilation rate (A), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) were estimated from the gas exchange measurements with the equations of von Caemmerer and Farquhar (1981). Intrinsic water-use efficiency was calculated as the ratio of A to g_s (A/g_s) (Düring, 1994). While measurements were being made, photosynthetic photon flux ranged between 1830 and 1960 µmol m^{-2} s^{-1}, air temperature ranged between 30.4 and 34.1 °C and air vapor pressure deficits were around 2.4 and 3.1 kPa, in the morning and afternoon, respectively.

Maximum photochemical efficiency of PSII in dark-adapted leaves (F_v/F_m), minimal (F_0) and maximal fluorescence (F_m) at open and closed PSII reaction centres, respectively, and variable fluorescence (F_v), were determined around midday on attached intact leaves similar to those used for gas exchange measurements with a portable chlorophyll fluorometer (Plant Stress Meter, BioMonitor SCI AB, Umeå, Sweden), as described by Öquist and Wass (1988). Before measurements, leaves were dark-adapted for 30 min in a clamp cuvette. A 5-s light pulse at 400 µmol m^{-2} s^{-1} was used. Eighteen measurements of chlorophyll a fluorescence parameters were performed per scion–rootstock combination.

5.3.4. Canopy Light Transmittance

Canopy light transmittance (LC_t) values were taken as an indirect indicator of canopy size (cf. Dufrêne and Bréda, 1995). Midday LC_t was calculated as the ratio of photosynthetic photon flux (PPFD) measured horizontally below and above the canopy with a Sunfleck Ceptometer (Model SF-80, Decagon Devices, Cambridge, U.K.), as described by Campbell (1986). Eight means of LC_t were determined per scion–rootstock combination. Each mean consisted of 10 measurements taken over the ground area shaded by the canopy.

5.3.5. Photosynthetic Pigments and Metabolites Assays

Leaf discs (1.57 cm^2) taken from fully expanded leaves of comparable physiological age were frozen in liquid N_2 and stored at –80 °C until analyzed. Chlorophyll a (Chl_a) and b (Chl_b) were extracted in 80% acetone and quantified spectrophotometrically (Sesták *et al.*, 1971). Total carotenoids were extracted with the chlorophylls and determined with the equations of Lichtenthaler (1987).

Total soluble sugars were extracted by heating leaf discs in 80% ethanol (Irigoyen *et al.*, 1992) and incubating 200 µl of the extract with 3 ml of fresh anthrone in a boiling water bath for 10 min. After cooling, the absorbance at 625 nm was determined. Starch was extracted from the ethanol-insoluble fraction with 30% perchloric acid, as described by Osaki *et al*. (1991), and the concentration determined by the anthrone method, as described above. Glucose was used as the standard for both total soluble sugars and starch.

The concentration of total phenolics in leaf extracts was determined on the same extract used for pigment analysis by the Folin-Ciocalteu procedure (Singleton and Rossi, 1965), with the modifications described by Heinonen *et al.* (1998).

Leaf discs for the photosynthetic pigment and metabolite assays were used to calculate leaf mass per unit area (LMA) as: LMA = DM/LA (g dm^{-2}) where DM is dry mass, measured after drying at 70 °C to constant mass, and LA is the leaf disc area. Eight leaves per scion–rootstock interaction were collected (July 8, 2002 and July 9, 2003) to determine photosynthetic pigment and metabolite concentrations. Values of these parameters were expressed on a DM basis.

5.3.6. Fruit Quality Indices

Sweet cherries from cultivars Burlat, Summit and Van were randomly harvested by hand in 2002 and 2003. For each scion–rootstock combination, maturity of 20 fruits was assessed by the following indices: mass, firmness (with an Effegi penetrometer, Model FT

327, Effegi Systems, Milan, Italy), soluble sugar concentration (by refractometry), titratable acidity (by titrimetry) and pH.

Skin color, which is the main criterion for assessing maturity and harvest time, was measured in 20 fruits with a tristimulus colorimeter (Minolta CR-200B Chroma Meter, Minolta, Japan) having an 8-mm-diameter viewing area. Chromatic analyses were carried out following the CIE (Commission International de l'Eclairage) system of 1976. Values of lightness, redness and greenness (a^* and $-a^*$) and yellowness and blueness (b^* and $-b^*$) on the hue circle (Voss, 1992) were measured to describe a three-dimensional color space. The vertical axis is a measure of lightness, where values range from completely opaque (0) to completely transparent (100). From the a^* and b^* values, the hue angle (H^*) or tonality, which expresses the color nuance, can be calculated from $H^* = \text{arctg}\ (b^*/a^*)$ (McLaren, 1980; Voss, 1992). The chroma (C^*), a measure of chromaticity indicating the purity or saturation of the color, is obtained as $C^* = (a^{*2} + b^{*2})^{1/2}$ (Voss, 1992). The values presented for each measurement date are the means of triplicate measures on equidistant points of each fruit.

5.3.7. Statistics

Fifteen treatments were laid out as a two-factor experiment with five rootstocks and three cultivars arranged in a randomized complete block design with two replications. Each replication comprised six trees per treatment. The measurements were made on two representative trees of each scion–rootstock combination. Data were subjected to analysis of variance (ANOVA) and means were separated by Duncan's significant difference test, when ANOVA indicated significant ($P < 0.05$) variable effects. The contribution of each factor to the global variation was calculated according to a fixed effects model (Snedecor and Cochran, 1967).

A Fisher correlation analysis including all parameters was also performed. A Principal Component Analysis (orthotran/varimax) applied to 20 variables allowed us to identify a subset of characters that separated the genotypes to the maximum and to identify the relative contribution of each variable to their separation.

5.4. Results

5.4.1. Water Relations

There were no significant differences ($P > 0.05$) in Ψ_{PD} values between rootstocks, cultivars or cultivar–rootstock combinations (Table 5.1), whereas there was high variation in Ψ_{MD} values between rootstocks ($P < 0.01$), cultivars ($P < 0.01$) and cultivar–rootstock

combinations ($P < 0.05$). For example, on July 9, 2003, Ψ_{MD} ranged from –1.17 MPa in Summit grafted on CAB 11E to –1.71 MPa in Van on Gisela 5 (Table 5.2). On average, Ψ_{MD} reached lower values (down to –1.71 MPa) in trees on the dwarfing rootstocks Edabriz and Gisela 5 than in trees on the invigorating rootstocks *Prunus avium*, CAB 11E and Maxma 14.

Table 5.1
Summary of factorial analysis of variance and respective percentage of total variation (in parenthesis) for stem water potential, leaf gas exchange, maximum photochemical efficiency of photosystem II (PSII), canopy light transmittance, photosynthetic pigments, leaf metabolites and fruit characteristics of three cherry cultivars, each grafted on five rootstocks during year 2003.

	Period	Parameter	Source of variation		
			Cultivar (C)	Rootstock (R)	C x R
Leaf physiology	Predawn	Ψ_{PD}	ns (2.7)	ns (0.0)	ns (15.8)
	Midday	Ψ_{MD}	** (15.1)	** (17.7)	* (16.3)
	Morning	A	ns (3.0)	*** (23.0)	* (12.2)
		g_s	ns (0.0)	*** (34.1)	ns (0.0)
		C_i	ns (0.0)	*** (42.6)	ns (0.0)
		A/g_s	ns (0.0)	*** (39.2)	ns (0.0)
	Afternoon	A	ns (2.7)	*** (47.2)	ns (5.9)
		g_s	ns (0.4)	*** (24.8)	ns (0.0)
		C_i	ns (0.0)	*** (17.8)	ns (0.0)
		A/g_s	ns (0.0)	*** (35.1)	ns (0.0)
	Midday	F_v/F_m	ns (0.0)	* (9.2)	ns (8.1)
		F_0	ns (1.1)	*** (34.1)	ns (3.5)
		F_m	ns (0.0)	*** (28.1)	** (16.4)
		F_v	ns (0.0)	*** (24.0)	** (17.5)
Canopy magnitude	Midday	LC_t	*** (21.6)	*** (27.6)	*** (16.6)
Leaf metabolites	Morning	LMA	*** (50.0)	* (3.5)	ns (0.0)
		Chl	*** (33.2)	** (7.6)	ns (0.1)
		Car	*** (33.1)	** (7.4)	** (11.0)
		$Chl_{a/b}$	* (4.9)	* (5.8)	* (12.5)
		Chl/Car	ns (0.0)	ns (2.2)	ns (4.4)
		SS_l	ns (1.3)	ns (4.3)	ns (10.4)
		St	*** (14.0)	* (5.4)	ns (3.7)
		TP	* (4.5)	*** (16.7)	** (16.1)
Fruit characteristics	Harvest	W	*** (78.3)	*** (1.1)	*** (6.2)
		F	*** (21.7)	*** (21.2)	*** (25.1)
		SS_f	*** (41.4)	*** (2.2)	*** (41.5)
		pH	*** (59.6)	*** (18.7)	*** (18.5)
		TA	*** (76.1)	*** (3.9)	*** (18.1)
		L^*	*** (17.3)	*** (22.7)	*** (21.4)
		C^*	*** (30.2)	*** (15.6)	*** (15.0)
		H^*	*** (29.6)	*** (17.7)	*** (10.2)

Abbreviations: Ψ_{PD} and Ψ_{MD} – predawn and midday stem water potential, respectively; A – net CO_2 assimilation rate; g_s – stomatal conductance; C_i – intercellular CO_2 concentration; A/g_s – intrinsic water-use efficiency; F_v/F_m – maximum PSII efficiency; F_0 – minimal fluorescence, F_m – maximal fluorescence; F_v – variable fluorescence; LC_t– canopy light transmittance; LMA – leaf mass per unit area; Chl – total chlorophyll; Car – total carotenoids; $Chl_{a/b}$ = ratio of chlorophyll a to chlorophyll b; SS_l – leaf total soluble sugars; St – starch; TP – total phenols; W – mass; F – firmness; SS_f – fruit total soluble sugars; TA – titratable acidity; L^* – lightness; C^* – chroma; H^* – hue angle.
ns indicates not significant; * indicates $P < 0.05$; ** indicates $P < 0.01$; *** indicates $P < 0.001$ by Duncan's test.

Among the cultivars, Summit had the highest Ψ_{MD}, except when grafted on Edabriz, where it was similar (–1.59 MPa) to that of the other cultivars. As expected, Ψ_{stem} decreased

from predawn to midday, and the low Ψ_{stem} values coincided with the time of maximum atmospheric evaporative demand. The greatest decreases in Ψ_{stem} from predawn to midday were recorded in Burlat and Summit grafted on Edabriz and in Van on Gisela 5.

Table 5.2
Effects of cherry cultivars and rootstocks on stem water potential at predawn (Ψ_{PD}) and midday (Ψ_{MD}) measured on July 9, 2003. Means (n = 8) followed by the same letter are not significantly different at $P < 0.05$ (Duncan's test).

Graft combinations		Ψ_{PD} (MPa)	Ψ_{MD} (MPa)
Cultivar	Rootstock		
Burlat	Edabriz	–0.16	–1.53 abcd
	Gisela 5	–0.28	–1.45 abcd
	Maxma 14	–0.39	–1.55 abc
	CAB 11E	–0.23	–1.55 abcd
	Prunus avium	–0.27	–1.35 bcde
Summit	Edabriz	–0.34	–1.59 ab
	Gisela 5	–0.20	–1.38 bcde
	Maxma 14	–0.27	–1.28 de
	CAB 11E	–0.22	–1.17 e
	Prunus avium	–0.25	–1.18 e
Van	Edabriz	–0.24	–1.49 abcd
	Gisela 5	–0.22	–1.71 a
	Maxma 14	–0.22	–1.38 bcde
	CAB 11E	–0.22	–1.47 abcd
	Prunus avium	–0.18	–1.32 cde

5.4.2. Gas Exchange and Chlorophyll a Fluorescence

Diurnal patterns of the physiological parameters measured were similar throughout the season and in the two studied years, so only the data obtained on July 9, 2003 are presented. There were significant differences ($P < 0.001$) in the leaf gas exchange parameters (A, g_s, C_i and A/g_s) between rootstocks, but not between cultivars or cultivar × rootstock combinations, except for A ($P < 0.05$) in the morning (Table 5.1). In both the morning and afternoon, higher values of A, g_s and C_i were observed in trees on the invigorating rootstocks *Prunus avium*, CAB 11E and Maxma 14 than in trees on the dwarfing rootstocks (Figure 5.1). Trees grafted on Edabriz were the exception, with morning A of trees grafted onto dwarfing rootstocks being similar to the values measured in trees grafted

on invigorating rootstocks. For every scion–rootstock combination studied, A, g_s and C_i were consistently higher in the morning than in the afternoon. The greatest diurnal decreases in A were in trees on dwarfing rootstocks Edabriz and Gisela 5: the maximum diurnal reduction in A was 53% in Van grafted on Gisela 5, which had a minimum value of 6 μmol m^{-2} s^{-1}. On the other hand, trees grafted on dwarfing rootstocks had the highest A/g_s of the graft types (Figure 5.1). Values of A/g_s increased from the morning to the afternoon, mainly in trees on Edabriz, ranging from 32 to 42 μmol mol^{-1}.

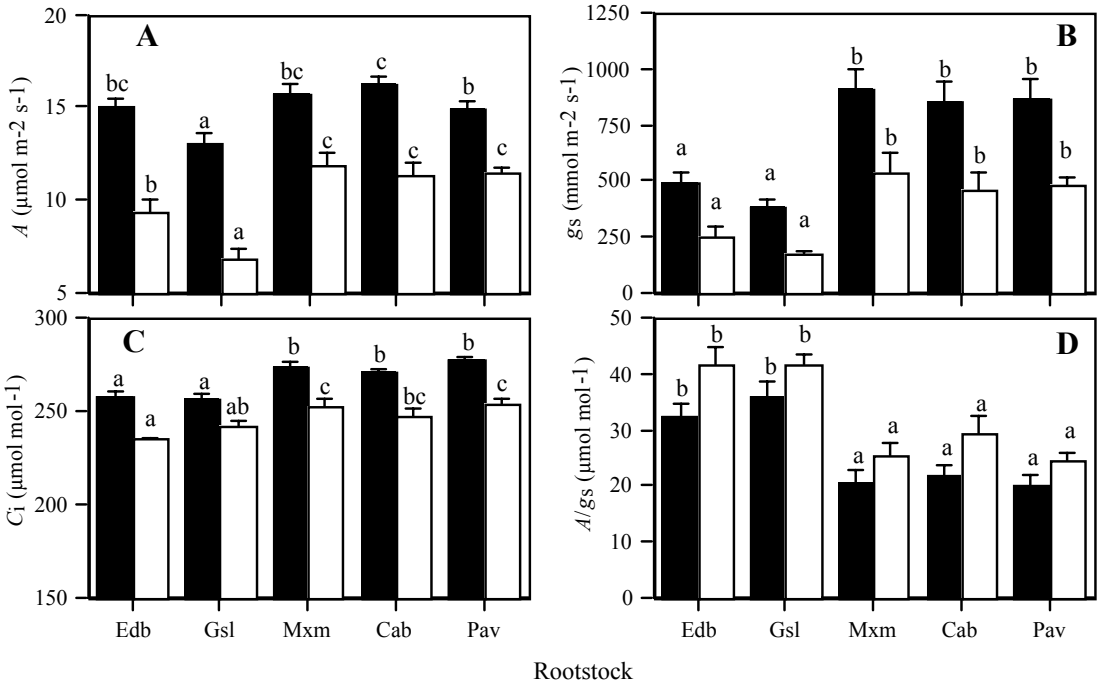

Figure 5.1
Net CO_2 assimilation (A) (A), stomatal conductance (g_s) (B), intercellular CO_2 concentration (C_i) (C) and intrinsic water-use efficiency (A/g_s) (D) of fully exposed leaves of three cherry cultivars, each grafted on five rootstocks (Edb = Edabriz, Gsl = Gisela 5, Mxm = Maxma 14, Cab = CAB 11E and Pav = *Prunus avium*) measured in the morning (solid columns) and afternoon (open columns) on July 9, 2003. Columns are means (n = 12) and vertical bars represent the SE. Measurements of each diurnal period followed by the same letter are not significantly different at $P < 0.05$ (Duncan's test).

There were significant differences ($P < 0.05$) in the F_v/F_m between rootstocks, but not between cultivars or cultivar × rootstock combinations (Table 5.1). Moreover, F_0, F_m and F_v varied significantly ($P < 0.001$) among rootstocks, and F_m and F_v differed ($P < 0.01$) among cultivar × rootstock combinations (Table 5.1). Generally, F_v/F_m, F_0, F_m and F_v were lower in trees on dwarfing Edabriz and Gisela 5 than in trees on invigorationg *Prunus avium*, CAB 11E and Maxma 14 (Table 5.3).

Table 5.3
Effect of rootstocks on maximum photochemical efficiency of photosystem II in dark–adapted leaves (F_v/F_m), minimal fluorescence (F_0), maximal fluorescence (F_m) and variable fluorescence (F_v) measured at midday on July 9, 2003. Means (n = 18) followed by the same letter are not significantly different at $P < 0.05$ (Duncan's test).

Rootstock	F_v/F_m	F_0	F_m	F_v
Edabriz	0.670 a	0.159 a	0.494 a	0.336 a
Gisela 5	0.684 ab	0.164 a	0.526 ab	0.361 ab
Maxma 14	0.692 ab	0.174 b	0.571 bc	0.397 bc
CAB 11E	0.700 b	0.179 bc	0.609 cd	0.430 cd
Prunus avium	0.708 b	0.188 c	0.653 d	0.465 d

5.4.3. Canopy Light Transmittance

Midday LC_t varied significantly ($P < 0.001$) between rootstocks, cultivars and cultivar–rootstock combinations (Table 5.1). Values of LC_t were always higher in cultivars grafted on the dwarfing rootstocks Edabriz and Gisela 5 than in cultivars grafted on invigorating rootstocks (Table 5.4). In all rootstocks, the highest values of LC_t were determined in Van. Among the scion–rootstock combinations, Van grafted on Edabriz had the highest LC_t (47%), whereas the lowest value was observed in Summit on CAB 11E (7%).

Table 5.4
Effects of cherry cultivars and rootstocks on canopy light transmittance (LC_t) (ANOVA after transformation in arc sine values) measured at midday on July 9, 2003. Means (n = 8) followed by the same letter are not significantly different at $P < 0.05$ (Duncan's test).

Graft combinations		LC_t (%)
Cultivar	Rootstock	
Burlat	Edabriz	19.16 cde
	Gisela 5	17.32 bcde
	Maxma 14	11.12 abc
	CAB 11E	10.90 abc
	Prunus avium	10.19 ab
Summit	Edabriz	12.32 abcd
	Gisela 5	22.51 e
	Maxma 14	10.21 ab
	CAB 11E	6.72 a
	Prunus avium	10.65 abc
Van	Edabriz	47.22 g
	Gisela 5	35.04 f
	Maxma 14	19.98 de
	CAB 11E	12.47 abcd
	Prunus avium	11.98 abc

5.4.4. Photosynthetic Pigments and Metabolites in Leaves

Among the rootstocks, significant differences ($P < 0.01$) in total chlorophyll and total carotenoid concentrations and in ($P < 0.05$) LMA and chlorophyll a to chlorophyll b ratio ($Chl_{a/b}$) were observed (Table 5.1). Higher values of total chlorophyll, total carotenoids and $Chl_{a/b}$ were always observed in the leaves of cultivars grafted on dwarfing rootstocks Edabriz and Gisela 5 (Table 5.5) than on invigorating rootstocks. Trees grafted on Maxma 14 had the highest LMA, but had 25 and 20% lower concentrations of total chlorophyll and total carotenoids, respectively, than trees grafted on Edabriz (Table 5.5).

There were significant differences in LMA, photosynthetic pigment concentrations ($P < 0.001$) and in the $Chl_{a/b}$ ratio ($P < 0.05$) between cultivars (Table 5.1). Van leaves had the highest concentrations of total chlorophyll and total carotenoids, approximately 30% higher than Burlat leaves, although Van had the lowest LMA (Table 5.5). On average, Summit had the highest $Chl_{a/b}$ (3.850) and Burlat the lowest (3.091). Cultivar, rootstock and cultivar–rootstock combinations had no influence on total chlorophyll/total carotenoids ratio (Table 5.1).

Table 5.5
Effects of cherry cultivars and rootstocks on leaf mass per unit area and concentrations of photosynthetic pigments measured on July 9, 2003. Means ($n = 40$ and $n = 24$ for cultivars and rootstocks, respectively) followed by the same letter are not significantly different at $P < 0.05$ (Duncan's test).

Source of variation	LMA (g dm^{-2})	Chl (mg g^{-1} DM)	$Chl_{a/b}$	Car (mg g^{-1} DM)	Chl/Car
Cultivar					
Burlat	0.754 c	5.372 a	3.091 a	1.067 a	5.048
Summit	0.713 b	6.218 b	3.850 b	1.265 b	5.066
Van	0.584 a	8.036 c	3.301 ab	1.570 c	5.112
Rootstock					
Edabriz	0.674 abc	7.624 c	3.504 ab	1.467 c	5.286
Gisela 5	0.709 bc	6.859 bc	4.070 b	1.419 bc	4.926
Maxma 14	0.719 c	5.691 a	3.353 ab	1.173 a	4.821
CAB 11E	0.653 a	6.286 ab	3.290 ab	1.252 ab	5.009
Prunus avium	0.660 ab	6.293 ab	2.850 a	1.204 a	5.319

Abbreviations: LMA = leaf mass per unit area; Chl = total chlorophyll; $Chl_{a/b}$ = ratio of chlorophyll a to chlorophyll b; Car = total carotenoids; DM = dry mass.

There were significant differences in starch concentrations between cultivars ($P < 0.001$) and rootstocks ($P < 0.05$) (Table 5.1). Total phenolic concentrations varied among cultivars ($P < 0.05$), rootstocks ($P < 0.001$) and cultivar–rootstock combinations ($P < 0.01$),

whereas total soluble sugar concentrations did not vary significantly ($P > 0.05$) with cultivar, rootstock or scion–rootstock combination (Table 5.1). Among the rootstocks, trees grafted on invigorating rootstocks had the highest starch and total phenolic concentrations (Table 5.6). Among the cultivars, starch was the main metabolite in leaves of Burlat and Summit, whereas Van leaves had similar concentrations of starch, total phenolics and total soluble sugars. Burlat leaves were richest in total soluble sugars, starch and total phenolics, whereas Van leaves had the lowest concentrations of total soluble sugars, starch and total phenols, with 47% less starch than Burlat (Table 5.6).

Table 5.6
Effects of cherry cultivars and rootstocks on concentrations of leaf metabolites measured on July 9, 2003. Means (n = 40 and n = 24 for cultivars and rootstocks, respectively) followed by the same letter are not significantly different at $P < 0.05$ (Duncan's test).

Source of variation	TP (mg g^{-1} DM)	SS_l (mg g^{-1} DM)	St (mg g^{-1} DM)
Cultivar			
Burlat	88.77 b	87.62	143.16 b
Summit	80.55 ab	75.61	105.44 a
Van	75.65 a	75.67	75.21 a
Rootstock			
Edabriz	66.64 a	93.57	91.23 a
Gisela 5	71.04 a	82.88	84.14 a
Maxma 14	88.79 b	81.74	125.44 ab
CAB 11E	91.12 b	74.77	95.98 a
Prunus avium	90.72 b	65.21	142.89 b

Abbreviations: TP = total phenols; SS_l = total soluble sugars; St = starch; DM = dry mass.

5.4.5. Fruit Quality

The basic characteristics of the cherries varied significantly ($P < 0.001$) with cultivar, rootstock and cultivar–rootstock combinations (Table 5.1). In general, the effect of cultivars accounted for the highest percentage of total variation in the fruit quality parameters (Table 5.1). Fruit mass was highest (> 10.3 g) in ripe cherries from Summit, whereas fruits of the other cultivars had similar masses (always < 7.8 g) (Table 5.7). At harvest, the epidermis of Burlat cherries had low firmness, whereas high firmness values were measured in Van fruits (Table 5.7). However, firmness varied with cultivar–rootstock combination, particularly in Burlat, which had soft fruits when grafted on CAB 11E (1.2 kgf cm^{-2}) and relatively firm fruits when grafted on Gisela 5 and *Prunus avium* (around 1.8 kgf cm^{-2}) (Table 5.7). The concentration of soluble sugars was highest in cherries from Van trees on Edabriz (21.9 °Brix), but was particularly low (< 13.5 °Brix) in Burlat grafted on dwarfing Gisela 5 and Edabriz and on invigorating *Prunus avium* (Table 5.7). Burlat cherries contained lower

titratable acidity (< 73 meq l^{-1}) than Van cherries (> 97 meq l^{-1}) and Summit fruits, which had intermediate acidity (Table 5.7). Rootstock had little effect on the titratable acidity and pH of the fruits. Among cultivar–rootstock combinations, fruits of Burlat and Summit grafted on invigorating rootstocks had the highest soluble sugar concentrations. Among Van fruits, the highest soluble sugar concentrations were found in Van grafted on dwarfing rootstocks (Table 5.7).

Table 5.7
Effects of cherry cultivars and rootstocks on quality indices of cherries measured at harvest in 2003. Means (n = 20) followed by the same letter are not significantly different at $P < 0.05$ (Duncan's test).

Graft combinations		W (g)	F (kgf cm^{-2})	SS_f (°Brix)	pH	TA (meq l^{-1})
Cultivar	Rootstock					
Burlat	Edabriz	6.33 a	1.48 b	13.38 a	4.16 fg	62.53 a
	Gisela 5	7.79 b	1.77 de	13.03 a	4.36 i	70.07 b
	Maxma 14	7.56 b	1.64 bcd	15.47 b	4.14 ef	72.57 b
	CAB 11E	7.31 b	1.18 a	15.36 b	4.30 h	70.37 b
	Prunus avium	7.60 b	1.74 de	13.47 a	4.20 g	67.43 ab
Summit	Edabriz	10.70 cd	2.40 f	13.87 a	4.11 de	82.73 c
	Gisela 5	10.32 c	1.81 de	15.59 b	4.14 ef	85.93 c
	Maxma 14	11.12 de	1.54 bc	18.05 d	4.01 b	92.10 d
	CAB 11E	11.57 ef	1.67 bcd	16.70 c	4.04 b	104.67 f
	Prunus avium	11.87 f	1.91 e	15.71 b	4.05 bc	93.00 d
Van	Edabriz	7.59 b	2.46 f	21.90 f	4.08 cd	102.37 ef
	Gisela 5	7.60 b	2.38 f	20.08 e	4.03 b	127.70 h
	Maxma 14	6.27 a	1.83 de	17.37 cd	3.94 a	97.70 de
	CAB 11E	6.42 a	1.76 de	15.24 b	4.12 def	97.433 de
	Prunus avium	7.48 b	1.70 cd	17.73 d	3.93 a	112.067 g

Abbreviations: W = mass; F = firmness; SS_f = total soluble sugars; TA = titratable acidity.

Fruit chromatic characteristics are shown in Table 5.8. There were significant differences in luminosity, chroma and hue angle ($P < 0.001$) among cultivars, rootstocks and cultivar–rootstock combinations (Table 5.1). At harvest, Van cherries always had lower luminosity, chroma and hue angle, representing redder and darker cherries than Summit and Burlat cherries (Table 5.8). Cherries from Burlat and Summit grafted on dwarfing Edabriz and Gisela 5 generally had higher luminosity than cherries from trees on invigorating rootstocks.

Table 5.8
Effects of cherry cultivars and rootstocks on chromatic parameters (L^* = luminosity; C^*= chroma and H^* = hue angle) of cherries measured at harvest in 2003. Means (n = 60) followed by the same letter are not significantly different at $P < 0.05$ (Duncan's test).

Graft combinations		L^*	C^*	H^*
Cultivar	Rootstock			
Burlat	Edabriz	31.82 e	32.68 g	19.65 d
	Gisela 5	36.46 h	40.15 i	24.84 g
	Maxma 14	30.49 d	29.17 ef	17.45 bc
	CAB 11E	29.84 bcd	28.19 e	18.07 c
	Prunus avium	30.14 cd	28.64 ef	18.01 c
Summit	Edabriz	34.60 g	35.68 h	22.20 e
	Gisela 5	36.12 h	38.77 i	23.47 f
	Maxma 14	29.33 abc	25.51 cd	16.78 bc
	CAB 11E	30.41 d	30.43 f	17.92 c
	Prunus avium	33.11 f	33.51 g	20.41 d
Van	Edabriz	28.74 a	21.06 a	14.83 a
	Gisela 5	30.33 d	26.12 d	16.48 b
	Maxma 14	29.04 ab	22.48 ab	13.73 a
	CAB 11E	30.65 d	27.25 de	16.44 b
	Prunus avium	29.36 abc	23.94 bc	14.64 a

5.4.6. Relationship Between Principal Physiological, Biochemical and Quality Parameters

A principal component analysis (orthotran/varimax) between physiological parameters (Ψ_{MD}, A, g_s, C_i, F_v/F_m, LC_t and A/g_s), photosynthetic pigments (total cholorophyll and total carotenoids), leaf metabolites (starch and total phenolics), LMA and fruit quality parameters (mass, firmness, titratable acidity, pH, soluble sugars, luminosity, chroma and hue angle) (Figure 5.2) measured in each cultivar–rootstock combination during the 2003 season was used to extract two main factors. The chromatic characteristics (luminosity, chroma and hue angle), pH and LMA, seemed to correlate well with factor 1, which accounted for about 27% of the overall variance, and were more dependent on cultivar genotype. The fruit variables mass, soluble sugars and titratable acidity were also more dependent on factor 1. Several physiological variables (A, g_s, C_i and Ψ_{MD}) of sweet cherry trees were strongly correlated with factor 2, which accounted for about 23% of the overall variance. These four physiological variables seemed to be associated with the vigor of the tree and were mainly influenced by rootstock genotype, being consistently higher in trees grafted on invigorating rootstocks than in trees grafted on dwarfing rootstocks (Figure 5.1, Table 5.2). Intrinsic water-use efficiency

was positioned on the opposite side to the A, g_s, C_i and Ψ_{MD} grouping. Trees on dwarfing Edabriz and Gisela 5 had the highest A/g_s values (Figure 5.1). Foliar concentrations of starch and total phenolics and LC_t correlated well with factor 2, and were highly dependent on rootstock genotype. The variables total chlorophyll, total carotenoids and firmness varied with both main factors. The F_v/F_m ratio varied almost independently of cultivar and rootstock genotype (Figure 5.2).

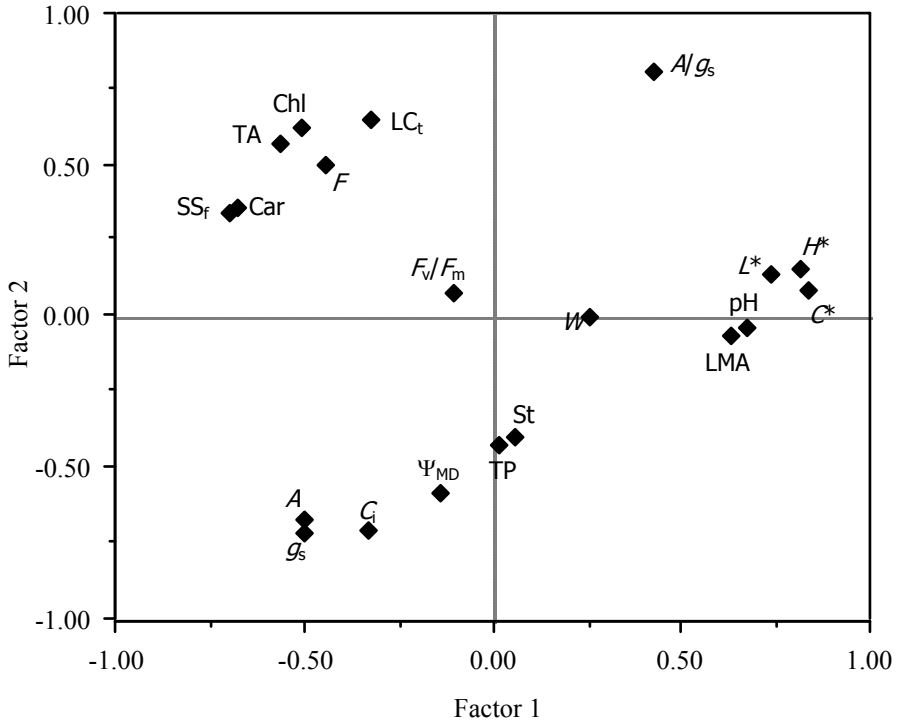

Figure 5.2
Principal Component Analysis (PCA) of 20 variables, comprising physiological parameters (Ψ_{MD} = midday stem water potential; A = net CO_2 assimilation rate; g_s = stomatal conductance; C_i = intercellular CO_2 concentration; A/g_s = intrinsic water-use efficiency; F_v/F_m = maximum photochemical efficiency of PSII in dark–adapted leaves; LC_t = canopy light transmittance), biochemical parameters of the leaves (Chl = total chlorophyll; Car = total carotenoids; TP = total phenols; St = starch), LMA = leaf mass per unit area and fruit quality parameters (W = mass; SS_f = total soluble sugars; TA = titratable acidity; pH; F = firmness; L^* = lightness; C^* = chroma; H^* = hue angle) in three sweet cherry cultivars grafted onto five roostocks and measured in the 2003 growing season.

5.5. Discussion

5.5.1. Effect of Size–controlling Rootstocks in the Physiology of Grafted Trees

Rootstock genotype significantly affected Ψ_{MD} of cherry cultivars but not Ψ_{PD} (Table 5.1), perhaps indicating complete overnight rehydration in all scion–rootstock combinations. Although the lowest LC_t was recorded in trees grafted on invigorating *Prunus avium* (Table 5.4) as a result of the dense canopy, these plants had the highest Ψ_{MD}. Trees on the most size–controlling rootstocks, Edabriz and Gisela 5, consistently had lower Ψ_{MD} (Table 5.2) than

trees on invigorating rootstocks, consistent with previous findings for cherry (Schmitt *et al.*, 1989; Gonçalves *et al.*, 2003), peach (Bongi *et al.*, 1994; Basile *et al.*, 2003) and apple (Giulivo and Bergamini, 1982). This phenomenon is likely related to low water absorption capability of the root system of dwarfed trees compared with the transpiration demand of the canopy, or the high hydraulic resistance of the root system or graft union. Olien and Lakso (1986) favored the hydraulic limitation hypothesis (Ryan and Yoder, 1997) to explain the lower Ψ_{MD} of apple trees on dwarfing rootstocks compared with trees on invigorating rootstocks. In apple orchards, Cohen and Naor (2002) reported that differences in hydraulic conductivity resulted in differences in water use. Another explanation for the lower Ψ_{MD} in cherry cultivars grafted on dwarfing rootstocks compared to the invigorating *Prunus avium* may be associated with differences in effective rooting depth among these rootstocks, and hence, water supply. This is consistent with the commonly held view that vegetatively propagated rootstocks establish a more spreading, shallower root system than seed propagated rootstocks (Shackel *et al.*, 1997). Some recent studies have identified a partial xylem discontinuity in the scion–rootstock grafting point of dwarfed trees that could be responsible for a reduced water supply to plant organs (Sekse, 1998).

At high Ψ_{MD}, leaves of cherry trees grafted on *Prunus avium*, CAB 11E and Maxma 14 exhibited consistently higher values of A and g_s than leaves of cherry trees grafted on dwarfing Edabriz or Gisela 5, but the cultivars on the dwarfing rootstocks had higher A/g_s (Figure 5.1, Table 5.2), which is in accordance with previous reports for cherry (Shackel *et al.*, 1997) and grapevine (Patakas *et al.*, 2003). In our study, Ψ_{MD} was positively correlated with A (r = 0.405, P < 0.01), g_s (r = 0.473, P < 0.001) and C_i (r = 0.504, P < 0.001) (Table 5.9) and negatively correlated with A/g_s (r = –0.442, P < 0.001) (Table 5.9). Shackel *et al.* (1997) also showed a positive correlation between Ψ_{MD} and A in cherry, and Naor (1998) and Naor *et al.* (1995) reported high correlations between Ψ_{MD}, g_s and productivity parameters in apple and other deciduous orchard species. As expected, A correlated positively with g_s (r = 0.821, P < 0.001) (Table 5.9), and the data best fitted a hyperbolic function (r^2 = 0.62), indicating that the linear relationship between the parameters varied with size–controlling rootstock used (Figure 5.3). The slopes of the first–order linear regression between A and g_s and the respective coefficients of determination increased from invigorating to dwarfing rootstocks, indicating that, for a given variation in g_s, the trees grafted on dwarfing Edabriz and Gisela 5 exhibited greater variation in A than trees on invigorating *Prunus avium*, CAB 11E and Maxma 14. This relationship can be used to assess the effect of size–controlling rootstocks on gas exchange of sweet cherry trees under comparable environmental conditions.

Table 5.9
Correlations (linear correlation coeficients) between physiological parameters and fruit quality characteristics of cherry cultivars Burlat, Summit and Van grafted on rootstocks Edabriz, Gisela 5, Maxma 14, CAB 11E and *Prunus avium*.

	Ψ_{MD}	A	g_s	C_i	A/g_s
A	0.405**				
g_s	0.473***	0.821***			
C_i	0.504***	0.481***	0.692***		
A/g_s	–0.442***	–0.777***	–0.851***	–0.859***	
W	0.403**	–0.152	–0.100	0.021	0.057
F	–0.261*	–0.296*	0.184	0.202	0.317*
L^*	0.008	–0.503***	–0.386**	–0.213	0.373**
C^*	0.019	–0.462***	–0.364**	–0.206	0.334**
SS_f	–0.026	0.087	0.073	0.011	0.012
TA	0.042	–0.003	0.053	0.047	–0.015

Statistical significance: * = $P < 0.05$; ** = $P < 0.01$; and *** = $P < 0.001$ by Fisher's test. Physiological parameters: Ψ_{MD} = midday stem water potential; A = net CO_2 assimilation rate; g_s = stomatal conductance; C_i = intercellular CO_2 concentration; A/g_s = intrinsic water-use efficiency. Fruit quality parameters: W = mass; F = firmness; L^* = luminosity; C^* = chroma; SS_f = total soluble sugars; and TA = titratable acidity.

Values of C_i, like Ψ_{MD}, were lower in trees grafted on the dwarfing rootstocks than in trees grafted on invigorating rootstocks. Low C_i, in association with high A/g_s values (Figure 5.1), indicates a greater effect of stomatal factors than non–stomatal factors on photosynthetic capacity (Flexas and Medrano, 2002). The high A/g_s values in association with low C_i and low Ψ_{MD} in trees grafted on dwarfing rootstocks suggests the involvement of other mechanisms such as root–shoot hormonal signals, namely abscisic acid (ABA) synthesis, in the stomatal regulation mechanism in these trees. Although this finding is consistent with the well–documented phenomenon of non–hydraulic signalling (Zhang *et al.*, 1997), it cannot be confirmed because we did not measure ABA concentration. Nevertheless, non–hydraulic signalling related to root–sourced ABA has been reported previously for *Prunus* (Fubeder *et al.*, 1992; Correia *et al.*, 1997).

The lower A in cultivars on dwarfing rootstocks was associated with slight reductions in F_v/F_m, F_0, F_m and F_v (Figure 5.1, Table 5.3) compared with cultivars on invigorating rootstocks, indicating that down–regulation of PSII efficiency was associated with a protective increase in non–radiative dissipation of light energy (Oberhuber and Bauer, 1991). Moreover, among the rootstocks, trees on dwarfing rootstocks had the highest concentrations of total carotenoids (Table 5.5), probably including xanthophyll cycle

pigments, which have a photoprotective role in thermal energy dissipation (Demmig-Adams, 1990; Ball *et al.*, 1994).

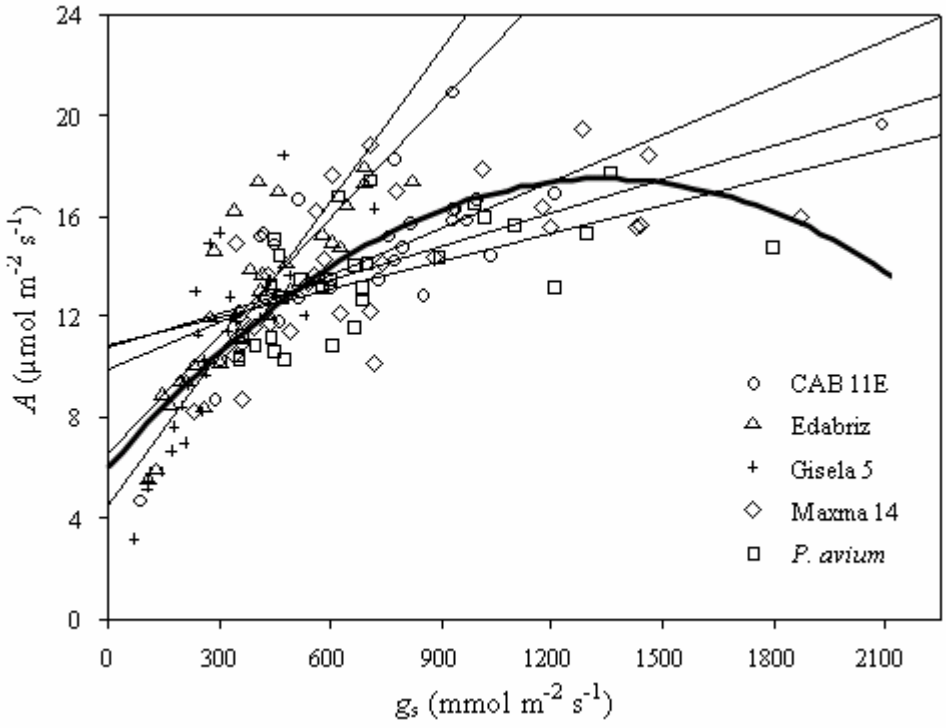

Figure 5.3
Relationship between photosynthesis (A) and stomatal conductance (g_s) in CAB 11E (y = 9.933 + 0.006 x, r^2 = 0.53, P < 0.001), Edabriz (y = 6.542 + 0.016 x, r^2 = 0.71, P < 0.001), Gisela 5 (y = 4.527 + 0.020 x, r^2 = 0.67, P < 0.001), Maxma 14 (y = 10.786 + 0.004 x, r^2 = 0.40, P < 0.001), *Prunus avium* (y = 10.888 + 0.004 x, r^2 = 0.35, P < 0.001) and all data (y = 6.213 + 0.017 x – 6.376E^{-6} x^2, r^2 = 0.62, P < 0.001). Measurements were taken throughout the diurnal period on July 9, 2003.

Concentrations of total chlorophyll, $Chl_{a/b}$, total carotenoids and total soluble sugars were highest in cherry trees grafted on dwarfing rootstocks, whereas starch and total phenolic concentrations were highest in trees on invigorating rootstocks (Tables 5.5 and 5.6). Despite having the highest concentrations of total chlorophyll, trees on dwarfing Edabriz and Gisela 5 presented the lowest A, which supports our assertion that stomatal control is the main cause of the variation in photosynthetic rates among the rootstocks.

5.5.2. Effect of the Physiology of the Scion–Rootstock Interaction on Fruits Physicochemical Characteristics

The physicochemical characteristics measured in cherries from the three cultivars suggest that regulation of fruit quality is more dependent on the cultivar than on the rootstock (expressed as the percentage of total variation). However, the effect of the rootstock was statistically significant (Table 5.1). Several studies have shown that the

rootstock affects fruit quality in other species. For example, in grapevine, the rootstock affected growth, yield and juice qualitative components (Paranychianakis *et al*., 2004). In sweet cherry trees, Moreno *et al.* (1996) reported that the invigorating rootstock Adara profoundly affected the fruiting response of Van compared with the less invigorating rootstocks studied, SL 64 and *Prunus mahaleb*. In our study, Summit, which had high values of Ψ_{MD} and *A*, had the highest fruit mass, particularly when grafted on the invigorating rootstocks Maxma 14, CAB 11E and *Prunus avium* (Table 5.7). Furthermore, Ψ_{MD} correlated positively with fruit mass (r = 0.403, P < 0.01), which means that heavier fruits were found in trees with better water status (Table 5.9). Stem water potential was also closely related to fruit growth in apple (Naor *et al*., 1995) and in pear (Shackel *et al*., 1997). In apple, in addition to fruit size, other important fruit quality factors were related to Ψ_{stem}; for example, fruit soluble solids content and yellow color increased linearly with decreasing Ψ_{MD} (Shackel *et al*., 1997).

The effect of size–controlling rootstocks on fruit firmness and soluble sugar concentration did not follow a specific trend among the three cultivars (Table 5.7). We observed a distinct effect of dwarfing and invigorating rootstocks only in cherries from Van. Van cherries had the highest firmness and soluble sugar concentrations when grafted on dwarfing rootstocks, which implies a high resistance to postharvest damage and high consumer acceptability. As expected, soluble sugar concentration, titratable acidity and skin color were low in Burlat (Tables 5.7 and 5.8), which is well known as an early–maturing cultivar with low sugar content and total acidity, and less red fruits. However, the quality of the fruits from Burlat was enhanced when Burlat was grafted on Maxma 14 or CAB 11E (Tables 5.7 and 5.8).

In our study, total soluble sugar concentration in leaves did not correlate with soluble sugar in fruits (data not shown). The early fruiting habit in cherry suggests that a substantial portion of the carbohydrates necessary for early crop growth come from stored reserves in roots and stems; however, the importance of reserve carbohydrates could depend on the degree to which sweet cherry leaves have the capacity to photosynthesize under marginal environmental conditions during early spring (Roper and Kennedy, 1986). Sweet cherry has a low light saturation point (Atkinson *et al*., 1997), which could be advantageous because CO_2 assimilation could occur on cloudy days.

We found that *A* was negatively correlated with luminosity (r = –0.503, P < 0.001) and chroma (r = –0.462, P < 0.001) (Table 5.9). These findings indicate that the trees on invigorating Maxma 14, CAB 11E and *Prunus avium* that have the highest *A* also produced darker and redder fruits than trees on dwarfing Edabriz and Gisela 5.

We conclude that, when climatic transpirational demand was high, the low ability of the hydraulic system of the dwarfing rootstocks to supply water to the leaves led to a decrease in plant water potential and low g_s. These responses control plant water deficits and maintain water potential in a range that does not endanger the hydraulic system (Tyree and Sperry, 1989). The reduction in water potential reduced photosynthetic carbon uptake and growth potential, which may be the mechanism underlying dwarfing of cherry trees by rootstocks, as found for apple (Higgs and Jones, 1990; Sperry, 2000). Limited carbon uptake, however, does not necessarily reduce fruit yield, because we observed that reduced photosynthetic productivity on dwarfing rootstocks resulted in good yields and reduced vegetative growth (Cohen and Naor, 2002).

Our results fit well with the two hypotheses presented in the Introduction. We conclude that physiological responses of sweet cherry trees are influenced by the rootstock, because trees on different size–controlling rootstocks had different water relations, gas exchange and vegetative growth. In addition, we observed that invigorating rootstocks led to improved water status and high photosynthetic capacity. However, the different physicochemical characteristics of cherries from the three cultivars suggest that regulation of fruit quality is more dependent on the cultivar, although the effects of rootstock genotype and cultivar × rootstock interaction were also statistically significant. We conclude that the scion–rootstock combination is an important parameter to consider in orchard planting strategies.

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5.6. References

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CAPÍTULO 6

EFFECT OF RIPENESS AND POSTHARVEST STORAGE ON THE PHENOLIC PROFILES OF CHERRIES (*Prunus avium* L.)

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6. EFFECT OF RIPENESS AND POSTHARVEST STORAGE ON THE PHENOLIC PROFILES OF CHERRIES (*Prunus avium L.*)

6.1. Abstract

The phenolic compounds hydroxycinnamates, anthocyanins, flavonols, and flavan-3-ols of sweet cherry cultivars Burlat, Saco, Summit, and Van harvested in 2001 and 2002 were quantified by HPLC-DAD. Phenolics were analyzed at partially ripe and ripe stages and during storage at 15 ± 5 °C (room temperature) and 1–2 °C (cool temperature). Neochlorogenic and *p*-coumaroylquinic acids were the main hydroxycinnamic acid derivatives, but chlorogenic acid was also identified in all cultivars. The 3-glucoside and 3-rutinoside of cyanidin were the major anthocyanins. Peonidin and pelargonidin 3-rutinosides were the minor anthocyanins, and peonidin-3-glucoside was also present in cvs. Burlat and Van. Epicatechin was the main monomeric flavan-3-ol with catechin present in smaller amounts in all cultivars. The flavonol rutin was also detected. Cultivar Saco contained the highest amounts of phenolics [227 mg 100 g^{-1} of fresh weight (fw)] and cv. Van the lowest (124 mg 100 g^{-1} of fw). Phenolic acid contents generally decreased with storage at 1–2 °C and increased with storage at 15 ± 5 °C. Anthocyanin levels increased at both storage temperatures. In cv. Van the anthocyanins increased up to 5-fold during storage at 15 ± 5 °C (from 47 to 230 mg 100 g^{-1} of fw). Flavonol and flavan-3-ol contents remained quite constant. For all cultivars the levels of phenolic acids were higher in 2001 and the anthocyanin levels were higher in 2002, which suggest a significant influence of climatic conditions on these compounds.

Key words: cherry, *Prunus avium*, ripeness, storage, HPLC–DAD, phenolics; anthocyanins; flavan-3-ols; flavonols; hydroxycinnamates.

6.2. Introduction

High fruit and vegetable consumption is associated with a reduced risk of major chronic diseases such as cancer, atherosclerosis and cardiovascular disease (Doll, 1990; Hertog *et al.*, 1993; Kinsella *et al.*, 1993; Hollman, 2001). Phenolic compounds have been widely identified in fruits and vegetables and partly related to those protective effects (Knekt *et al.*, 1996; Rim *et al.*, 1996; Santos-Buelga and Scalbert, 2000).

The phenolic composition of plant foods is dependent on plant genetic information (Harborne and Turner, 1984) and environmental factors during growth and postharvest.

Cherries are considered a major source of phenolic compounds, which are also responsible for their color and taste, and presumably also their antioxidant properties. Cherry phenols include flavonoids such as anthocyanins, flavan-3-ols, and flavonols in addition to the nonflavonoid compounds, hydroxycinnamic acids and hydroxybenzoic acids (Macheix *et al.*, 1990; Gao and Mazza, 1995).

Fruit tissues are able to biosynthesize phenolic compounds and changes in this content can be induced by biotic and abiotic stress conditions (Harborne and Turner, 1984; Kataoka *et al.*, 1996). Water availability and soil composition (mineral and organic nutrients) have a marked effect on the phenolic content of plants (McClure, 1975) and on the ability of plant products to suffer browning and other phenolic-related physiological disorders that appear during the maturity stage and postharvest (Tomás-Barberán and Espín, 2001). Storage at low temperatures might have positive or negative effects on phenolics and in turn on fruit quality, depending on the commodity and the storage temperature (Tomás-Barberán, 2001; Tomás-Barberán and Espín, 2001).

Cherries are nonclimacteric fruits that are usually picked at peak maturity for optimal taste and appearance. However, in Portugal, and in other countries that produce sweet cherries for fresh consumption, the cherries are often stored for up to 3–4 weeks at cold temperatures to increase the seasonal supply. To our knowledge, information about the changes in phenolic content of cherries during maturity and postharvest storage is limited; however, this information is relevant to the understanding of the parameters that affect fruit color and stability and the potential health protective effects of the phenolics. The aims of this study were to (1) identify and quantify individual phenolic compounds in sweet cherries at partially ripe and ripe stages, (2) evaluate the effects of postharvest storage on the phenolic composition of sweet cherries, and (3) assess the variations caused by natural harvest fluctuations in different harvest years.

6.3. Materials and Methods

6.3.1. Sample Preparation

Sweet cherries from the cultivars, Burlat, Saco, Summit and Van grown in Vila Real, Portugal, were randomly harvested by hand in 2001 and 2002, at two different stages of ripeness: partially ripe and ripe. Skin color is the main criterion used for indicating maturity for cherry picking. For each cultivar the maturity was assessed for 20 fruits by the following indices: weight, skin color (by a Minolta colorimeter), soluble solids content (°Brix by a refractometer), titratable acidity (by an automatic titration system), and pH (by a pH-meter). The ranges of these indices for each cultivar are shown in Table 6.1.

Table 6.1
Quality indices of cherries at two ripeness stages (partially ripe and ripe). Means ± SD (n = 20) for the year 2001 and 2002, followed by the same letter are not significantly different at $P < 0.05$ (Duncan's test).

cultivar	stage	weight (g)	skin color *a** value	soluble solids (ºBrix)	titratable acidity (meq L^{-1})	pH
			year 2001			
Burlat	partially ripe	6.2 ± 0.6 d	36.4 ± 6.8 bc	10.4 ± 0.1 a	79.3 ± 7.6 b	3.70 ± 0.02 b
	ripe	7.4 ± 0.9 e	36.2 ± 5.9 bc	13.4 ± 0.1 c	70.3 ± 13.9 ab	3.74 ± 0.01 c
Saco	partially ripe	4.3 ± 0.4 a	42.0 ± 2.1 bc	14.7 ± 0.2 d	89.3 ± 1.2 c	4.21 ± 0.02 f
	ripe	5.0 ± 0.6 b	35.8 ± 10.3 e	15.1 ± 0.1 e	68.7 ± 1.2 a	4.27 ± 0.01 g
Summit	partially ripe	6.6 ± 0.8 d	38.5 ± 3.0 ab	12.5 ± 0.0 b	112.3 ± 0.6 e	3.82 ± 0.02 d
	ripe	9.0 ± 1.2 f	34.2 ± 10.2 cd	18.6 ± 0.1 g	99.3 ± 1.2 d	4.16 ± 0.00 e
Van	partially ripe	5.6 ± 0.6 c	40.9 ± 2.3 a	15.2 ± 0.2 e	133.7 ± 1.5 f	3.64 ± 0.01 a
	ripe	7.4 ± 0.7 e	32.8 ± 11.3 de	16.5 ± 0.1 f	143.3 ± 2.1 f	3.66 ± 0.01 a
			year 2002			
Burlat	partially ripe	4.2 ± 0.6 a	43.2 ± 4.8 f	11.9 ± 1.4 a	58.7 ± 1.6 b	3.92 ± 0.01 d
	ripe	7.2 ± 0.8 c	18.5 ± 6.5 a	16.3 ± 2.1 cd	67.2 ± 1.7 c	3.91 ± 0.04 cd
Saco	partially ripe	4.3 ± 0.8 a	36.3 ± 7.8 e	15.8 ± 1.1 c	76.2 ± 0.6 d	3.95 ± 0.03 d
	ripe	5.2 ± 0.8 b	26.6 ± 6.6 c	17.6 ± 0.9 e	81.4 ± 1.8 ef	3.84 ± 0.02 ab
Summit	partially ripe	5.3 ± 0.7 b	41.0 ± 4.7 f	13.6 ± 1.4 b	74.3 ± 2.3 d	3.87 ± 0.02 bc
	ripe	7.0 ± 0.8 c	33.5 ± 4.6 d	16.7 ± 2.1 cde	79.9 ± 0.3 e	3.87 ± 0.02 bc
Van	partially ripe	5.5 ± 0.6 b	23.4 ± 12.9 b	17.3 ± 1.3 de	48.7 ± 2.2 a	4.16 ± 0.04 e
	ripe	7.0 ± 1.2 c	21.5 ± 5.4 b	19.2 ± 2.0 f	83.6 ± 1.4 f	3.80 ± 0.02 a

The phenolic analyses were made on days 0, 5, 10, 15, 20, 25 and 30 in the fruits under storage at cold treatment [1–2 ºC and 90% relative humidity (RH)] and on days 0, 3 and 6 for fruits subjected to room temperature treatment (15 ± 5 ºC). Cherries were cut in half (the stone was taken off), and the cherry halves were frozen in liquid nitrogen, crushed, and freeze-dried prior to analysis.

6.3.2. Extraction of Phenolic Compounds

Pitted and freeze-dried cherry samples (0.5 g) were mixed in 5 mL of 60% MeOH, flushed with N_2, and extracted during shaking using a thermostated (25 ± 1 °C) water bath, at 200 rpm for 10 min. The suspension was initially filtered under vacuum through one layer of Whatman no. 1 filter paper. The extract was then filtered through a 0.45 µm hydrophilic Durapore filter (Millipore Corp., Bedford, MA), flushed with N_2, and the filtrate was injected into the HPLC, after a period not exceeding 24 h, for the separation and quantification of phenolic compounds. The cherry samples were submitted to a second and a third extraction and analyzed by HPLC, and the total content was determined as the sum of the three values.

6.3.3. HPLC–DAD Analyses

Samples of 10 µL of extracts were analyzed using an HPLC system equipped with a diode array detector (DAD) (Hewlett-Packard 1100 system, Waldbronn, Germany) operated by HP ChemStation software with a Nova-Pak C18 column (3.9 x 150 mm, Waters) at 40 °C. The mobile phase was made of three solvents delivered in a gradient system at a flow rate of 0.5 mL min^{-1} essentially as described by Lamuela-Raventós and Waterhouse (1994).

6.3.4. Identification and Quantification of Phenolic Compounds

The phenolic compounds in cherry extracts were identified by their spectral and retention time characteristics, recorded with a diode array detector, and, wherever possible, by spectral chromatographic comparisons with authentic markers (Markham, 1982).

The quantities of the different phenolic compounds were assessed from peak areas and calculated as equivalents of seven representative standard compounds (from standard, linear regression curves of authentic standards) as follows: at 280 nm (flavan-3-ols), catechin and epicatechin, respectively; at 316 nm (hydroxycinnamates), neochlorogenic acid, chlorogenic acid, and other hydroxycinnamaic acids as chlorogenic acid equivalents and *p*-coumaroylquinic acid as *p*-coumaric acid equivalents; at 365 nm (flavonols), quercetin glucosides as rutin equivalents; at 520 nm (anthocyanins), cyanidin-3-glucoside and cyanidin-3-rutinoside, respectively, and other anthocyanins as cyanidin-3-rutinoside. Coefficients of variation on the HPLC quantifications were < 5%. Concentrations were expressed as milligrams per 100 g of fresh weight (fw).

6.3.5. Determination of Total Phenolics

The concentration of total phenolics in cherry extracts was determined on the same extract used for HPLC analysis, according to the Folin–Ciocalteu's procedure (Singleton and Rossi, 1965) and was expressed as mg L^{-1} gallic acid equivalents (GAE), and then transformed into mg gallic acid 100 g^{-1} fw.

6.3.6. Statistics

The analyses of data were performed as analysis of variance using the Super ANOVA software (1.11 Abacus Concepts Inc., 1991). Significances of differences were established from a Duncan's test ($P < 0.05$).

6.4. Results

6.4.1. Phenolic Content

The total phenolic content obtained by using the Folin–Ciocalteu procedure of the different sweet cherry cultivars is shown in Table 6.2. The analysis of variance revealed significant differences ($P < 0.001$) in the total content of phenolic compounds among the four cultivars. The levels of total phenols in cv. Saco were consistently higher than the levels in the other cultivars, which were at the relatively same levels (Table 6.2). However, levels depended on ripeness stage, harvest year, and storage conditions ($P < 0.001$). Higher values of total phenolics were always observed in ripe cherries; for instance, 264 mg 100 g^{-1} of fw was observed in cv. Saco *versus* cv. Van in the partially ripe stage, which contained only 69 mg 100 g^{-1} of fw (Table 6.2), independent of the years. Between years, levels in 2001 were always greater than those of 2002 ($P < 0.001$), except for cv. Burlat cherries after storage at 15 ± 5 °C.

Table 6.2
Total phenolic content of cherry cultivars determined according to Folin–Ciocalteu's method at two ripeness stages (partially ripe and ripe), after storage at room (15 ± 5 °C) and cool temperature (1–2 °C). Means ± SD ($n = 3$) of each cultivar and year followed by the same letter are not significantly different at $P < 0.05$ (Duncan's test).

cultivar /stage	storage conditions	total phenolics mg 100 g^{-1} fw	
		year 2001	year 2002
Burlat			
partially ripe	day 0	108 ± 2.2 a	91.6 ± 1.0 a
partially ripe	1–2 ºC; day 30	135 ± 2.5 c	92.5 ± 0.2 a
ripe	day 0	141 ± 2.0 d	119 ± 0.7 b
ripe	15 ± 5 ºC; day 6	124 ± 1.8 b	144 ± 1.1 c
ripe	1–2 ºC; day 30	167 ± 1.3 e	118 ± 1.7 b
Saco			
partially ripe	day 0	169 ± 0.8 a	123 ± 1.2 a
partially ripe	1–2 ºC; day 30	---[a]	123 ± 7.6 a
ripe	day 0	264 ± 0.9 c	171 ± 10.6 b
ripe	15 ± 5 ºC; day 6	278 ± 2.0 d	223 ± 16.7 c
ripe	1–2 ºC; day 30	222 ± 3.9 b	129 ± 1.3 a
Summit			
partially ripe	day 0	120 ± 1.6 b	74.7 ± 2.2 a
partially ripe	1–2 ºC; day 30	132 ± 1.0 c	84.5 ± 4.4 a
ripe	day 0	159 ± 2.1 d	92.7 ± 9.4 ab
ripe	15 ± 5 ºC; day 6	160 ± 0.6 d	110 ± 14.6 b
ripe	1–2 ºC; day 30	104 ± 2.8 a	88.2 ± 0.6 a
Van			
partially ripe	day 0	115 ± 3.4 a	69.0 ± 0.5 a
partially ripe	1–2 ºC; day 30	158 ± 3.0 c	111 ± 0.4 c
ripe	day 0	144 ± 2.3 b	94 ± 2.4 b
ripe	15 ± 5 ºC; day 6	209 ± 3.8 d	187 ± 1.4 e
ripe	1–2 ºC; day 30	152 ± 7.0 bc	134 ± 0.3 d

[a]Data not available

Regarding the influence of storage on total phenolic contents, it was observed that in partially ripe cherries levels increased with storage at low temperature. During cool storage, the total phenolic levels in the ripe cv. Saco decreased slightly (both years $P < 0.001$), and in 2001 they also decreased in the cv. Summit ($P < 0.001$). The phenolic levels of the other cultivars showed only small variations during cool storage, although the contents in cv. Van apparently increased during the cool storage, especially for the 2002 harvest ($P < 0.001$; Figure 6.1). At 15 ± 5 °C, a slight increase in phenolics was noted in cvs. Van and Saco in the two years of study ($P < 0.001$ in all cases). In contrast, cv. Burlat showed a slight but significant decrease in the total phenols between days 3 and 6 at ambient storage in 2001. The others did not differ between the sampling dates for the two years (Figure 6.1).

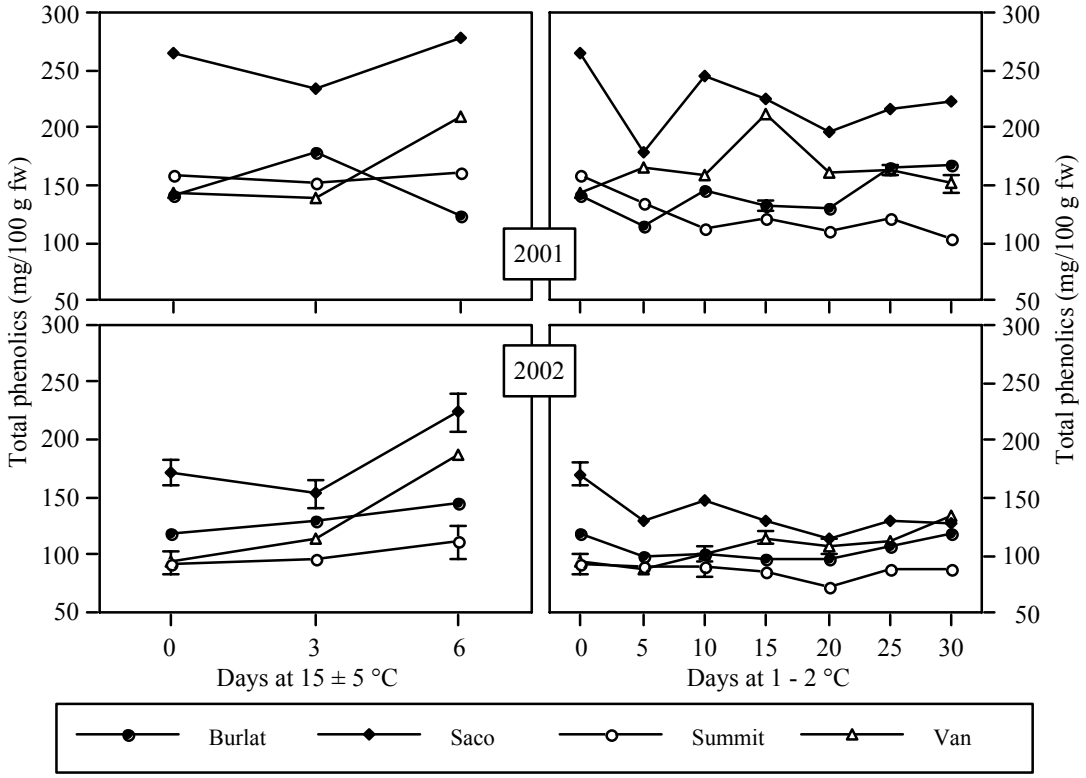

Figure 6.1
Total phenolics (by Folin–Ciocalteu's procedure) of the four sweet cherry cultivars in mg 100 g^{-1} of fresh weight during storage at room temperature (15 ± 5 °C) and cool temperature (1–2 °C) in 2001 and 2002. Values are the mean ± SD (n = 3). SD bars are not shown if they are smaller than the symbols.

The data presented here as well as those in the literature suggest that cultivar, ripeness stage, harvest year, and storage conditions have a major influence on the quantitative individual phenolic composition of cherries. It is important to note, however,

that during short-term storage at ambient temperature, the phenolics increase significantly in certain cultivars, whereas cold storage can induce small decreases in the phenols of certain cherry cultivars.

6.4.2. HPLC–DAD Analysis of Cherry Phenolics

The methanol extracts of cherries were analyzed by HPLC–DAD, and example chromatograms recorded at 280 nm are shown in Figure 6.2. Generally, the same phenolic compounds were present in all cultivars, but the relative levels varied among the four cultivars. Four groups of phenolic compounds were identified: the hydroxycinnamic acids, anthocyanins, flavan-3-ols, and flavonols (Figure 6.2). The chromatograms showed three peaks (Figure 6.2) with UV spectra characteristic of caffeic acid derivatives. In cvs. Saco and Van, the main hydroxycinnamic acid was identified as neochlorogenic acid (3-*O*-caffeoylquinic acid) (Table 6.3, peak 1 in Figure 6.2), followed by *p*-coumaroylquinic acid (Table 6.3, peak 2 in Figure 6.2); note that in the cv. Burlat, the levels of neochlorogenic acid and *p*-coumaroylquinic acid were at approximately the same levels (Table 6.3). The third compound was identified as chlorogenic acid (5-*O*-caffeoylquinic acid) (peak 3, Figure 6.2). Other minor compounds with the characteristic spectra of hydroxycinnamic acid derivatives were detected, but their exact identities could not be ascertained by DAD analysis, which is why they are designated "other hydroxycinnamic acids" in Table 6.3.

The chromatograms revealed that the cherries of the four cultivars contained at least five different anthocyanins (Figure 6.2). They had similar UV-vis spectra with a maximum around 515 nm in the spectra recorded with the diode array detector. The main pigments were identified as cyanidin-3-rutinoside (peak 5), followed by cyanidin-3-glucoside (peak 4). Other minor anthocyanins, peonidin-3-glucoside (peak 6), pelargonidin-3-rutinoside (peak 7), and peonidin-3-rutinoside (peak 8), were also detected. In addition were identified two peaks (Figure 6.2) with UV spectra of flavan-3-ols (maximum at 280 nm). The major compound of this phenolic group was identified as epicatechin (peak 10), followed by catechin (peak 9). One flavonol peak (Figure 6.2) was detected, and the UV spectra suggest it to be rutin (quercetin-3-*O*-rutinoside) (peak 11) glycosylated at the hydroxyl in the 3-position (Mabry *et al.*, 1970).

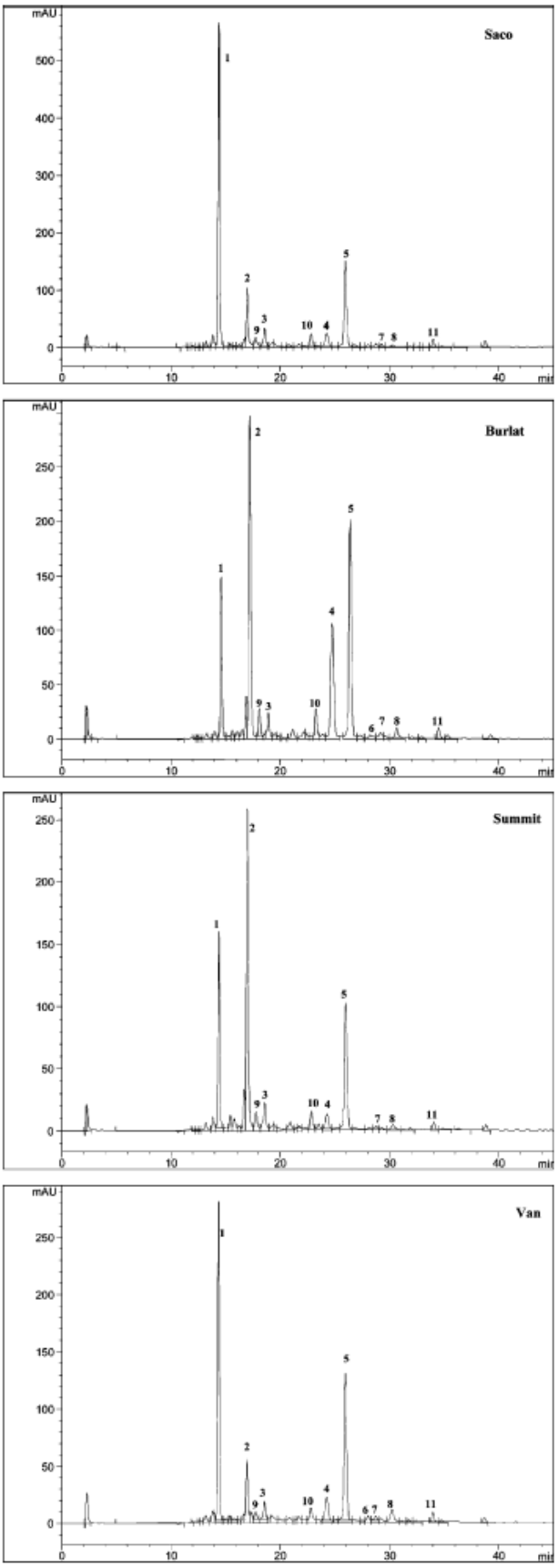

Figure 6.2
HPLC chromatograms of the four sweet cherry cultivar extracts recorded at 280 nm. Hydroxycinnamic acid derivative peaks: (1) neochlorogenic acid; (2) *p*-coumaroylquinic acid; (3) chlorogenic acid. Anthocyanin peaks: (4) cyanidin-3-glucoside; (5) cyanidin-3-rutinoside; (6) peonidin-3-glucoside; (7) pelargonidin-3-rutinoside; (8) peonidin-3-rutinoside. Flavan-3-ols peaks: (9) catechin; (10) epicatechin. Flavonol peak: (11) rutin.

6.4.3. Phenolic Acids Contents

The hydroxycinnamic acids content of the cherry cultivars is shown in Table 6.3. The levels of phenolic acids differed between cultivars, ripeness stage, year, and storage conditions ($P < 0.001$). On average, hydroxycinnamic acids in cv. Saco (156 mg 100 g^{-1} of fw) were higher than in the other cultivars, the lowest being in cv. Burlat (53 mg 100 g^{-1} of fw). However, levels depended on maturity stage, year, and storage conditions ($P < 0.001$). At harvest, cv. Saco partially ripe cherries presented the highest values of phenolic acids (147 mg 100 g^{-1} of fw in 2001 and 140 mg 100 g^{-1} of fw in 2002), and the lowest values were observed in cv. Burlat (51 mg 100 g^{-1} of fw) in 2001 and in cv. Van (55 mg 100 g^{-1} of fw) in 2002 (Table 6.3). When picked at the ripe stage, cv. Saco cherries also had the highest values (222 and 137 mg 100 g^{-1} of fw in 2001 and 2002, respectively), and cv. Burlat showed the lowest values (52 and 53 mg 100 g^{-1} of fw, in 2001 and 2002, respectively) (Table 6.3). The majority of the sampling dates revealed higher levels of hydroxycinnamic acids in 2001. Storage period induced some variations in phenolic acids, although the final tendency was a reduction of these levels in cherries storage at 1–2 °C and an increase in cherries stored at 15 ± 5 °C except for cv. Burlat (Table 6.3).

With regard to the individual hydroxycinnamic acid derivatives, it was noted that neochlorogenic acid was the major compound, varying from 22 to 190 mg 100 g^{-1} of fw in ripe fruits, and represented 19 and 71% of the phenolics, respectively, and from 19 to 126 mg 100 g^{-1} of fw in partially ripe fruits representing 24–72% of the phenolics. The contents of *p*-coumaroylquinic and chlorogenic acids presented similar values during ripeness and storage for the same cultivar (Table 6.3). The *p*-coumaroylquinic acid content varied from 4 to 34 mg 100 g^{-1} of fw and represented 4% of the total phenolics in cherry extract in cv. Van and 27% in cv. Summit, respectively (Table 6.3). The chlorogenic acid content ranged from 3 to 12 mg 100 g^{-1} of fw and represented 2% of the total phenolics in cherry extract cv. Van and 4% in cv. Saco, respectively. Other hydroxycinnamic acids represented < 2% of the total phenolics (Table 6.3).

6.4.4. Anthocyanins Content

The anthocyanin levels differed among the cherry cultivars, ripeness stage, year of study, and storage conditions ($P < 0.001$). Levels of anthocyanins in partially ripe cherries were very low (from 5 to 23 mg 100 g^{-1} of fw) when compared to ripe fruits (19 to 96 mg 100 g^{-1} of fw) (Table 6.4). At harvest, cv. Burlat ripe cherries showed the largest content, totaling 96 mg 100 g^{-1} of fw in 2002, and cv. Van showed the lowest, 20 mg 100 g^{-1} of fw in 2001 (Table 6.4). Higher values of anthocyanins were always determined in the ripe picking

stage for all cultivars. In general, the total anthocyanins increased during storage at room and cool temperature for all of the cherry cultivars, but usually more at room temperature storage. For instance, a huge increase of 5-fold in total anthocyanins from 47 to 230 mg 100 g^{-1} of fw was observed in cv. Van cherries, in 2002, after 6 days of storage at 15 ± 5 °C (Table 6.4; Figure 6.3), whereas in 2001, the increase was 3-fold (Table 6.4).

The major anthocyanin was cyanidin-3-rutinoside, which ranged from 18 to 61 mg 100 g^{-1} of fw and represented 13 and 37% of the total phenolics in cherry extract of cvs. Van and Burlat, respectively. These were the two cultivars having the highest anthocyanin contents at harvest. During storage, these two cherry cultivars also became redder than other cultivars (as evaluated visually). The cv. Burlat also had higher levels of cyanidin-3-glucoside, 15 and 32 mg 100 g^{-1} of fw (13 and 19% of the total phenolics), in 2001 and 2002, respectively. In the other cultivars the values were much lower, representing < 6% in the total phenolics. The glucoside and rutinoside of peonidin and pelargonidin-3-rutinoside represented < 5% of the total phenolics. Peonidin-3-glucoside was identified only in very low amounts in cvs. Burlat and Van (Table 6.4). Climatic conditions in 2002 clearly induced higher anthocyanin levels in both ripe and partially ripe fruits.

6.4.5. Flavan-3-ols and Flavonols Content

The flavan-3-ols, catechin and epicatechin, and the flavonol (rutin) content of the different cultivars are shown in Table 6.5. There were significant differences ($P < 0.001$) in the flavonol content among the cherry cultivars, but not among harvest years. Cv. Saco was the richest in this compound, containing 14 mg 100 g^{-1} of fw, and cvs. Burlat and Summit had lower values, 3 mg 100 g^{-1} of fw that represented 5 and 3% of the total phenolics contents, respectively (Table 6.5). The levels of flavonol (rutin) showed a slight increase from partially ripe to ripe stage, and a higher increase was observed in cv. Saco, which varied from 3 to 10 mg 100 g^{-1} of fw (3-fold), in 2002. The flavonol content of cherries remained quite constant during the storage period of ripe and partially ripe cherries at both temperatures.

The levels of flavan-3-ols, catechin, and epicatechin, differed among the cherry cultivars and the year ($P < 0.001$). At harvest, mean contents ranged from 7 mg 100 g^{-1} of fw (in 2002) in cv. Van to 27 mg 100 g^{-1} of fw (in 2001) in cv. Saco. In general, the flavan-3-ols content was higher in 2001, except for cv. Burlat. Epicatechin was found to be the dominant flavan-3-ol in sweet cherry cultivars. Slight variations of total flavonol and flavan-3-ols were observed during storage period (Table 6.5).

Table 6.3
Hydroxycinnamic acid derivative levels (milligrams per 100 g of fresh weight) in cherry cultivars at two ripeness stages (partially ripe and ripe), after storage at room (15 ± 5 °C) and cool temperature (1–2 °C) in 2001 and 2002.

cultivar/stage	storage conditions	neochlorogenic acid		*p*-coumaroylquinic acid		chlorogenic acid		other hydroxycinnamic acids		total phenolic acids	
		2001	2002	2001	2002	2001	2002	2001	2002	2001	2002
Burlat											
partially ripe	day 0	18.7 (24.5)	26.2 (24.1)	29.0 (37.5)	25.9 (23.9)	3.27 (4.4)	4.20 (3.6)	nd[a]	0.65 (0.2)	51.0	57.0
partially ripe	1–2 ºC; day 30	29.6 (30.9)	24.1 (21.9)	29.4 (30.3)	20.9 (19.7)	4.59 (4.8)	4.30 (3.5)	2.33 (1.2)	0.39 (0.1)	65.9	49.7
ripe	day 0	21.7 (18.6)	25.9 (13.6)	26.3 (19.9)	23.1 (12.4)	3.65 (3.1)	3.96 (1.8)	nd	0.33 (0.1)	51.6	53.2
ripe	15 ± 5 ºC; day 6	18.1 (19.1)	25.5 (8.6)	25.4 (27.9)	23.2 (8.4)	2.34 (2.5)	3.83 (1.2)	1.04 (0.4)	0.32 (0.1)	46.9	52.8
ripe	1–2 ºC; day 30	34.1 (21.6)	23.3 (11.3)	32.3 (20.4)	20.0 (10.0)	5.39 (3.2)	3.33 (1.4)	2.54 (0.9)	0.35 (0.1)	74.4	47.0
Saco											
partially ripe	day 0	126 (72.1)	121 (74.1)	12.6 (7.9)	11.5 (7.4)	7.99 (4.6)	6.49 (4.0)	nd	0.63 (0.2)	147	140
partially ripe	1–2 ºC; day 30	––– [b]	122 (68.6)	–––	13.5 (8.9)	–––	6.06 (3.4)	–––	4.23 (1.7)	–––	145
ripe	day 0	190 (70.5)	117 (52.8)	15.2 (5.5)	9.16 (4.2)	12.0 (4.2)	7.56 (3.3)	5.27 (1.3)	3.48 (1.1)	222	137
ripe	15 ± 5 ºC; day 6	186 (73.7)	132 (40.0)	16.9 (5.2)	12.5 (4.0)	11.1 (3.5)	7.28 (2.2)	7.35 (0.8)	5.54 (1.2)	221	158
ripe	1–2 ºC; day 30	136 (46.5)	93.4 (47.7)	12.2 (4.4)	8.45 (4.7)	9.73 (3.5)	5.58 (2.9)	6.39 (1.5)	3.49 (0.8)	164	111
Summit											
partially ripe	day 0	33.3 (35.6)	35.0 (37.9)	27.5 (30.2)	26.0 (30.4)	8.58 (9.0)	5.84 (5.9)	2.91 (1.2)	1.61 (0.7)	72.3	68.4
partially ripe	1–2 ºC; day 30	37.2 (37.2)	31.3 (32.2)	28.9 (30.7)	28.3 (31.9)	7.63 (7.5)	5.64 (5.0)	4.99 (2.3)	2.29 (0.9)	78.7	67.5
ripe	day 0	40.4 (31.6)	28.3 (25.8)	34.0 (27.3)	20.9 (20.4)	9.73 (9.9)	4.60 (4.1)	1.86 (0.5)	2.21 (0.8)	86.0	56.0
ripe	15 ± 5 ºC; day 6	62.5 (42.8)	30.7 (19.2)	27.7 (18.2)	23.5 (16.8)	9.81 (6.3)	4.80 (2.8)	nd	2.19 (0.6)	100	61.2
ripe	1–2 ºC; day 30	24.4 (24.9)	26.7 (25.1)	18.5 (19.4)	22.0 (21.6)	5.57 (5.6)	4.38 (3.7)	2.06 (0.8)	0.93 (0.3)	50.5	54.1
Van											
partially ripe	day 0	59.2 (70.0)	47.3 (56.1)	5.27 (6.8)	2.83 (4.3)	5.07 (5.9)	3.81 (4.2)	0.87 (0.4)	0.65 (0.3)	70.4	54.6
partially ripe	1–2 ºC; day 30	96.5 (65.6)	76.3 (63.4)	8.29 (6.3)	6.15 (6.8)	7.96 (5.3)	5.35 (3.8)	2.82 (0.8)	1.82 (0.6)	116	89.6
ripe	day 0	84.9 (64.2)	46.2 (37.5)	7.25 (6.2)	3.97 (3.5)	6.32 (4.6)	3.19 (2.4)	2.02 (0.9)	0.75 (0.3)	101	54.2
ripe	15 ± 5 ºC; day 6	98.4 (48.0)	72.5 (19.2)	9.89 (5.3)	5.33 (1.6)	7.08 (3.7)	4.67 (1.0)	1.98 (0.4)	1.21 (0.1)	117	83.8
ripe	1–2 ºC; day 30	81.2 (53.7)	49.1 (25.5)	7.54 (5.9)	3.72 (2.2)	5.80 (3.7)	3.38 (1.7)	1.14 (0.3)	0.56 (0.1)	95.7	56.8

[a] nd: not detected. [b] Data not available. The relative amount of each compound in each type of cherry sample is shown in parenthesis.

Table 6.4
Anthocyanin levels (milligrams per 100 g of fresh weight) in cherry cultivars at two ripeness stages (partially ripe and ripe), after storage at room (15 ± 5 °C) and cool temperature (1–2 °C) in 2001 and 2002.

cultivar/stage	storage conditions	cyanidin-3-glucoside		cyanidin-3-rutinoside		peonidin-3-glucoside		pelargonidin-3-rutinoside		peonidin-3-rutinoside		total anthocyanins	
		2001	2002	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002
Burlat													
partially ripe	day 0	1.23 (1.6)	4.27 (3.7)	4.21 (5.5)	18.3 (18.2)	nd [a]	0.02 (0.01)	nd	0.04 (0.01)	0.31 (0.27)	0.56 (0.54)	5.75	23.18
partially ripe	1–2 ºC; day 30	2.56 (2.6)	5.06 (4.9)	13.0 (13.7)	29.6 (29.4)	nd	0.02 (0.01)	0.02 (0.01)	0.10 (0.1)	0.47 (0.32)	1.09 (1.13)	16.08	35.84
ripe	day 0	14.5 (12.6)	31.94 (19.0)	28.5 (24.6)	60.6 (36.5)	nd	0.26 (0.1)	nd	0.27 (0.1)	1.67 (1.56)	2.61 (1.67)	44.67	95.68
ripe	15 ± 5 ºC; day 6	9.85 (10.3)	48.5 (18.6)	30.6 (31.0)	125 (50.4)	0.13 (0.1)	0.40 (0.1)	0.15 (0.1)	0.59 (0.2)	2.06 (2.16)	5.63 (2.37)	42.83	180.00
ripe	1–2 ºC; day 30	11.2 (6.9)	27.2 (14.6)	43.0 (28.7)	86.1 (48.4)	0.15 (0.06)	0.26 (0.1)	0.17 (0.1)	0.48 (0.3)	1.92 (1.20)	4.33 (2.30)	56.41	118.40
Saco													
partially ripe	day 0	0.25 (0.1)	0.33 (0.3)	6.22 (3.2)	5.97 (3.6)	nd	nd	nd	0.02 (0.01)	nd	0.05 (0.01)	6.47	6.37
partially ripe	1–2 ºC; day 30	*b*	1.36 (0.4)	*b*	16.7 (8.9)	*b*	nd	*b*	0.05 (0.01)	*b*	0.55 (0.21)	*b*	18.60
ripe	day 0	1.94 (0.6)	8.23 (3.8)	24.5 (8.5)	52.6 (23.9)	nd	nd	0.09 (0.01)	0.23 (0.1)	nd	0.80 (0.23)	26.51	61.86
ripe	15 ± 5 ºC; day 6	3.14 (0.9)	14.4 (4.2)	81.7 (22.8)	122 (39.4)	nd	nd	0.57 (0.1)	0.69 (0.24)	0.84 (0.02)	4.16 (1.38)	86.25	141.65
ripe	1–2 ºC; day 30	11.2 (3.8)	8.58 (4.4)	62.9 (22.1)	55.8 (29.1)	nd	nd	0.43 (0.1)	0.28 (0.10)	0.55 (0.14)	1.23 (0.68)	75.08	65.91
Summit													
partially ripe	day 0	0.18 (0.1)	0.53 (0.9)	5.72 (5.3)	8.09 (8.9)	nd	nd	nd	0.03 (0.01)	nd	0.04 (0.02)	5.90	8.67
partially ripe	1–2 ºC; day 30	0.30 (0.2)	0.66 (1.0)	8.05 (7.8)	13.5 (14.8)	nd	nd	0.03 (0.01)	0.05 (0.02)	0.14 (0.05)	0.31 (0.22)	8.52	14.49
ripe	day 0	1.08 (0.6)	3.8 (3.8)	20.1 (14.5)	31.8 (30.3)	nd	nd	0.10 (0.03)	0.15 (0.10)	0.08 (0.02)	0.90 (0.55)	21.39	36.56
ripe	15 ± 5 ºC; day 6	1.31 (0.6)	3.97 (3.1)	44.9 (17.8)	3.97 (44.2)	nd	nd	0.30 (0.1)	0.35 (0.17)	0.78 (0.29)	3.59 (2.67)	47.31	72.12
ripe	1–2 ºC; day 30	1.03 (1.2)	2.38 (2.5)	31.5 (33.7)	2.38 (31.8)	nd	nd	0.16 (0.1)	0.16 (0.11)	0.72 (0.52)	0.96 (0.99)	33.41	35.06
Van													
partially ripe	day 0	0.09 (0.04)	0.75 (1.5)	4.86 (5.6)	13.0 (18.6)	nd	nd	nd	0.06 (0.03)	0.02 (0.01)	0.30 (0.26)	4.97	14.06
partially ripe	1–2 ºC; day 30	0.65 (0.2)	0.50 (1.0)	18.6 (12.6)	12.0 (11.8)	nd	nd	nd	0.04 (0.01)	0.52 (0.26)	0.54 (0.32)	19.75	13.05
ripe	day 0	0.85 (0.4)	5.97 (6.0)	18.2 (13.4)	38.2 (38.4)	nd	0.04 (0.01)	0.10 (0.03)	0.19 (0.13)	0.18 (0.08)	2.80 (2.57)	19.37	47.14
ripe	15 ± 5 ºC; day 6	2.16 (0.7)	33.4 (10.2)	60.6 (30.3)	180 (57.6)	nd	0.33 (0.1)	0.60 (0.2)	1.00 (0.38)	2.41 (0.85)	15.55 (5.29)	65.80	230.33
ripe	1–2 ºC; day 30	1.46 (0.6)	13.5 (7.3)	36.0 (24.7)	88.1 (51.2)	nd	0.11 (0.04)	0.21 (0.12)	0.48 (0.20)	1.48 (0.70)	6.94 (4.02)	39.17	109.10

[a] nd: not detected. [b] Data not available. The relative amount of each compound in each type of cherry sample is shown in parenthesis.

Table 6.5
Flavonol and flavan-3-ol levels (milligrams per 100 g of fresh weight) in cherry cultivars at two ripeness stages (partially ripe and ripe), after storage at room (15 ± 5 °C) and cool temperature (1–2 °C) in 2001 and 2002.

cultivar/stage	storage conditions	rutin		catechin		epicatechin		total flavonol + flavan-3-ols	
		2001	2002	2001	2002	2001	2002	2001	2002
Burlat									
partially ripe	day 0	2.22 (2.8)	2.41 (2.7)	7.33 (9.2)	9.18 (8.9)	6.54 (8.5)	10.08 (9.4)	16.08	21.67
partially ripe	1–2 ºC; day 30	2.01 (2.2)	3.22 (3.1)	5.97 (6.2)	6.15 (5.9)	7.55 (7.8)	8.29 (7.5)	15.53	17.66
ripe	day 0	3.06 (2.9)	6.46 (3.2)	5.73 (6.0)	8.68 (4.8)	4.51 (4.0)	8.98 (4.5)	13.30	24.12
ripe	15 ± 5 ºC; day 6	2.13 (2.6)	8.40 (3.2)	2.41 (1.7)	8.02 (3.0)	3.18 (2.2)	8.09 (2.6)	7.72	24.51
ripe	1–2 ºC; day 30	4.26 (2.8)	6.44 (3.2)	9.38 (5.8)	6.81 (3.5)	8.75 (5.2)	6.55 (3.0)	22.39	19.79
Saco									
partially ripe	day 0	6.67 (4.5)	2.78 (2.0)	9.01 (4.9)	4.79 (3.0)	5.92 (2.8)	10.23 (5.4)	21.59	17.79
partially ripe	1–2 ºC; day 30	––– [a]	4.35 (2.5)	–––	4.87 (2.9)	–––	5.80 (2.6)	–––	15.02
ripe	day 0	13.69 (4.7)	9.90 (4.3)	14.90 (5.0)	6.11 (2.7)	11.78 (4.0)	8.72 (3.7)	40.37	24.73
ripe	15 ± 5 ºC; day 6	13.30 (4.2)	8.14 (2.8)	12.25 (3.8)	6.48 (2.1)	15.00 (3.9)	9.02 (2.5)	40.55	23.64
ripe	1–2 ºC; day 30	13.50 (5.6)	7.22 (3.9)	9.80 (3.3)	5.25 (2.8)	27.04 (9.0)	5.89 (3.0)	50.34	18.36
Summit									
partially ripe	day 0	2.17 (2.1)	2.39 (2.9)	6.68 (7.2)	3.87 (4.4)	8.71 (9.2)	6.48 (6.5)	17.56	12.79
partially ripe	1–2 ºC; day 30	4.84 (4.3)	1.86 (2.2)	5.82 (5.5)	3.99 (4.4)	5.40 (4.6)	4.06 (3.4)	16.06	9.91
ripe	day 0	2.81 (2.5)	3.39 (3.1)	7.32 (3.8)	4.36 (4.0)	11.28 (9.1)	5.18 (4.3)	21.41	12.93
ripe	15 ± 5 ºC; day 6	3.39 (2.7)	3.04 (2.1)	8.13 (5.0)	4.47 (3.1)	9.89 (6.3)	5.26 (3.5)	21.41	12.77
ripe	1–2 ºC; day 30	4.07 (3.8)	3.31 (3.3)	4.27 (4.5)	4.01 (4.0)	5.59 (5.6)	4.62 (4.0)	13.92	11.94
Van									
partially ripe	day 0	2.86 (3.6)	4.70 (9.3)	3.26 (4.0)	2.21 (3.5)	3.18 (3.7)	2.87 (2.1)	9.29	9.78
partially ripe	1–2 ºC; day 30	3.54 (3.4)	3.89 (4.7)	4.85 (3.8)	4.13 (4.8)	4.03 (1.8)	5.02 (2.7)	12.42	13.04
ripe	day 0	4.06 (3.1)	3.92 (3.9)	4.61 (3.6)	2.42 (2.1)	4.95 (3.5)	4.09 (3.1)	13.62	10.43
ripe	15 ± 5 ºC; day 6	8.28 (4.6)	9.26 (3.0)	6.56 (3.8)	3.33 (0.6)	4.97 (2.2)	6.36 (1.1)	19.80	18.96
ripe	1–2 ºC; day 30	3.79 (3.1)	5.20 (3.2)	4.89 (4.1)	3.05 (2.0)	5.30 (3.1)	4.60 (2.6)	13.99	12.85

[a] Data not available. The relative amount of each compound in each type of cherry sample is shown in parenthesis.

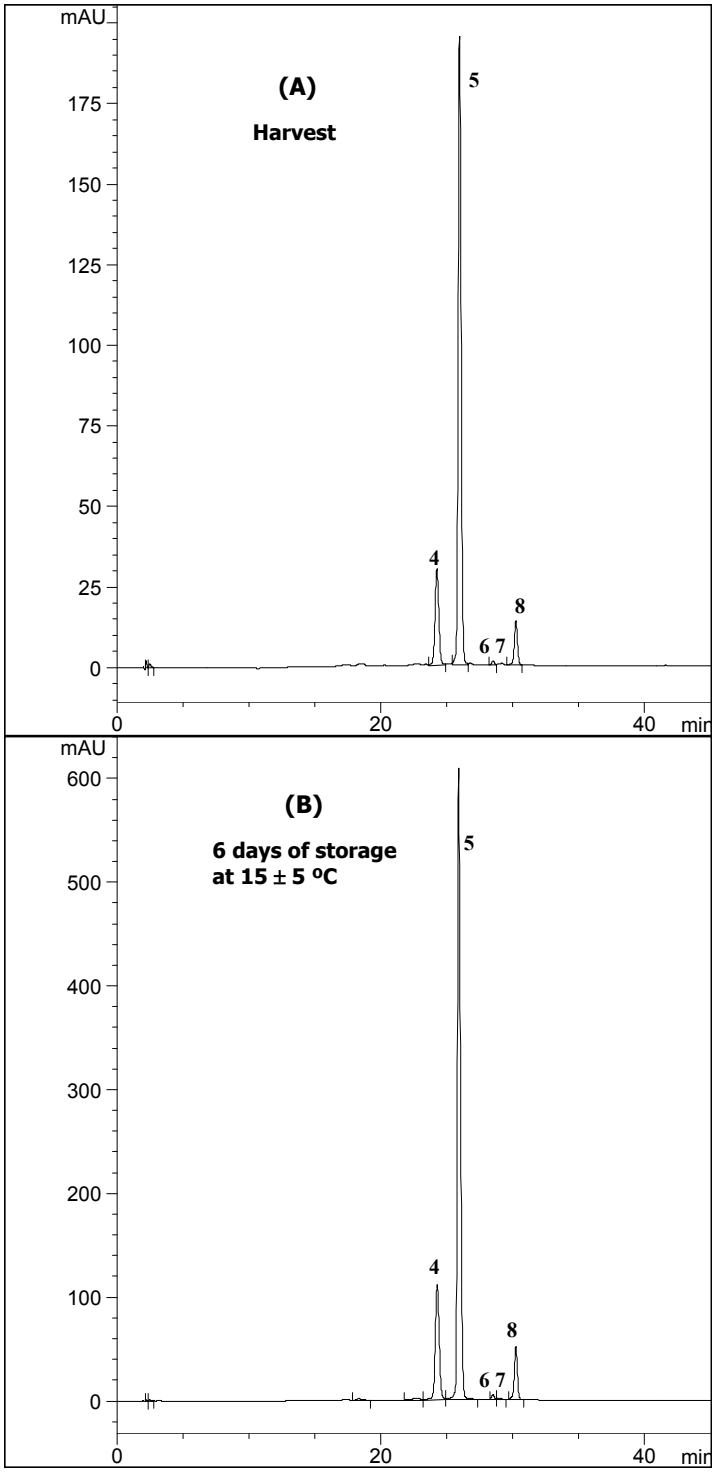

Figure 6.3
HPLC separation of anthocyanins in a methanolic extract of cv. Van cherries, in 2002, monitored at 520 nm. Peaks with the retention times (RT) for the two chromatograms **A** and **B**, are, respectively: (4) cyanidin-3-glucoside (RT = 24.26 and 24.28 min); (5) cyanidin-3-rutinoside (RT = 25.98 and 25.91 min); (6) peonidin-3-glucoside (RT = 28.52 and 28.51 min); (7) pelargonidin-3-rutinoside (RT = 28.93 and 28.92 min); (8) peonidin-3-rutinoside (RT = 30.25 and 30.25 min). Different scales were used to stress differences within each chromatogram.

6.5. Discussion

Previous studies on cherry phenolics showed that neochlorogenic acid and *p*-coumaroylquinic acid were the main hydroxycinnamic derivatives (Möller and Herrmann, 1983; Gao and Mazza, 1995). The results of this study confirm that the two acids are the main hydroxycinamates in both partially ripe and ripe cherries, with smaller amounts of chlorogenic acid. In ripe fruits the levels of neochlorogenic acid were higher (22–190 mg 100 g^{-1} of fw) and the levels of *p*-coumaroylquinic acid were lower (4–34 mg 100 g^{-1} of fw) than the values obtained by Gao and Mazza (1995), who found neochlorogenic acid in a range between 24 and 128 mg 100 g^{-1} in pitted cherry and *p*-coumaroylquinic acid from 23 to 131 mg 100 g^{-1}. These differences could be due to the studied cultivars and to the influence of extraction and analytical procedures. Because these three hydroxycinnamates have been described to be involved in the copigmentation with anthocyanins (Asen *et al.*, 1972; Mazza and Brouillard, 1990), they are likely to have a major importance in the color of cherries.

Initial studies have found cyanidin-3-rutinoside and cyanidin-3-glucoside as the main anthocyanin pigments in sweet cherries (Robinson and Robinson, 1931; Li and Wagenknecht, 1958; Lynn and Luh, 1964; Okombi, 1979). In addition, other studies (Lynn and Luh, 1964; Casoli *et al.*, 1967) reported the presence of peonidin and two of its glycosidic derivatives in cv. Bing cherries. However, Fouassin (1956), Harborne and Hall (1964), and Olden and Nybom (1968) found only cyanidin derivatives and no peonidin glycosides in cultivars of *Prunus avium*. Tanchev *et al.* (1973) and Tanchev (1977) claimed that cyanidin-3-sophoroside was present in cvs. Lambert, Helmsdorf, Somaya, Kozerskia, and Bing cherries, but this particular anthocyanin was not identified by Gao and Mazza (1995) in any of the cherry cultivars they studied, and it was not detected in our study either. Another minor anthocyanin, peonidin-3-rutinoside, detected for the first time in sweet cherries by Gao and Mazza (1995), was also found in the four cherry cultivars studied here. Hence, our results are generally consistent with the results reported in the literature, which identify the 3-rutinoside and 3-glucoside of cyanidin as the major anthocyanins and the same glycosides of peonidin and pelargonidin-3-rutinoside as the minor anthocyanins. In other stone fruits, such as peaches and nectarines that belong to the same botanical family as sweet cherries, cyanidin-3-glucoside is the major anthocyanin, followed by cyanidin-3-rutinoside (Tomás-Barberán *et al.*, 2001).

In general, storage at 1–2 ºC or 15 ± 5 °C resulted in an overall increase in anthocyanins in both ripe and in partially ripe cherries, which is in agreement with other findings, for other types of ripe fruits maintained at cool temperature, with high anthocyanin content, such as strawberries (Gil *et al.*, 1997; Sanz *et al.*, 1999), blueberries (Kalt and

McDonald, 1996), grapes (Cantos *et al*., 2000), and pomegranates (Holcroft *et al*., 1998). We are currently examining the relationship between quality indices, notably color of cherries and pH, and anthocyanin contents.

The data in Tables 6.3 and 6.4 suggest that the quantities of phenolic acids and anthocyanins were influenced by the climatic conditions of the two years of study. In 2001, in the last third of the fruit growth stage higher temperature (average 19 °C) and higher solar irradiation (231 h) were observed, when compared to 2002 (15 °C and 171 h, respectively), which favored the biosynthesis of phenolic acids, which tend to reach highest levels in the late stage of final maturity as referred to by Stöhr *et al.* (1975) and Melin *et al.* (1977) (cf. ref Herrmann, 1989). However, data from several more harvest years or different weather conditions are required before any firm conclusions can be drawn on these issues.

With regard to flavan-3-ols, epicatechin was found to be the major compound with smaller amounts of catechin, which is in complete agreement with other findings in cherries (Macheix *et al*., 1990).

The occurrence of quercetin glucosides has been reported in sour cherries, but not in sweet cherries (Friedrich *et al*., 1998). However, rutin was detected in this study. In fact, in some of the samples, several other quercetin glucoside derivatives were also found (data not shown). Because quercetin glucosides, especially in onions, are known to possess significant antioxidant potency, the presence of quercetin glucoside derivatives and other phenolics, such as catechin, epicatechin, and anthocyanins in sweet cherries, may contribute to make sweet cherries a beneficial source of health protective antioxidants. Limited research on the health effects of cherries has been conducted. However, on the basis of the present data, which confirm that sweet cherry is rich in phenolics, it is likely that cherries will provide the types of health benefits associated with fruits and vegetables in general.

This study showed that the cherry cultivars have the same phenolic pattern, however, with large variations on content. Levels of phenolics are always higher in ripe than in partially ripe cherries. Both cool and room temperature storage increase phenolics levels; however, levels for cold storage cherries will never be as high as for room temperature storage cherries. The levels of the individual phenolic substances in cherries generally vary during storage, but the variation in phenolic profiles is less during a month at cool storage (where the levels may go up or down) than at storage for a few days at room temperature (15 ± 5 °C), where notably the anthocyanins and phenolic acid contents may increase. Finally, our evaluation of cherries harvested in two different years indicates that the influence of weather conditions during cherry growth may have a profound influence on the phenolics levels.

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CAPÍTULO 7

EFFECT OF RIPENESS AND POSTHARVEST STORAGE ON THE EVOLUTION OF COLOUR AND ANTHOCYANINS IN CHERRIES (*Prunus avium* L.)

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7. EFFECT OF RIPENESS AND POSTHARVEST STORAGE ON THE EVOLUTION OF COLOUR AND ANTHOCYANINS IN CHERRIES (*Prunus avium* L.)

7.1. Abstract

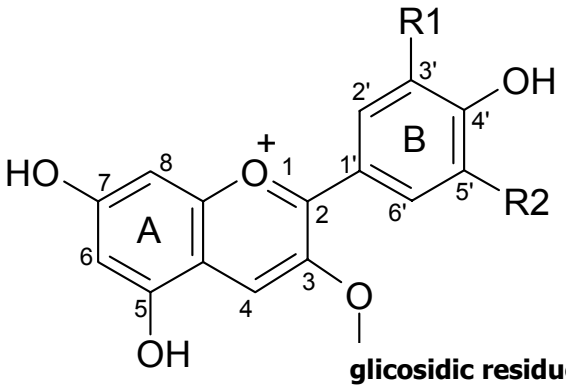
The relationship between colour parameters and anthocyanins of four sweet cherry cultivars, Burlat, Saco, Summit and Van was studied. The colour (*L**, *a**, *b**, chroma and hue angle parameters) and anthocyanins were analysed during two different years at two different ripening stages (partially ripe and ripe, respectively). The cherries were analysed at harvest and after storage at 1.5 ± 0.5 °C and 15 ± 5 °C for 30 and 6 days, respectively. The colour was measured by tristimulus colourimetry (CIELAB system) directly on the fruits, while anthocyanins were quantified by HPLC–DAD analysis on methanolic extracts of freeze-dried samples of the fresh cherries and on the differently stored cherries. *L**, chroma and hue angle were always lower for the ripe than for the partially ripe cherries. All of the cultivars were found to contain cyanidin-3-rutinoside and cyanidin-3-glucoside as the major anthocyanins. The total anthocyanin content in fruits of the different cultivars varied in the order Burlat > Saco > Van > Summit. The concentration of anthocyanins increased at both temperatures of storage in both ripe and partially ripe cherries, but the extent of increase varied among cultivars. Cherries stored at 15 ± 5 °C showed higher reduction of *L**, chroma and hue angle than fruits stored at 1.5 ± 0.5 °C. *L**, *a**, *b**, chroma and hue angle correlated negatively with the total anthocyanins levels ($P < 0.001$) and with the total phenols ($P < 0.01$). These results show that chromatic functions of chroma and hue correlate closely with the evolution of colour and anthocyanins levels during storage of sweet cherries and indicate that colour measurements can be used to monitor pigment evolution and anthocyanin contents of cherries (and *vice versa*).

Key words: Anthocyanins, cherries, chromatic coordinates, chroma, colour evolution, hue angle, ripeness stage, storage.

7.2. Introduction

Colour is one of the most important indicators of maturity and quality of fresh, stored and processed cherries (Drake *et al.*, 1982). In cherries, colour is mainly influenced by the concentration and distribution of different anthocyanins in the skin (Gao and Mazza, 1995) as well as pH and levels and types of colourless phenolics in the fruits and other factors such as light, temperature, oxygen, metal ions and enzymes (Delgado-Vargas and Paredes-López, 2003).

In a previous study we showed that levels of anthocyanins in four sweet cherry cultivars, Burlat, Saco, Summit and Van, ranged from ~ 5 to 86 mg 100 g^{-1} of fresh weight (fw) in 2001, and from ~ 6 to 230 mg 100 g^{-1} of fw in 2002 (Gonçalves *et al.*, 2004). In general, the total anthocyanins levels were higher in 2002 than in 2001, but the profiles of anthocyanins were similar both among the two years and among all four cultivars (Gonçalves *et al.*, 2004). The major anthocyanins in sweet cherries include the 3-*O*-glucoside and 3-*O*-rutinoside (-rhamnosyl-D-glucopyranose) of cyanidin, with peonidin-3-*O*-rutinoside and -glucoside, as well as pelargonidin-3-*O*-rutinoside occurring in much lower amounts (Figure 7.1) (Gao and Mazza, 1995; Esti *et al.*, 2002; Gonçalves *et al.*, 2004). Gao and Mazza (1995) reported that the total anthocyanin content ranged from 82 to 297 mg 100 g^{-1} for dark cherries and from 2 to 41 mg 100 g^{-1} for the light coloured cherries. The cyanidin-3-rutinoside and the cyanidin-3-glucoside contents in pitted, sweet cherry cultivars have been found to range from 4 to 44 mg 100 g^{-1} of fw and from 2 to 243 mg 100 g^{-1} of fw, respectively (Gao and Mazza, 1995).



R^1	R^2	Anthocyanin	Glucoside	Colour
H	H	Pelargonidin	Rutinoside	Red
OH	H	Cyanidin	Glucoside	Orange-red
OH	H	Cyanidin	Rutinoside	Red-purple
OCH_3	H	Peonidin	Glucoside	Orange-red
OCH_3	H	Peonidin	Rutinoside	Orange-red

Figure 7.1
The basic chemical structure of the five most commonly occurring anthocyanins in sweet cherries. The structures shown the hydroxylation and methoxylation substitution pattern, type of glycosidic residue, and colour of the flavylium ion form (prevailing at acidic pH).

As expected, the total levels of anthocyanins are higher in ripe cherries than in partially ripe ones (Gonçalves *et al.*, 2004). In freshly harvested, fully ripe cherries, the levels

of cyanidin-3-rutinoside were in our previous study found to represent 63–94 % by weight of the total anthocyanins. In both years, cherries of cv. Burlat showed the highest anthocyanin levels, particularly with respect to the cyanidin-3-glucoside levels. The levels of anthocyanins increased during storage, and after storage the total anthoycanins represented around 50% of total phenolics. The cherries from cv. Van exhibited the most profound increase in total anthocyanin levels during storage, and the increase in total anthocyanin levels was mainly attributable to increases in the cyanidin-3-rutinoside level (Gonçalves *et al.*, 2004). Studies for anthocyanin levels in mature grapes stored at 0 ºC supported our findings, since a slight increase, from 4500 to 6000 mg kg^{-1} of peel fw, was shown previously for these compounds (Cantos *et al.*, 2000).

The colorimetric CIE system, Commission International d'Eclairage, is widely used in the quantification and characterization of anthocyanin chromatic properties and in the assessment of colour quality and colour changes during maturity and processing of plant foods (Dodds *et al.*, 1991; Heredia *et al.*, 1998).

Fresh sweet cherries represent an important, but fragile, commodity in the Portuguese agricultural export market. The harvesting season is very short, and cold storage is used to stretch the supply period in the season. However, the effects of different storage conditions on cherry quality, including colour development, is not well studied and the available knowledge on anthocyanins levels *versus* colour development during cherry storage appears somewhat confusing. In a study of two Italian sweet cherry cultivars, Sciazza and Ferrovia, it was found that the content of cyanidin-3-rutinoside and cyanidin-3-glucoside in the cherries decreased several fold during cold storage for 15 days at 1 ºC (Esti *et al.*, 2002). Nevertheless, the colour attributes of the same cherries, measured as CIE: *L**, *a**, and *b** values of the cherry skins and pulp, did not change significantly during storage independent of the storage temperature – the only exception being a decrease in *L** observed for the skin of the cv. Ferrovia cherries (Esti *et al.*, 2002). In contrast, as discussed above, we previously observed increases in anthocyanin levels during storage suggesting that colour attributes would change during storage (Gonçalves *et al.*, 2004). This study was therefore undertaken to a) determine the evolution of colour in sweet cherries during postharvest storage at different temperatures, b) assess the relationship between colour attributes and anthocyanins content in cherries, and c) unravel any potential differences in colour attributes and anthocyanin-colour relationships among four different cherry cultivars.

7.3. Materials and Methods

7.3.1. Cherry Raw Material

Four sweet cherry cultivars, Burlat, Saco, Summit and Van from an orchard in Vila Real, Portugal, were randomly hand harvested in 2001 and 2002, both years as partially ripe and ripe. The fruit analysis were made at harvest and during storage at typical, industrially used fruit storage conditions, that is at 1.5 ± 0.5 ºC and 90% RH (cool temperature) for 30 days or at 15 ± 5 ºC (room temperature) for 6 days. The details on the quality criteria decisive for picking as well as an elaborate examination of the evolution of quality parameters in the four different cherry cultivars during the storage (in addition to effects of maturity stage at picking) have been reported separately (Gonçalves *et al.*, 2004).

7.3.2. Colour Analyses

Ground colour was measured on 20 fruits using a tristimulus colorimeter (Minolta CR-200B Chroma Meter, Minolta, Japan) having an 8 mm diameter viewing area. Chromatic analyses were carried out following the CIE (Commission International de l'Eclairage) system of 1976. Values of L^*, a^* and b^* were measured to describe a three-dimensional colour space and interpreted as follows: L^* indicates lightness read from 0 (completely opaque or 'black') to 100 (completely transparent or 'white'). A positive a^* value indicates redness ($-a^*$ is greenness) and positive b^* value yellowness ($-b^*$ is blueness) on the hue-circle (Voss, 1992; Hutchings, 1994). The hue angle (º), hue = arctg (b^*/a^*), expresses the colour nuance (Voss, 1992) and values are defined as follows: red-purple: 0º, yellow: 90º, bluish-green: 180º, and blue: 270º (McGuire, 1992). The chroma, obtained as $(a^{*2} + b^{*2})^{1/2}$, is measure of chromaticity (C^*), which denotes the purity or saturation of the colour (Voss, 1992). The data of each measurement are the average of triplicate measures on equidistant points of each fruit.

7.3.3. Chemicals and Reagents

Anthocyanin-glucosides were purchased from Polyphenols A/S (Stavanger, Norway) and the HPLC grade acetonitrile was purchased from Merck (Darmstadt, Germany).

7.3.4. Extraction of Anthocyanins

Pitted and freeze-dried cherry samples (0.5 g) were contacted with 60% v/v aqueous MeOH (5 ml); flushed with N_2, and extracted for 10 min using a shaking (200 rpm) water bath at 25 ºC. The individual samples were then filtered through one layer of Whatman No. 1 filter paper (using vacuum suction during the filtration) and the solvent contacting was

repeated twice on the residue. Each filtrate was then filtered through a 0.45 µm syringe-tip hydrophilic Durapore filter (Millipore Corp., Bedford, MA) prior to high performance liquid chromatography (HPLC) analyses. The reported values are the sums of the values obtained from each extraction and each HPLC analysis.

7.3.5. Anthocyanins and Phenols Analyses

HPLC analysis was carried out according to the procedure described by Lamuela-Raventós and Waterhouse (1994) using a Hewlett-Packard 1100 system (Waldbronn, Germany) equipped with a diode array detector (DAD), a Nova-Pak C18 column (3.9 x 150 mm, Waters) at 40 ºC, and controlled by a PC with HPChem station Software. The solvent flow rate was 0.5 ml min^{-1} and the injected volume was 10 µl. The anthocyanins were identified by spectral and retention time analysis. The quantities of the different phenolic compounds were assessed from peak areas and calculated as equivalents of standard compounds from linear regression curves of authentic standards. The anthocyanins were quantified at 520 nm, in mg 100 g^{-1} of fw, as cyanidin-3-glucoside and cyanidin-3-rutinoside, respectively, while the quantities of peonidin-3-glucoside, peonidin-3-rutinoside, and pelargonidin-3-rutinoside were calculated as cyanidin-3-rutinoside equivalents.

7.3.6. Statistics

Analyses of variance were accomplished by use of the Super ANOVA software (1.11 Abacus Concepts Inc., 1991). Significances of differences were established from a Duncan's Test ($P < 0.05$). A Fisher correlation analysis including all the parameters was also performed. Possible differences between cultivars in the correlation between two parameters were analyzed by comparison of regression lines.

7.4. Results and Discussion

7.4.1. HPLC-DAD Analysis of Cherry Anthocyanins

The HPLC chromatograms of sweet cherry extracts obtained in the visible spectral region (520 nm) revealed five peaks, which corresponded to five anthocyanins (Figure 7.2): cyanidin-3-glucoside (peak 1), cyanidin-3-rutinoside (peak 2), peonidin-3-glucoside (peak 3), pelargonidin-3-rutinoside (peak 4) and peonidin-3-rutinoside (peak 5). As discussed below these findings are in complete agreement with what has been reported on anthocyanins in sweet cherries (Gao and Mazza, 1995). The details on the evolution of anthocyanins in the four different cherry cultivars during storage (in addition to effects of maturity stage at picking and year) are reported separately (Gonçalves *et al.*, 2004).

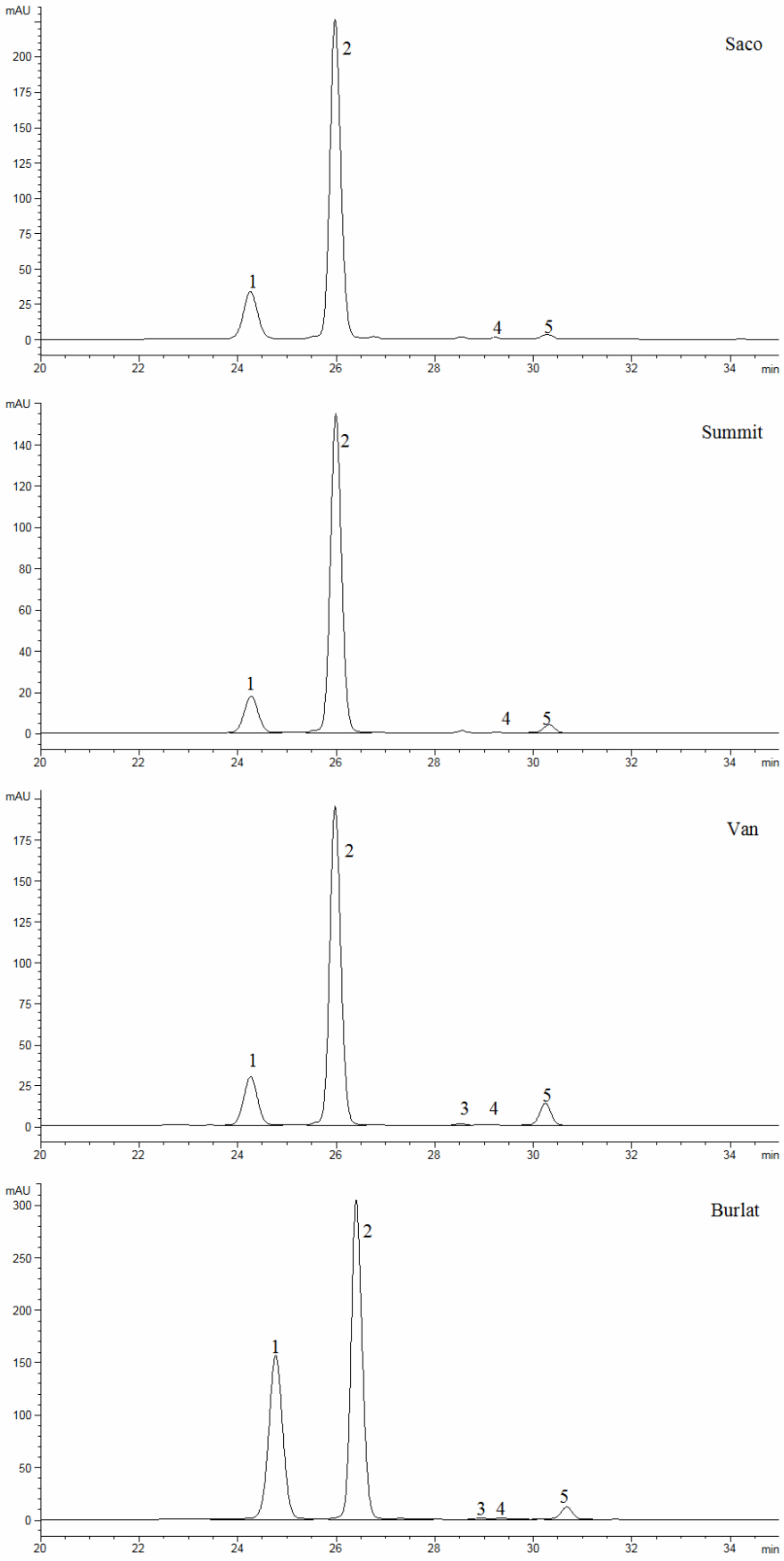


Figure 7.2
HPLC separation of anthocyanins in a methanolic extract of the four sweet cherry cultivars, monitored at 520 nm.
Peaks:
1, cyanidin-3-glucoside;
2, cyanidin-3-rutinoside;
3, peonidin-3-glucoside;
4, pelargonidin-3-rutinoside;
5, peonidin-3-rutinoside.

7.4.2. Effect of Ripeness and Storage Temperature on Cherry Colour

The chromatic characteristics of the fruits studied are shown in Tables 7.1 and 7.2. There were significant differences in *L**, chroma and hue angle ($P < 0.001$) between the four cultivars, ripeness stage, year and storage. At harvest, Burlat cherries always showed lower hue angle and *L** and higher anthocyanin levels in both years, a synonym of redder and darker cherries. Summit fruits showed higher *L** and chroma, which is in accordance with the observation that Summit fruits are both less red and lighter in colour than the other cherry cultivars. *L**, chroma and hue angle of partially ripe cherries were always higher than in the ripe cherries, which means a less red fruit, and was correlated with lower anthocyanin content (Tables 7.1–7.3; Gonçalves *et al.*, 2004).

Table 7.1
CIE 1976 (*L*a*b**) colour space (CIELAB) of cherry cultivars at two ripeness stages (partially ripe and ripe) and influenced by storage at 15 ± 5 ºC (room temperature) and 1.5 ± 0.5 ºC (cool temperature) for year 2001. Values are means ± SD ($n = 60$). Means flanked by the same letter are not significantly different at $P < 0.05$ (Duncan's test).

cultivar	storage	chromatic coordinates			chroma	hue angle
		*L**	*a**	*b**	*C**	*H**
Burlat						
partially ripe	day 0	51.8 ± 7.1 j	36.4 ± 6.8 def	21.2 ± 2.3 hi	42.4 ± 4.9 e	31.0 ± 7.9 j
partially ripe	1.5 ± 0.5 ºC; day 30	42.2 ± 6.1 h	37.0 ± 3.4 efg	15.6 ± 2.5 f	40.2 ± 3.7 d	22.9 ± 2.9 de
ripe	day 0	37.3 ± 4.8 de	36.2 ± 5.9 def	15.5 ± 4.4 f	39.4 ± 7.1 d	22.6 ± 3.1 de
ripe	15 ± 5 ºC; day 6	31.4 ± 3.5 a	25.8 ± 7.9 a	9.2 ± 4.7 bc	27.5 ± 8.7 a	18.7 ± 6.1 abc
ripe	1.5 ± 0.5 ºC; day 30	30.4 ± 2.6 a	25.4 ± 6.5 a	7.8 ± 3.4 a	26.6 ± 7.2 a	16.3 ± 3.2 a
Saco						
partially ripe	day 0	52.2 ± 6.8 j	35.8 ± 10.3 de	29.4 ± 4.9 j	47.6 ± 4.1 f	40.5 ± 13.5 k
partially ripe	1.5 ± 0.5 ºC; day 30	41.3 ± 5.6 gh	41.9 ± 3.6 i	20.1 ± 4.5 h	46.6 ± 4.4 f	25.3 ± 4.6 fg
ripe	day 0	38.5 ± 2.7 ef	42.0 ± 2.1 i	20.4 ± 3.2 h	47.6 ± 4.1 f	25.8 ± 2.9 fgh
ripe	15 ± 5 ºC; day 6	30.2 ± 1.3 a	31.3 ± 3.2 b	9.6 ± 2.1 c	32.7 ± 3.7 b	16.8 ± 1.9 ab
ripe	1.5 ± 0.5 ºC; day 30	33.2 ± 3.1 b	34.8 ± 5.7 cde	12.3 ± 3.9 d	36.9 ± 6.7 c	19.0 ± 3.2 bc
Summit						
partially ripe	day 0	55.3 ± 7.1 k	34.2 ± 10.2 cd	29.2 ± 3.5 j	46.0 ± 4.8 f	41.8 ± 13.0 k
partially ripe	1.5 ± 0.5 ºC; day 30	46.4 ± 5.8 i	38.3 ± 4.5 fg	20.8 ± 2.6 hi	43.8 ± 2.8 e	28.8 ± 6.0 ij
ripe	day 0	41.1 ± 5.3 gh	38.5 ± 3.0 fg	20.4 ± 4.1 h	43.7 ± 3.8 e	27.7 ± 4.4 ghi
ripe	15 ± 5 ºC; day 6	35.4 ± 2.1 c	37.0 ± 3.3 efg	16.2 ± 2.9 f	40.4 ± 4.1 d	23.4 ± 2.2 ef
ripe	1.5 ± 0.5ºC; day 30	35.7 ± 2.7 cd	34.4 ± 2.6 cd	14.1 ± 2.0 e	37.2 ± 3.0 c	22.2 ± 2.0 de
Van						
partially ripe	day 0	54.1 ± 8.4 k	32.8 ± 11.3 bc	31.0 ± 4.3 k	46.5 ± 4.8 f	44.8 ± 14.5 e
partially ripe	1.5 ± 0.5 ºC; day 30	39.4 ± 5.2 fg	38.9 ± 3.3 gh	18.7 ± 4.3 g	43.3 ± 4.2 e	25.4 ± 4.7 fg
ripe	day 0	41.6 ± 4.2 h	40.9 ±2.3 hi	21.8 ± 3.0 i	46.4 ± 2.7 f	28.0 ± 3.2 hi
ripe	15 ± 5 ºC; day 6	29.9 ± 1.4 a	26.5 ± 4.4 a	8.1 ± 2.3 ab	27.7 ± 4.8 a	16.7 ± 2.2 ab
ripe	1.5 ± 0.5 ºC; day 30	33.2 ± 2.6 b	34.1 ± 3.9 cd	12.9 ± 3.0 de	36.5 ± 4.7 c	20.4 ± 2.4 cd

*L**, chroma and hue angle of ripe and partially ripe cherries were always higher in 2001 (Tables 7.1 and 7.2). These parameters decreased during storage, mostly at room temperature, but the extent of change varied among the cultivars. In general, at 15 ± 5 ºC, chroma was the parameter that showed higher reduction in 2001 and 2002. The decrease in chroma means an increase in the tonality of the fruit colour. A reduction of 19 units was measured in Van cherries in 2001 (Table 7.1). Rodríguez-Saona *et al.* (1999) observed a reduction of 10 units in chroma, in radish-coloured juices after storage at 16 days at 25 ºC.

A decrease in chroma during fruit storage was also observed with stored strawberries (Abers and Wrolstad, 1979). The decrease could have been caused by an increase in total anthocyanins and a decrease in chlorophyll and carotenoids, and, according to Abers and Wrolstad (1979), by development of dark, pigmented compounds, which tend to mask colour.

Table 7.2
CIE 1976 (*L*a*b**) colour space (CIELAB) of cherry cultivars at two ripeness stages (ripe and partially-ripe) and influenced by storage at 15 ± 5 ºC (room temperature) and 1.5 ± 0.5 ºC (cool temperature) for year 2002. Values are means ± SD (*n* = 60). Means flanked by the same letter are not significantly different at $P < 0.05$ (Duncan's test).

cultivar	storage	*chromatic coordinates*			chroma	hue angle
		*L**	*a**	*b**	*C**	*H**
Burlat						
partially ripe	day 0	45.1 ± 5.3 h	43.2 ± 4.8 l	23.4 ± 2.3 i	49.2 ± 4.7 l	28.5 ± 2.8 i
partially ripe	1.5 ± 0.5 ºC; day 30	38.1 ± 4.4 g	36.1 ± 3.3 j	16.0 ± 2.7 g	39.5 ± 4.0 k	23.7 ± 2.3 gh
ripe	day 0	27.8 ± 2.4 cd	18.5 ± 6.5 e	5.0 ± 2.9 bc	19.2 ± 7.0 e	14.1 ± 3.2 bcde
ripe	15 ± 5 ºC; day 6	26.5 ± 0.7 bc	9.3 ± 3.2 ab	2.0 ± 0.8 a	9.5 ± 3.3 ab	11.8 ± 1.9 abc
ripe	1.5 ± 0.5 ºC; day 30	24.4 ± 1.3 a	9.8 ± 4.1 ab	2.4 ± 1.3 a	11.4 ± 11.0 b	15.1 ± 9.9 bcd
Saco						
partially ripe	day 0	46.9 ± 8.1 i	36.3 ± 7.8 j	24.7 ± 6.2 j	44.9 ± 3.4 l	34.8 ± 12.5 k
partially ripe	1.5 ± 0.5 ºC; day 30	29.8 ± 2.9 de	32.1 ± 4.9 i	10.7 ± 3.5 e	33.9 ± 5.7 i	18.1 ± 2.9 f
ripe	day 0	30.5 ± 2.7 e	26.6 ± 6.6 h	8.2 ± 3.7 d	27.9 ± 7.3 h	16.5 ± 3.7 def
ripe	15 ± 5 ºC; day 6	26.1 ± 0.7 abc	12.2 ± 2.9 cd	2.2 ± 0.8 a	12.4 ± 3.0 cd	10.1 ± 1.3 a
ripe	1.5 ± 0.5 ºC; day 30	25.4 ± 1.2 ab	13.6 ± 3.9 d	2.8 ± 1.4 a	13.9 ± 4.1 d	11.3 ± 2.2 ab
Summit						
partially ripe	day 0	46.3 ± 5.4 hi	41.0 ± 4.7 k	25.7 ± 3.1 j	48.6 ± 3.1 e	32.3 ± 6.1 jk
partially ripe	1.5 ± 0.5 ºC; day 30	39.0 ± 4.3 g	36.4 ± 2.4 j	17.5 ± 2.9 h	40.4 ± 3.0 k	25.5 ± 3.3 h
ripe	day 0	34.4 ± 3.6 f	33.5 ± 4.6 i	13.9 ± 4.1 f	36.4 ± 5.7 j	22.0 ± 3.9 g
ripe	15 ± 5 ºC; day 6	28.0 ± 1.6 cd	17.8 ± 3.7 e	4.4 ± 1.5 b	18.3 ± 3.9 e	13.6 ± 2.3 bcd
ripe	1.5 ± 0.5 ºC; day 30	27.9 ± 2.6 cd	23.7 ± 5.5 g	7.5 ± 2.7 d	24.9 ± 6.0 g	16.9 ± 2.9 ef
Van						
partially ripe	day 0	59.1 ± 8.4 j	23.4 ± 12.9 fg	30.1 ± 5.4 k	40.3 ± 4.3 k	53.8 ± 19.2 l
partially ripe	1.5 ± 0.5 ºC; day 30	39.3 ± 14.3 g	25.8 ± 9.0 h	16.7 ± 10.5 gh	33.1 ± 6.0 i	31.5 ± 21.6 j
ripe	day 0	28.6 ± 1.7 d	21.5 ± 5.4 f	5.9 ± 2.5 c	22.3 ± 5.9 f	14.6 ± 2.8 cde
ripe	15 ± 5 ºC; day 6	26.2 ± 0.6 abc	7.8 ± 2.5 a	1.4 ± 0.5 a	7.9 ± 2.6 a	10.5 ± 1.9 a
ripe	1.5 ± 0.5 ºC; day 30	26.1 ± 1.0 abc	10.7 ± 3.5 bc	2.3 ± 0.9 a	11.0 ± 3.6 bc	12.2 ± 1.8 abc

All treatments induced a reduction in hue angle values, when compared to the values observed at harvest (Tables 7.1 and 7.2). The loss of lightness was reflected by a reduction of *L** (the photometric parameter proportional to the light reflected by the object) and was directly related to the humidity during storage (humidity data not shown). Hence, the cherries of all four cultivars became a little darker as well as a little redder during storage, as shown by decreases in L* values and in hue abgle values, during both the cool (1.5 ± 0.5 ºC) and the ambient (15 ± 5 ºC) storage periods. However, the changes in colorimetric parameters varied depending on the storage temperature and the anthocyanins composition in the different cherry cultivars. Refrigerated temperatures greatly improved colour stability of the cherries. Differences in hue angle could be attributed to both differences in anthocyanin and phenols composition, and to interaction of anthocyanins with other compounds at the relatively lo pH of the fruits (intermolecular co-pigmentation). Co-

pigmentation is optimal in the range pH 3–5 (Brouillard *et al.*, 1991), and is known to decrease with increasing temperature (Baranac *et al.*, 1996). In addition, the low pH increases the stability of anthocyanins (Eiro and Heinonen, 2002). On this basis we ascribe the fact that cherries can be stored for at least one month at 1.5 ± 0.5 ºC without degradation of anthocyanins to be due to stabilization by pH and co-pigmentation. However, to firmly establish the mechanism behind the stabilization of the colour of the cherries during storage, further chemical analysis including LC–MS and NMR analysis are warranted.

7.4.3. Influence of pH on Cherry Colour

The decrease in chromatic parameters was associated with a weak increase in the pH (Tables 7.1 and 7.2; Gonçalves *et al.*, 2004). The same results were observed by Heredia *et al.* (1998), who found that the chroma defined in the uniform colour spaces, underwent a linear decrease as pH increased. The influence of pH on fruit colour is well known. As pH increases, the colour of anthocyanins moves to the non-spectral purple and approaches a progressive loss of colour.

7.4.4. Correlations and Regressions between Anthocyanins Content and Colour

With all the cherry cultivars, the levels of anthocyanins correlated negatively with each of the colour parameters *L**, *a**, *b**, chroma and hue angle (Table 7.3). The steepest and most significant correlations were found for the cultivars having highest levels of anthocyanins, namely Burlat and Van. The different correlation coefficients obtained for the different cherry cultivars means that the evolution of colour during storage varied among the different cherry cultivars. Some of the main aims of the present work were to assess the evolution of colour in relation to anthocyanin profiles in different cherry cultivars, and notably to unravel any differences in the anthocyanin-colour relationships among different cherry cultivars. For this reason the number of samples taken from each cultivar is not sufficient to build a valid mathematical function to robustly predict the colour evolution during storage of each different cultivar. Thus, for a more comphreensive mathematical function describing the colour development in individual cherry cultivars, collection of more data from a large number of samples is recommended. The next step in such studies will be to predict the colour evolution and select the optimal storage mode for individual cherry cultivars.

When lumping the data for all four cultivars, all the negative correlations obtained statistical significance. The chromatic parameters *L**, *a**, *b**, chroma and hue angle correlated negatively ($P < 0.001$) with the total anthocyanins levels, and with total phenols

(P < 0.01) (Table 7.4). In all cases, the lowest values of chroma and hue angle corresponded to the samples having the highest anthocyanins content. It seems logic that yellowness (b*) and lightness (L*), and consequently hue and chroma values may correlate negatively with the anthocyanins levels, but it is more complex to understand why an increase in pigments causing redness gives lower redness value readings, i.e. decreased a* values. This phenomenon was investigated and discussed in an early study on dark coloured fruit beverages by Eagerman, Clydesdale and Francis (1973). They clearly demonstrated the presence of an 'inversion area', where the increase anthocyanin pigment concentration, cyanidin-3-glucoside, where both the L*, a* and b* values failed to correlate as expected to increases in the pigment concentration. In brief, this phenomenon is presumed to occur when increased pigment concentration both darkens the sample (e.g., the fruit or the fruit beverage) and increases the chroma. When this occurs, the colour scales are no longer tied linearly to the luminous transmittance (Eagerman *et al.*, 1973). In our present work, the anthocyanins did indeed darken the cherries as their concentration increased, and therefore the chromaticity responses were no longer linear, and might, in fact, have reverted to correlate negatively. The darker the cherries, the more negative the correlation to anthocyanins levels. According to Little (1975) and McGuire (1992), hue angle and chroma give more information about spatial distribution of colours. Indeed, better correlation between these parameters and pigment concentrations have been obtained than when pigment concentrations were compared directly with the values from the colorimeter (McGuire, 1992).

Table 7.3
Highlighting the different correlations between total anthocyanins and evolution of colour parameters (L*, a*, b*), chroma (C*) and hue angle (H*) of ripe cherries during storage among cultivars.

	L*	a*	b*	C*	H*
Total anthocyanins					
Burlat	–0.739	–0.878*	–0.819*	–0.871*	–0.843*
Saco	–0.702	–0.685	–0.734	–0.699	–0.768
Summit	–0.675	–0.721	–0.733	–0.729	–0.774
Van	–0.665	–0.839*	–0.727	–0.823*	–0.744
Sum anthocyanins all cultivars	–0.675**	–0.791***	–0.729***	–0.784***	–0.745***

* indicates P < 0.05; ** indicates P < 0.01; *** indicates P < 0.001 by Fisher's test.

For each relationship between total anthocyanins levels and chromatic parameters of the four sweet cherry cultivars, the regressions with highest determination coefficients and with significant (P < 0.05) regression coefficients were selected (Figure 7.3). The best fit-

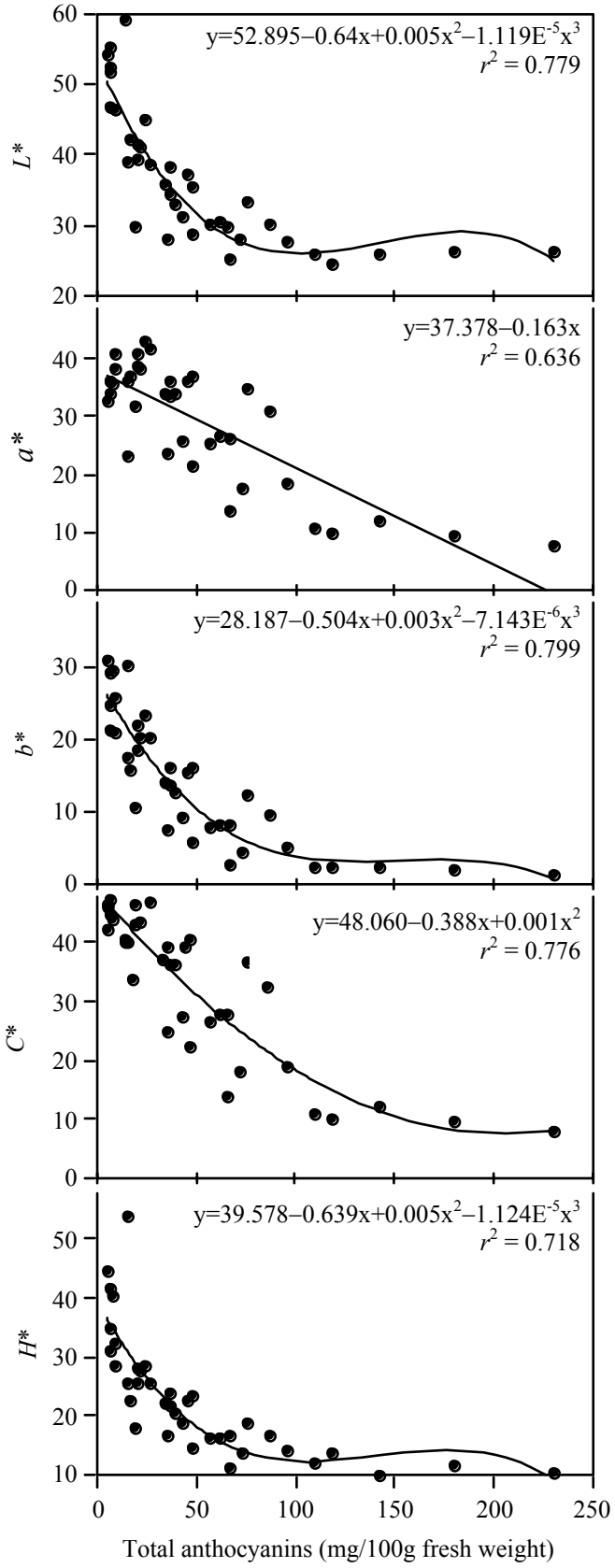


Figure 7.3

Relationships between total anthocyanins and chromatic parameters of the four sweet cherry cultivars (n = 39). All the regression coefficients were significant (P < 0.05).

adjustment (lower scattering) was found in samples having less than 100 mg 100 g^{-1} of fw of total anthocyanins. This evidence suggested that the measure of chromatic parameters could be a good tool to predict the levels of anthocyanins in storage cherries and predict the beneficial human health effects of each sweet cherry cultivar, since anthocyanins exert antioxidant activity (Lapidot *et al.*, 1999; Wang *et al.*, 1999; Matsumoto *et al.*, 2002). Therefore, for cherries for human consumption, it seems important to have a simple and non-destructive technique for anthocyanins content determination, and in this way easily and quickly assess and monitor cherry quality on a large number of cherries.

Table 7.4
Correlation matrix between anthocyanins (total and individual anthocyanins) and total phenols with chromatic coordinates (*L**, *a**, *b**), chroma (*C**) and hue angle (*H**) of the four cherry cultivars during storage.

	*L**	*a**	*b**	*C**	*H**
Cyanidin-3-glucoside	–0.548***	–0.709***	–0.629***	–0.729***	–0.548***
Cyanidin-3-rutinoside	–0.710***	–0.779***	–0.772***	–0.831***	–0.702***
Peonidin-3-glucoside	–0.449**	–0.660***	–0.529***	–0.657***	–0.435**
Peonidin-3-rutinoside	–0.533***	–0.732***	–0.624***	–0.742***	–0.539***
Pelargonidin-3-rutinoside	–0.684***	–0.724***	–0.735***	–0.776***	–0.667***
Total anthocyanins	–0.697***	–0.797***	–0.768***	–0.842***	–0.692***
Total phenols	–0.533***	–0.408**	–0.506***	–0.475**	–0.520***

** indicates $P < 0.01$; *** indicates $P < 0.001$ by Fisher's test.

7.5. Conclusions

The chromatic parameters *L**, *a**, *b**, chroma and hue angle correlated negatively with the total anthocyanins levels and with the total phenols. In stored cherries the evolution of colour is a direct result of an increase in the levels of anthocyanins, particularly in the dominant anthocyanins cyanidin-3-rutinoside and -glucoside. Despite the variation on the anthocyanin levels in different cherry cultivars, which induces some variation in the degree of correlation between total anthocyanins and colour parameters, it is clear that colour measurements provide an easy assessment of the relative levels and changes of anthocyanins in different cherry cultivars during storage – and *vice versa*.

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CAPÍTULO 8

STORAGE AFFECTS THE PHENOLIC PROFILES AND ANTIOXIDANT ACTIVITIES OF CHERRIES (*Prunus avium* L.) ON HUMAN LOW DENSITY LIPOPROTEINS

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8. STORAGE AFFECTS THE PHENOLIC PROFILES AND ANTIOXIDANT ACTIVITIES OF CHERRIES (*Prunus avium* L.) ON HUMAN LOW DENSITY LIPOPROTEINS

8.1. Abstract

Four sweet cherry cultivars (cvs.), Burlat, Saco, Summit and Van, were analysed at harvest and after storage at 1–2 ºC and 15 ± 5 ºC for 30 and 6 days, respectively. Phenolic profiles in methanolic extracts of freeze-dried samples of the fresh and differently stored cherries were quantified by high-performance liquid chromatography. Hydroxycinnamates dominated in all samples and represented 60–74% by weight of the phenols in the fresh and stored samples of the cvs. Saco, Summit and Van, and 45% by weight of the phenols in the cv. Burlat samples, which were richer in anthocyanins. The relative and total levels of hydroxycinnamates, anthocyanins, flavonols and flavan-3-ols varied among cultivars and during storage. Storage at 15 ± 5 ºC increased the phenol levels, particularly the cyanidin-3-rutinoside concentration. Cold storage induced decreased total phenol levels in the cvs. Summit and Van but increased total phenol levels in the cvs. Burlat and Saco. Phenolic cherry extracts inhibited low-density lipoprotein oxidation *in vitro* in a dose-dependent manner. Extracts of freshly harvested cherries exhibited significantly higher antioxidant activities than extracts of stored samples. The cv. Summit samples had the highest antioxidant activity. Differences in the antioxidant effects of the cherry samples were positively correlated with their levels of *p*-coumaroylquinic acid ($P < 0.1$) but negatively correlated with their cyanidin-3-rutinoside levels ($P < 0.05$).

Key words: hydroxycinnamates, anthocyanins, *p*-coumaroylquinic acid, catechin, cherries, storage, antioxidant activity.

8.2. Introduction

Epidemiological studies strongly suggest that consumption of plant foods rich in flavonoids and other phenolic phytochemicals is associated with reduced chronic disease risk, including lowered risk of certain cancers (Steinmetz, 1991), less atherogenesis, reduced incidence of heart disease, and lowered heart disease mortality (Criqui and Ringel, 1994; Hertog *et al.*, 1995; Knekt *et al.*, 1996; Ness and Powles, 1997). Oxidative modification of low-density lipoproteins (LDL) is considered conducive to atherogenic plaque formation, and LDL oxidation has been proven to be an important step in the development of cardiovascular disease (Steinberg, 1997). Thus protection of LDL against oxidation appears to be a

particularly important mechanism for prevention of heart disease (ITFPCHD, 1998). The health-protective effects of flavonoids and other phenolic phytochemicals may therefore be at least partly due to the antioxidant properties of these components (Hertog *et al.*, 1995; Knekt *et al.*, 1996, Ness and Powles, 1997).

Sweet cherries contain approximately 1500 mg total phenols kg^{-1} fresh weight, where the phenols comprise mainly hydroxycinnamates, anthocyanins, flavan-3-ols (catechins) and flavonols, with hydroxycinnamates constituting the main portion of the phenolics in the commonly consumed varieties (Herrmann, 1989; Gao and Mazza, 1995; Heinonen *et al.*, 1998).

The types of phenolic compounds found in cherries have all been demonstrated individually to inhibit lipid peroxidation of human LDL *in vitro*, with certain phenolics being significantly more potent antioxidants than others (Nardini *et al.*, 1995; Teissedre *et al.*, 1996; Meyer *et al.*, 1998ab). Furthermore, the antioxidant potency of wines, grape juices, fresh grapes and various berry extracts on human LDL in test tube assays has been shown to correlate with the presence of distinct types of phenols and, in turn, with their relative abundance in the particular sample being tested (Frankel *et al.*, 1995; Ghiselli *et al.*, 1998; Heinonen *et al.*, 1998). A number of reports have documented that the antioxidant activity of phenolic mixtures may exceed the expected activity as calculated from the sum of the antioxidant activities of the individual phenols, which indicates that synergistic effects may occur among phenolics in mixtures (Miller and Rice-Evans, 1997). Antagonistic interactions cannot be ruled out either (Meyer *et al.*, 1998a). However, despite differences in their phenolic composition, phenolic extracts of two different varieties of fresh cherries, cv. Bing and cv. Burlat, were shown to exert similar antioxidant activities on human LDL oxidation *in vitro* when tested at equimolar, micromolar concentrations of total phenols (Heinonen *et al.*, 1998). In contrast, on lecithin liposomes the antioxidant potency of the same cherry extracts varied significantly (Heinonen *et al.*, 1998). Although it was shown that the overall antioxidant potency of different berry extracts *in vitro* on human LDL correlated with the presence of anthocyanins but not with the levels of hydroxycinnamates (Heinonen *et al.*, 1998), there were not enough evaluations on the extracts of sweet cherries to establish any relationship between their antioxidant potency and their particular phenolic composition. In addition, all previous studies on the phenolic composition and antioxidant activity of sweet cherries were done with extracts of freshly picked cherries. In commerce, however, cherries are often stored at cold temperatures, e.g., 2–5 ºC, for up to several weeks prior to wholesale and consumption in order to stretch the short, seasonal supply for the fresh

market. Very little is known about how such storage affects the phenolic profiles and potential antioxidant and health protective effects of cherries.

The purpose of this work was to study the antioxidant effects of cherry phenolics using a copper catalysed human LDL oxidation system *in vitro*. A main objective was to identify any possible differences in the antioxidant potency of four different cherry cultivars used commercially, and notably to determine the influence of storage of these cherries on their phenolic profile and antioxidant activity. Hence the overall aim was to relate any eventual differences in antioxidant activity to the phenolic composition and to any changes occurring therein during storage of cherries.

8.3. Materials and Methods

8.3.1. Cherry Samples

Four sweet cherry (*Prunus avium* L.) cultivars (cvs.), Burlat, Saco, Summit and Van, were harvested by hand from an orchard in Vila Real, Portugal. The cherries were harvested from different locations on the trees and randomly reorganised into three groups: group I, for immediate analysis; group II, for cold storage at 1–2 ºC and 90% RH for 30 days (cool temperature); group III, for storage at room temperature (15 ± 5 ºC) for 6 days (room temperature). Details on the quality criteria decisive for picking as well as a more elaborate examination of the evolution of phenolics in the four different cherry cultivars during storage, in addition to effects of maturity stage at picking, are reported separately (Gonçalves *et al.*, 2004).

8.3.2. Chemicals and Reagents

Chlorogenic acid, *p*-coumaric acid, catechin, epicatechin, gallic acid, rutin and human LDL were obtained from Sigma-Aldrich Chemicals (St Louis, MO, USA). Anthocyanin glucosides were purchased from Polyphenols A/S (Stavanger, Norway). Folin–Ciocalteu's phenol reagent, copper sulphate, buffer salts used in the LDL oxidation and in high performance liquid chromatography (HPLC) analysis, as well as HPLC-grade acetonitrile were purchased from Merck (Darmstadt, Germany).

8.3.3. Extraction of Phenols

Whole, individual cherries were cut in half, the pits were removed and the samples were then frozen in liquid nitrogen, crushed and lyophilised. For extraction of phenols, freeze-dried cherry samples (0.5 g) were contacted with 60% v/v aqueous methanol (5 ml), flushed with N_2 and extracted for 10 min using a shaking (200 rpm) water bath at 25 ºC.

The individual samples were then first filtered through one layer of Whatman No 1 filter paper (using vacuum suction during the filtration) and subsequently through a 0.45 μm syringe-tip hydrophilic Durapore filter (Millipore Corp, Bedford, MA, USA) prior to HPLC analysis and LDL antioxidation assessment.

8.3.4. Phenol Analyses

HPLC analysis was carried out according to the procedure described by Lamuela--Raventós and Waterhouse (1994) using a Hewlett Packard (Houston, TX, USA) 1100 system equipped with a diode array detector and a Nova-Pak C18 column (3.9 mm × 150 mm, Waters, Milford, MA, USA) and controlled by a personal computer with HPChemstation software. The phenolic compounds were identified by spectral and retention time analysis. The quantities of the different phenolic compounds were assessed from peak areas and calculated as equivalents of seven representative standard compounds (linear regression curves of authentic standards) as follows: at 280 nm (flavan-3-ols), catechin and epicatechin respectively; at 316 nm (hydroxycinnamates), neochlorogenic and chlorogenic acid as chlorogenic acid equivalents and *p*-coumaroylquinic acid as *p*-coumaric acid equivalents; at 365 nm (flavonols), quercetin glucosides as rutin equivalents; at 520 nm (anthocyanins), cyanidin-3-glucoside and cyanidin-3-rutinoside, respectively. The concentration of total phenols in the cherry extracts was determined on the same extract as that used for HPLC analysis and quantified according to the Folin–Ciocalteu procedure with phenols expressed as mg dm^{-3} gallic acid equivalents (GAE).

8.3.5. Inhibition of Human LDL Lipid Peroxidation

Antioxidant activities were assessed by direct spectrophotometrical monitoring of the retardation of conjugated diene lipid hydroperoxide formation at 234 nm during copper-induced oxidation of human LDL by a procedure slightly modified from Esterbauer *et al.* (1989). Absorbances were recorded every 30 s for 5 h with LDL standardised to 0.05 mg protein ml^{-1} in 0.01 M phosphate-buffered saline (0.15 M NaCl) pH 7.4 (PBS), at 37.0 ºC, 5 μM $CuSO_4$. All experiments were performed with LDL from the same lot. Prior to oxidation the LDL was diluted in PBS but not dialysed (Scheek *et al.*, 1995). To determine their antioxidant activity, the cherry extracts were evaluated randomly at equimolar concentrations of 10.0, 12.5, 15.0 and 20.0 μM GAE (by Folin–Ciocalteu) in the LDL assay. For all cherry samples and for gallic acid used as a positive control the different concentrations tested were diluted in doubly distilled water to add equal sample sizes of 10 μl to the reaction mixture (1.8 ml). The results were calculated after duplicate analysis and

expressed as the net prolongation of the lag time relative to the lag time of the control with no cherry extract added. The influence of differences in phenolic composition on antioxidant activity was determined by correlating the relative percentage of inhibition exerted by the different cherry extracts at 12.5 μM with the concentrations of individual phenolic components in the LDL assay by linear regression analysis by a methodology modified from that used by Frankel *et al*. (1995). In brief, the relative percentage inhibition of oxidation was calculated by setting the highest net lag time prolongation value (79.9 min) as 100% and adjusting the other inhibition data accordingly. Linear correlation analysis was then done for each individual phenolic compound in each cherry extract by comparing the level of that compound in the LDL assay mixture (when added at 12.5 μM GAE) with the relative inhibition exerted by the corresponding cherry extract.

8.3.6. Statistics

Differences in antioxidant activities were determined by one-way analysis of variance (Minitab Statistical Software, Addison-Wesley, Reading, MA, USA). The statistical significance of the correlations was established by the dose–response F-test (Berry and Lindgren, 1996).

8.4. Results and Discussion

8.4.1. Phenolic Composition of Cherry Cultivars

The concentrations of total phenols in the extracts of freshly picked cherries ranged from ~ 525 to 1000 mg dm^{-3} (Table 8.1). Assuming that the phenolic extraction was complete, these levels corresponded to a range of 525–1000 mg kg^{-1} fresh, pitted cherries (wet weight). Among the four cherry cultivars studied, the cv. Saco was richest in total phenols, with the extract of the freshly harvested cherries containing 1043 mg dm^{-3}, while the extracts of the other three cultivars contained almost equal levels (526–647 mg dm^{-3}) at day 0 prior to storage (Table 8.1). The Folin–Ciocalteu method is principally a measurement of reducing capacity of phenolic hydroxyl groups rather than an absolute quantitative measurement of phenolic substances (Singleton and Rossi, 1965). Nevertheless, the total levels of phenols as determined by HPLC were generally in good agreement with the total phenol levels obtained by the Folin–Ciocalteu method. The slight differences observed between the estimates obtained by the two different methods are presumably due to the principal differences between the two methodologies.

Except in the extracts of the cv. Burlat, where the levels of anthocyanins and hydroxycinnamates were almost equal, hydroxycinnamates were the main type of phenols in the cherry extracts. In the extracts of the cvs. Saco, Summit and Van, neochlorogenic acid

was the main compound, while in the cv. Burlat extracts *p*-coumaroylquinic acid was the dominant hydroxycinnamate, constituting 20–25% by weight of the phenolics (Table 8.1). The extracts of the cv. Summit also contained comparatively high levels of *p*-coumaroylquinic acid (16–25% by weight of the total phenols), and the relative and absolute levels were highest at day 0. Chlorogenic acid was also present in the extracts of all four cultivars, but at relatively low levels (Table 8.1). At a concentration averaging more than ~ 600 mg dm^{-3}, the contents of neochlorogenic acid were particularly high in the extracts of the cv. Saco fruits, while the relative contents of *p*-coumaroylquinic acid were low. The extracts of the cv. Van contained almost the same relative distribution of hydroxycinnamates as those of the cv. Saco, although the absolute levels of hydroxycinnamates were lower in the cv. Van extracts than in the cv. Saco extracts (Table 8.1). The major colourless phenolics in sweet cherries have previously been shown to be neochlorogenic acid (24–128 mg 100 g^{-1}) and *p*-coumaroylquinic acid (23–131 mg 100 g^{-1} pitted cherries) (Gao and Mazza, 1995). Hence the observed presence of neochlorogenic, *p*-coumaroylquinic and chlorogenic acids in sweet cherries, including the dominance of neochlorogenic and *p*-coumaroylquinic acids, fully agrees with previously published data on phenols in sweet cherries (Herrmann, 1989; Gao and Mazza, 1995).

Table 8.1
Levels (mg dm^{-3})[a] of main phenolic compounds[b] in cherry cultivars as influenced by storage at room temperature (15 ± 5 ºC) and cool temperature (1–2 ºC).

	Hydroxycinnamic acids			Anthocyanins		Flavonol	Flavan-3-ols		Total[c]	
	neochlorogenic	*p*-coumaroyl quinic	chlorogenic	cy-3-glu	cy-3-rut	rutin	catechin	epicatechin	HPLC[‡]	F–C[d]
Burlat										
day 0	98 (18.7)	121 (22.9)	17 (3.2)	66 (12.5)	130 (24.7)	14 (2.6)	25 (4.8)	21 (3.9)	526	611
15 ± 5 ºC; day 6	82 (18.5)	114 (25.9)	11 (2.4)	45 (10.2)	140 (31.6)	10 (2.1)	11 (2.5)	15 (3.4)	441	529
1–2 ºC; day 30	154 (21.5)	146 (20.2)	25 (3.4)	51 (7.0)	192 (26.7)	19 (2.7)	43 (5.9)	40 (5.6)	719	806
Saco										
day 0	682 (65.4)	54 (5.2)	43 (4.1)	7.2 (0.7)	89 (8.5)	50 (4.8)	56 (5.4)	43 (4.1)	1043	919
15 ± 5 ºC; day 6	694 (53.1)	62 (4.8)	40 (3.1)	12 (0.9)	309 (23.6)	25 (1.0)	46 (3.5)	57 (4.4)	1306	1011
1–2 ºC; day 30	599 (47.0)	53 (4.1)	43 (3.3)	50 (3.9)	277 (21.8)	57 (4.5)	43 (3.4)	121 (9.5)	1273	922
Summit										
day 0	197 (30.7)	165 (25.8)	47 (7.3)	5.3 (0.8)	98 (15.3)	14 (2.1)	36 (5.6)	55 (8.6)	641	757
15 ± 5 ºC; day 6	308 (35.9)	138 (16.0)	49 (5.7)	6.4 (0.7)	230 (26.8)	16 (1.9)	41 (4.7)	49 (5.7)	858	763
1–2 ºC; day 30	123 (23.9)	92 (17.9)	28 (5.5)	5.2 (1.0)	157 (30.7)	21 (4.1)	21 (4.1)	29 (5.6)	513	508
Van										
day 0	412 (63.6)	34 (5.3)	31 (4.8)	4.3 (0,7)	89 (13.8)	20 (3.1)	22 (3.4)	24 (3.8)	647	683
15 ± 5 ºC; day 6	314 (48.8)	31 (4.8)	22 (3.4)	6.9 (1.1)	193 (29.9)	26 (4.1)	20 (3.1)	16 (2.5)	645	613
1–2 ºC; day 30	341 (54.7)	31 (4.9)	25 (3.9)	6.3 (1.0)	151 (24.2)	15 (2.5)	20 (3.2)	23 (3.7)	623	607

[a] Values in parenthesis indicate relative amount (%) of compound in cherry extract.
[b] As determined by high-performance liquid chromatography (HPLC).
[c] Sum of all phenolic compounds.
[d] Folin–Ciocalteu procedure.

With respect to anthocyanins, cyanidin-3-rutinoside was found to be the main type of anthocyanin in all four cultivars, with the contents in the extracts varying between ~ 90 and 300 mg dm^{-3}. Cyanidin-3-glucoside was identified in the extracts of all cultivars, but in much lower amounts (Table 8.1). The glucoside and rutinoside of peonidin and the pelargonidin-3-rutinoside were also identified but represented less than 5% of the total phenolics (Macheix *et al.*, 1990). The contents of flavonols and flavan-3-ols were generally highest in the extracts of the cv. Saco cherries, although in the cv. Summit extracts the absolute as well as the relative contents of both catechin and epicatechin were also rather high, especially at day 0 (Table 8.1).

The findings on the qualitative occurrence of hydroxycinnamates, anthocyanins and flavan-3-ols agree well with the available data on cherry phenols (Macheix *et al.*, 1990; Gao and Mazza, 1995). However, the illustration that the levels of certain phenolics can vary up to 4–5-fold among cultivars—where especially *p*-coumaroylquinic acid and cyanidin-3-glucoside contents were found to vary significantly—is noteworthy.

8.4.2. Changes in Phenolic Profiles During Storage

Storage of cherries for 6 days at 15 ± 5 ºC increased the levels of phenols extracted from the cvs. Saco and Summit but had hardly any influence on the total levels of phenols extracted from the other two cultivars. The increases in the cv. Saco and cv. Summit extracts were mainly due to tripling and doubling respectively of the cyanidin-3-rutinoside levels extracted (Table 8.1). However, with the cv. Summit the extracted levels of neochlorogenic acid increased after storage at 15 ± 5 ºC (Table 8.1). After cold storage for 1 month a similar increase in the levels of anthocyanins as with storage at 15 ± 5 ºC was observed (Table 8.1). The changes in hydroxycinnamates among the four cherry cultivars were not consistent. Notably, the neochlorogenic acid level in the cv. Burlat increased, while cold storage induced decreases in the levels of this compound in the cvs. Saco, Summit and Van (Table 8.1). A similar evolution was observed for the *p*-coumaroylquinic and chlorogenic acid levels with cold storage. In the cv. Summit the decreases in the extracted levels of hydroxycinnamates penetrated to an overall decrease in the total phenols in the extracts after cold storage (Table 8.1). The levels of rutin varied little with storage, except in the cv. Saco extracts, where the rutin levels ranged from 25 to 57 mg dm^{-3}. In the other extracts the total and relative levels of rutin equivalents remained below 26 mg dm^{-3} and thus made up less than 4% by weight of the total phenols in the extracts (Table 8.1). In the cv. Saco the levels of rutin equivalents decreased with ambient storage but remained constant at ~ 50 mg dm^{-3} after cold storage. In contrast, the extracted level of epicatechin decreased in

the cv. Summit after cold storage, while it increased markedly in the cv. Saco after cold storage.

8.4.3. Antioxidant Activities of the Cherry Extracts

The control runs had an average induction time of ~ 22 min. The extracts of the fresh cherries exerted a dose-dependent antioxidant effect in the LDL assay at all the tested addition levels (Figure 8.1). Addition of 20 μM GAE completely blocked the oxidation, but the antioxidant potency varied strongly among the different cultivars, as illustrated in Figure 8.1 for the samples of freshly harvested cvs. Burlat and Summit. When compared at equimolar addition levels of 12.5 μM GAE, the extracts of the freshly harvested cherries consistently exerted the highest antioxidant activity, with the extracts of the cv. Summit cherries standing out as the best, and the cv. Van extracts having the weakest antioxidant potency (Table 8.2).

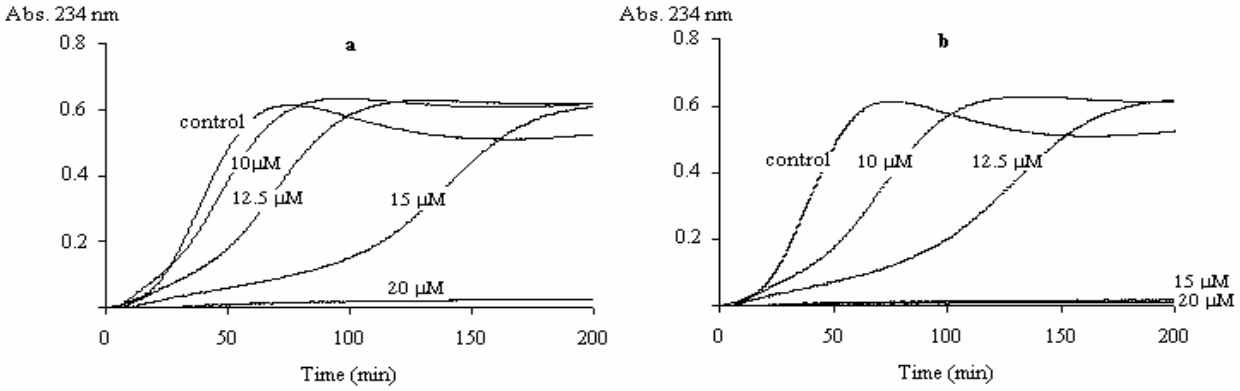

Figure 8.1
Kinetics of inhibition of conjugated diene lipid hydroperoxide formation by cv. Burlat (a) and cv. Summit (b) cherry extracts in copper-catalysed human LDL oxidation.

All cherry extracts were less potent than pure gallic acid added at 12.5 μM. There was no consistent difference in the antioxidant activities of extracts from cherries stored for 6 days at 15 ºC *versus* those of extracts obtained from cherries cold stored for 30 days (Table 8.2). However, with the cv. Van, storage apparently led to a prooxidant activity of the extract. Prooxidant activity on human LDL oxidation *in vitro* was previously observed with white grape juice concentrate (Landbo and Meyer, 2001). The prooxidant effect is a result of reduction of Cu^{2+} to the more prooxidative Cu^{+} by redox-active compounds in the cv. Van extract. This type of redox activation may also occur in the other cherry extracts, but it might have been outcompeted by high radical-scavenging efficiency and metal chelation of the apparently more potent antioxidant phenolics present in the other cherry extracts.

Table 8.2
Antioxidant effect of cherry extracts expressed as net prolongation of lag time relative to control: influence of cherry cultivar and storage at equimolar total phenol concentration (12.5 μM total phenols; LDL oxidation control lag time 21.77 ± 1.21 min).

	Net lag time prolongation ± SD[a] (min)
Burlat	
day 0	32.73 ± 5.70 b
15 ± 5 ºC; day 6	15.97 ± 4.58 c
1–2 ºC; day 30	29.80 ± 2.18 bc
Saco	
day 0	36.25 ± 7.84 b
15 ± 5 ºC; day 6	11.68 ± 2.72 c
1–2 ºC; day 30	18.03 ± 2.77 c
Summit	
day 0	79.90 ± 9.11 a
15 ± 5 ºC; day 6	12.30 ± 0.74 c
1–2 ºC; day 30	3.56 ± 3.24 cd
Van	
day 0	6.34 ± 5.98 cd
15 ± 5 ºC; day 6	Prooxidant
1–2 ºC; day 30	Prooxidant
Gallic acid (positive control)	> 280

[a]Values are average of duplicate assay runs ± standard deviation (pooled SD = 4.58). Different letters indicate significantly different values at $P < 0.05$.

8.4.4. Relations between Antioxidant Activity and Phenolic Composition of the Cherry Extracts

When the cherry extracts were compared on an equimolar basis of total phenols, any differences in antioxidant potency among the extracts must be due to differences in the concentration of individual phenolic constituents in the extracts. Correlation of the relative LDL antioxidant activities obtained at 12.5 μM addition levels of total phenols with the concentration of individual phenols by linear regression analysis revealed that the relative antioxidant potency of cherry extracts correlated positively with their levels of *p*-coumaroylquinic acid ($r = 0.5$, $P < 0.1$) but negatively with their levels of cyanidin-3-rutinoside ($r = -0.6$, $P < 0.05$). In addition, the antioxidant activity tended to correlate positively with the catechin levels, but this correlation did not reach statistical significance ($r = 0.45$, $P > 0.1$). No correlation between antioxidant activity and the levels in the cherry extracts of cyanidin-3-glucoside, epicatechin, rutin and the dihydroxycinnamates was found. Taken together, the data thus indicate that the antioxidant potency of the cherry phenols was distributed among several different phenolic compounds. Nevertheless, the obtained correlations fit well with the finding that the freshly harvested cv. Summit cherries, which

exerted the maximal antioxidant activity, had particularly high levels of *p*-coumaroylquinic acid (albeit only medium levels of catechin) as compared with the other cultivars (Table 8.1). The correlations of antioxidant activity with the presence of particularly high levels of *p*-coumaroylquinic acid also agree well with the following observations (Steinmetz, 1991). The reduction in antioxidant activity of the cv. Burlat extracts was lower after storage at cool temperature than that of the other extracts, which is in accordance with the presence of relatively high levels of *p*-coumaroylquinic acid and catechin in the cv. Burlat extract after cold storage of the cherries (Table 8.1) (Criqui and Ringel, 1994). The extracts of the cold-stored cv. Burlat cherries were also richer in both *p*-coumaroylquinic acid and catechin as well as in neochlorogenic acid (and cyanidin-3-rutinoside) than the extracts of the cv. Burlat cherries stored at room temperature, and the antioxidant activity of the extracts at cool temperature was higher than that of the extracts at room temperature (Hertog *et al.*, 1995). The extracts of the cold-stored cv. Summit cherries had markedly decreased levels of *p*-coumaroylquinic acid and catechin — as well as low neochlorogenic acid, chlorogenic acid and epicatechin concentrations — and exhibited very low antioxidant activity (Knekt *et al.*, 1996). The extracts of the cv. Van cherries all had very low levels of *p*-coumaroylquinic acid and catechin, both absolutely and relatively (Table 8.1), and these extracts exhibited only weak or no antioxidant activity (Table 8.2).

The negative correlation between antioxidant activity and cyanidin-3-rutinoside levels in the cherry extracts was surprising, since cyanidin (i.e., the aglycone) was previously demonstrated to exert potent antioxidant activity against human LDL oxidation *in vitro* (Meyer *et al.*, 1998*a*). The increases in the cyanidin-3-rutinoside levels were not accompanied by concomitant decreases in the levels of any of the other phenolics (Table 8.1). Nevertheless, the negative correlation between cyanidin-3-rutinoside levels and antioxidant activity may be a consequence of decreases in other, non-analysed, antioxidant compounds in the cherry extracts rather than due to a prooxidative effect of the cyanidin-3-rutinoside itself. Hence we propose that the relation is due to declines in the levels of other antioxidant compounds which escaped our HPLC analysis but nonetheless were linked to the increase in the extracted cyanidin-3-rutinoside levels from stored cherries. In accordance with previously reported data on cherries (Heinonen *et al.*, 1998), ascorbic acid was not detected in any of the cherry extracts. The contribution of ascorbic acid to the antioxidant activity of the phenolic cherry extracts could therefore not be considered in this work. Accordingly, the decreased antioxidant activity observed with extracts from stored cherries and, in turn, the negative correlation of antioxidant activity with cyanidin-3-rutinoside levels were presumably not a result of decreases in ascorbic acid levels during storage of the

cherries. Rather, the negative correlation of antioxidant activity with cyanidin-3-rutinoside levels might be related to an expense of non-analysed, polymeric dihydroflavonols or other anthocyanidin precursors. Thus, polymeric dihydroflavonols are presumed to be anthocyanin precursors (Goodwin and Mercer, 1990) and these dihydroflavonols will therefore disappear with an increase in the cyanidin-3-rutinoside levels. Even though the HPLC method employed here can analyse dimeric flavanols, e.g., various procyanidins, larger polyphenolic polymers presumably escape analysis. Assuming that the relevant dihydroflavonol polymers were extracted and that they exerted antioxidant activity, their decrease would be coupled to an increase in the cyanidin-3-rutinoside levels in the extracts of the stored cherries. The negative correlation with antioxidant activity of increased levels of cyanidin-3-rutinoside would thus be explained, especially because the antioxidant activity was always higher for the extracts from freshly harvested cherries (Table 8.2) where the cyanidin-3-rutinoside levels were consistently lowest (Table 8.1).

On the contrary, the finding that the antioxidant activity of cherry extracts tended to correlate positively with the levels of catechin in the mixtures was not surprising, as catechin is known to be one of the most important antioxidant phenols in red wines, exhibiting high antioxidant potency towards human LDL oxidation *in vitro* (Frankel *et al.*, 1995). When tested as a pure compound, catechin also exhibits higher antioxidant activity on human LDL oxidation *in vitro* than pure cyanidin, caffeic acid and quercetin (Meyer *et al.*, 1998a). Catechin contains a 3',4'-*O*-hydroxy group and is therefore expected to exert potent antioxidant activity, as this structure has a particularly high hydrogen donating ability and may also be important for metal chelation (Frankel *et al.*, 1995; Meyer *et al.*, 1998a; Meyer and Frankel, 2001). In contrast, *p*-coumaroylquinic acid has only one hydroxyl group and would therefore not be expected to exert as high an antioxidant activity as the dihydroxy cinnamates neochlorogenic acid and chlorogenic acid, or to exert higher antioxidant activity than the other dihydroxy phenolics present in the cherry extracts (Meyer *et al.*, 1998ab). Hence, when tested individually, the antioxidant activities of free (unconjugated) hydroxycinnamates on *in vitro* human LDL oxidation usually decrease in the order caffeic > sinapic > ferulic > *p*-coumaric acid, i.e., in relation to the decrease in hydroxylation and methylation degree of the aromatic ring (Meyer and Frankel, 2001). The results and set-up of the present study cannot exclude the possibility that the correlation of *p*-coumaroylquinic acid concentration with antioxidant activity reflects a mechanism where *p*-coumaroylquinic acid is important for regenerating the dihydroxyl groups of the other phenolics (e.g., of the neochlorogenic and chlorogenic acids) by direct hydrogen donation. If this is the case, the correlation of antioxidant activity with *p*-coumaroylquinic acid concentration is in fact a

picture of the differences among the cherry samples in such regeneration ability rather than an indication that *p*-coumaroylquinic acid is a more potent antioxidant on LDL than the other phenols in the mixture. This importance of apparently 'minor' antioxidants has been demonstrated previously in the case of ascorbic acid *versus* α-tocopherol in the inhibition on human LDL oxidation (Esterbauer *et al*., 1992). However, as previously pointed out in several reports (Frankel *et al*., 1995; Teissedre *et al*., 1996) the antioxidant activity on human LDL may depend on several factors other than hydrogen-donating ability and metal chelation, notably partitioning efficiency of the antioxidant molecules as well as protein-binding ability (Teissedre *et al*., 1996; Meyer and Frankel, 2001). The latter issue relates to the unique feature of the oxidation of the LDL substrate that the lipid peroxidation of LDL lipids is in fact initiated by oxidative degradation of tryptophan or histidine residues in the LDL apolipoprotein B rather than directly by radical formation in the lipids (Giessauf *et al*., 1995; Retsky *et al*., 1999). Hence structural features conferring differences in the abilities of antioxidants to bind to apolipoprotein B, and hence sterically block the copper access to tryptophans and histidines, may affect the antioxidant activity of phenolics on LDL. No data are available on the antioxidant efficacy of purified *p*-coumaroylquinic acid, but esterification of ferulic and *p*-coumaric acids to acid or sugar moieties was previously shown to enhance their antioxidant activity on LDL, and these results were suggested to result from structural differences conferring differences in molecular mobilities and protein binding of the antioxidant molecules (Ohta *et al*., 1997; Meyer *et al*., 1998b). Lastly, the possibility that slightly different potencies of the extracts could be a result of the discrepancy between the Folin–Ciocalteu determination of total phenols, i.e., assessment of total reducing potential, and the true sum of phenolic antioxidant compounds in the mixtures cannot be excluded. Clearly, further understanding of the antioxidant mechanisms of phenolic fruit phytochemicals on human LDL *in vitro* and *in vivo* is warranted.

Data on the absorption of naturally occurring fruit phenolics and on the possible protective effects *in vivo* of consumption of fruits and other plant foods rich in phenolics are gradually appearing in the literature (Bourne and Rice-Evans, 1999; Stein *et al*., 1999; Keevil *et al*., 2000; Murkovic *et al*., 2000; Manners *et al*., 2003; Nielsen *et al*., 2003). However, limited research on the health effects of cherries has been conducted. Based on the available data on the phytochemical content of cherries, including the data presented in this study, there is a high likelihood that cherries can provide the types of health benefits associated with fruit and vegetable consumption in general. The findings in the present study, that relatively large fluctuations in phenolic contents among different cherry cultivars exist, and that storage also affects the phenol levels and decreases the antioxidant potency of the

cherry phenols, may be useful in the selection of suitable cultivars and in the design of optimal storage for production of cherries with high levels of potentially health-protective phytochemicals.

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CAPÍTULO 9

CONSIDERAÇÕES FINAIS E PERSPECTIVAS DE TRABALHO FUTURO

9. CONSIDERAÇÕES FINAIS E PERSPECTIVAS DE TRABALHO FUTURO

Na investigação apresentada nos capítulos anteriores procurou-se estudar a ecofisiologia de quinze combinações cultivar/porta-enxerto, destacando particularmente o efeito do porta-enxerto e o efeito da cultivar nas características morfo-anatómicas, bioquímicas e fisiológicas de árvores ananicantes *versus* árvores vigorosas. Deste trabalho pôde concluir-se que o porta-enxerto tem uma grande influência sobre a árvore adulta e que as árvores ananicantes têm um comportamento fisiológico distinto do das árvores vigorosas (Capítulos 2, 3 e 5). Estas árvores, comparativamente às árvores ananicantes, apresentaram maiores valores de potencial hídrico (Capítulo 5), devido a uma maior capacidade de absorção pelo sistema radicular (Capítulo 2) associada a maior condutividade hidráulica das raízes e caules (Capítulo 3). As árvores de porta-enxertos mais vigorosos apresentaram também maior capacidade fotossintética, mas menor eficiência intrínseca do uso da água (Capítulo 5). As árvores enxertadas em porta-enxertos ananicantes, apesar da taxa fotossintética e dos crescimentos mais reduzidos, não foram aparentemente afectadas na sua produtividade. Embora a cerisicultura moderna procure porta-enxertos que imprimam menor vigor às árvores do que o tradicional *Prunus avium*, por razões de operacionalidade no pomar, a escolha dos porta-enxertos ananicantes deve ser bem ponderada. De facto, os porta-enxertos ananicantes em estudo (Edabriz e Gisela 5) permitem aumentar a densidade de plantação e a produtividade e diminuir os custos com a mão-de-obra. No entanto, também exigem maiores cuidados de manutenção hídrica para não prejudicar o desenvolvimento da árvore e dos frutos.

Para melhor compreender o efeito do porta-enxerto e da cultivar na fisiologia da cerejeira optou-se pela análise isolada destas origens de variação. Assim, no Capítulo 3, cujo objectivo era avaliar exaustivamente a arquitectura hidráulica da árvore e os seus efeitos funcionais, verificou-se que as raízes, relativamente aos caules das combinações Van/porta-enxerto, apresentaram vasos xilémicos de maior diâmetro, com efeitos directos na condutividade hidráulica relativa. Relativamente ao efeito do porta-enxerto, registou-se nas árvores ananicantes uma maior frequência de vasos xilémicos, mas de menor diâmetro, que levaram à redução da condutividade hidráulica, o que por sua vez terá afectado negativamente o potencial hídrico, as trocas gasosas e o desenvolvimento vegetativo.

Relativamente ao efeito da cultivar nas variações morfo-anatómicas, composição química, trocas gasosas e potencial hídrico das cultivares Burlat e Summit (porte erecto, copa densa) e Van (porte semi-erecto, copa aberta) enxertadas em Edabriz (Capítulo 4), concluiu-se que, apesar das três cultivares apresentarem diferenças significativas ao nível morfo-anatómico e bioquímico, apenas se registaram diferenças ligeiras quer ao nível das

trocas gasosas quer ao nível do estado hídrico devido a uma compensação entre os diversos parâmetros analisados para cada cultivar.

Na segunda parte deste estudo, mais orientada para os parâmetros de qualidade dos frutos, pretendeu-se analisar o efeito da cultivar, do estado de maturação, das condições climáticas do ano e da conservação na qualidade sensorial e nutricional da cereja, através de análises físicas e químicas. Os resultados obtidos revelaram que as características físico-químicas dos frutos são mais dependentes da cultivar, do que do efeito do porta-enxerto (Capítulo 5). De facto, a Summit apresentou frutos extremamente atractivos, com boa coloração, mais pesados e maiores, independentemente do porta-enxerto. A cultivar Burlat produziu frutos menos firmes e com características de acidez e doçura normalmente próprias de cultivares mais temporãs. Todavia, como os seus frutos são dos primeiros a aparecer no mercado, poderão atingir preços mais elevados, levando a uma maior rentabilidade para o produtor. Em contrapartida, as cerejas Saco tinham calibre inferior e maior firmeza, o que as torna mais resistentes ao manuseamento e conservação. A Van, que é uma cultivar de maturação mais tardia, apresentou frutos mais vermelhos e escuros e com maior doçura e acidez, o que lhe confere características qualitativas muito valorizadas pelo consumidor.

No que diz respeito ao efeito da conservação, verificou-se uma diminuição mais acentuada dos parâmetros qualitativos (massa, firmeza e cor) em ambiente natural, evidenciando a imperatividade da utilização de sistemas de refrigeração com atmosfera controlada (1–2 ºC e 90–95% H.R., 2,5% de O_2, 20% de CO_2) logo após a colheita (Capítulo 6).

Relativamente à composição fenólica das cerejas das cultivares Burlat, Summit, Van, e Saco, os estudos realizados (Capítulos 6, 7 e 8) permitiram identificar 4 grupos de compostos fenólicos: ácidos hidroxicinâmicos (ácido neoclorogénico, ácido *p*-cumaroilquínico e ácido clorogénico), antocianinas (cianidina-3-glucósido, cianidina-3-rutinósido, peonidina-3-glucósido, pelargonidina-3-rutinósido e peonidina-3-rutinósido), flavanóis (epicatequina e catequina) e flavonóis (rutina). O conjunto dos três ácidos hidroxicinâmicos revelou-se como a maior fracção de compostos fenólicos presentes nos extractos de cereja, excepto na Burlat, onde as concentrações dos ácidos hidroxicinâmicos e das antocianinas foram similares. Especificamente, a conservação provocou variações nas concentrações de ácidos hidroxicinâmicos, embora a tendência final seja a de uma redução das concentrações quando as cerejas foram armazenadas em atmosfera refrigerada e a de um aumento quando estiveram sujeitas à temperatura ambiente. Igualmente, o teor em antocianinas aumentou durante o período de conservação. Em contrapartida, as concentrações de rutina, epicatequina e catequina permaneceram bastante estáveis durante esse período.

A cultivar Saco apresentou frequentemente os valores mais elevados de fenóis totais. Em geral, as cerejas no estado óptimo de maturação tiveram sempre maior concentração de fenóis totais do que as cerejas ainda parcialmente maduras, as quais, mesmo após o período de conservação a que foram submetidas, nunca atingiram as concentrações das cerejas maduras (Capítulo 6). Isto reforça a ideia de que estes frutos, sendo não-climactéricos, deverão ser colhidos no estado óptimo de maturação para potenciar as suas qualidades organolépticas.

A composição fenólica variou com o ano de colheita, registando-se maiores concentrações de ácidos hidroxicinâmicos em 2001 do que em 2002 (Capítulo 6). O contrário foi verificado para a concentração de antocianinas, o que nos pareceu ser justificado pelas condições climáticas do último terço do crescimento do fruto. Com efeito, neste período, a temperatura média foi de 19 ºC e a insolação de 231 h, bastante acima dos valores verificados em 2002, com temperatura média de 15 ºC e insolação de 171 h. Em 2001, aquelas condições induziram a biossíntese de ácidos hidroxicinâmicos em detrimento das antocianinas, enquanto, em 2002, as temperaturas mais baixas e a menor insolação favoreceram a síntese de antocianinas em prejuízo dos ácidos hidroxicinâmicos.

No Capítulo 7 deu-se particular destaque ao estudo dos parâmetros cromáticos das cerejas das quatro cultivares estudadas. A primeira conclusão foi que esses parâmetros se correlacionaram negativamente com o teor em antocianinas totais (mas não com os fenóis totais). O estudo revelou que as funções cromáticas do croma (C^*) e do ângulo hue (H^*) podem ser utilizadas para prever a evolução da cor e os níveis de antocianinas durante o armazenamento da cereja e, possivelmente, de outros frutos ricos nestes pigmentos. Sendo a determinação da cor uma técnica simples e não-destrutiva, a sua utilização rotineira permitirá inferir sobre o teor em antocianinas num número elevado de amostras.

O último objectivo deste estudo consistiu em avaliar a actividade antioxidante dos extractos de cerejas das quatro cultivares, à colheita e após a sua conservação (Capítulo 8). Os resultados obtidos permitiram realçar a presença significativa de rutina e de outros compostos fenólicos, como a catequina, epicatequina e antocianinas, características estas que fazem deste fruto uma fonte importante de antioxidantes, sobretudo quando consumidas em fresco. Este efeito foi constatado através do grau de inibição da oxidação das lipoproteínas de baixa densidade *in vitro*. Neste âmbito, as cerejas Summit destacaram-se pela sua maior capacidade antioxidante.

As conclusões desta dissertação respondem integralmente aos objectivos que foram traçados. No entanto, novos ensaios, se possível com maior envolvimento pluridisciplinar, são fundamentais para que algumas tendências referidas, assim como outras questões que

entretanto se nos depararam, possam ser devidamente esclarecidas e aprofundadas. Como perspectivas de trabalhos futuros consideramos ser importante: (a) identificar o(s) gene(s) responsáveis pelo efeito ananicante; (b) avaliar as hormonas que estão envolvidas na regulação do efeito ananicante das árvores; (c) estudar anatomicamente a zona de enxertia; (d) determinar as causas de incompatibilidade em combinações cultivar/porta-enxerto; (e) determinar as melhores condições de conservação, incluindo a melhor composição gasosa, de maneira, a aumentar o período de vida útil deste fruto; (f) analisar a actividade antioxidante total de extractos de cereja, *in vitro*, usando diferentes metodologias; (g) estabelecer a biodisponibilidade de vários compostos fenólicos da cereja no homem através da determinação da actividade antioxidante do soro e urina.

ANEXO I

CARACTERÍSTICAS GERAIS DOS PORTA-ENXERTOS ESTUDADOS

Quadro I
Porta-enxertos de cerejeira utilizados neste trabalho.

Porta-enxerto	Espécie/cruzamento	Origem	Vigor
Prunus avium	*Prunus avium*	Europa[1]	Vigoroso[1] (*Vigor 9* [12])
CAB 11E	*Prunus cerasus* [6,8]	Itália[6,8]	Semi-vigoroso[11]
Maxma 14	*Prunus mahaleb* x *Prunus avium* [2,3,8-10]	EUA[2,3,8-10]	Semi-ananicante[2,6,10] (*Vigor 6* [12])
Gisela 5	*Prunus cerasus* x *Prunus canescens* [10]	Alemanha[10]	Ananicante[13] (*Vigor 2–3* [12])
Edabriz	*Prunus cerasus* [7,10]	França[7,10]	Ananicante a muito ananicante[3-5,8,10] (*Vigor 2* [12])

Fonte: [1]Breton (1980); [2]DELBARD (1986); [3]Edin e Tronel (1988); [4,5]Edin (1989ab); [6]Mansergas (1990); [7]Edin (1993a); [8]Godini (1993); [9]INRA (1993); [10]Kappel (1993), [11]Sansavini *et al*. (1993); [12]Edin *et al.* (1997); [13] Saunier *et al.* (1998).

Nota: As referências bibliográficas do Anexo I encontram-se no final da Introdução Geral.

Prunus avium

Porta-enxerto franco encontrado em numerosas regiões da Europa. É classificado como vigoroso, podendo originar árvores de 25 m de altura (IDF, 1980). O sistema radicular é forte, mais ou menos perfurante, consoante as condições do solo sejam mais ou menos favoráveis (Felipe, 1989; Perry, 1990). Breton (1980) considera excelente a ancoragem das cerejeiras neste porta-enxerto, embora em solos pouco profundos o sistema radicular seja pouco perfurante e neste caso a árvore torna-se bastante vulnerável aos ventos fortes. Tem boa adaptação ao solo, mas não tolera os muito asfixiantes e secos (Scheer e Juergenson, 1976). Manifesta boa compatibilidade com as cultivares de cerejeira doce (Breton, 1980) e também ácida (Druart e Trefois, 1991). É, todavia muito heterogéneo, e a entrada em produção é lenta (Edin, 1993b). De acordo com Felipe (1989), é bastante resistente ao frio, mas é sensível aos nemátodes *Pratylenchus vulnus*, assim como à podridão do colo devido a *Phytophthora*, ao *Verticillium* e ao *Agrobacterium tumefaciens*.

CAB 11E

O CAB 11E é um clone de *Prunus cerasus* seleccionado em Bolonha, Itália (Mansergas, 1990; Godini, 1993). É classificado como semi-vigoroso, levando a uma diminuição do crescimento do tronco de 40% quando comparado com o franco. Evidencia-se uma antecipação da época de maturação de alguns dias bem como uma época de maturação dos frutos mais concentrada. Lugli *et al*. (1989) verificaram que o peso do fruto e o seu teor em açúcar são superiores quando se trata do CAB 11E, comparando com o F12.1, e a firmeza da polpa não varia significativamente com os porta-enxertos CAB, F 12.1 e Colt. Todos os clones da série CAB são caracterizados por uma emissão de pôlas muito marcada, o que constitui o principal factor limitante à sua difusão (Sansavini *et al*., 1993).

Maxma 14

Em Forest Grove, Oregon, nos Estados Unidos, Lyle Brooks obteve o Brokforest – Maxma Delbard® 14, por hibridação interspecífica entre *Prunus mahaleb* (SL 64) e *Prunus avium* (DELBARD, 1986; Edin e Tronel, 1988; Godini, 1993; INRA, 1993; Kappel, 1993). Este cavalo tem sido difundido por todos os países produtores, pois é facilmente multiplicado *in vitro*; contudo as técnicas tradicionais de multiplicação dão resultados bastante aleatórios. A compatibilidade é boa com quase todas as cultivares (Edin e Tronel, 1988). É classificado como semi-ananicante e forma árvores de 40–60% do tamanho das árvores em porta-enxertos F 12.1 (Saunier *et al.*, 1998). A sua entrada em produção é rápida e abundante, ao 4º–5º ano (Sansavini *et al.*, 1993; Simon *et al.*, 2004). Kappel (1993) descreve como outras vantagens deste porta-enxerto a boa ancoragem, e a ausência de produção de pôlas. A grande capacidade da adaptação a diferentes tipos de solos, resistência à secura e aos terrenos pesados, são também referidos por Moreira (1996). Este autor aponta-lhe ainda resistência ao *Agrobacterium tumefaciens* e alguma tolerância à clorose, ao *Crown gall*, bem como ligeira sensibilidade à *Phytophthora*.

Gisela 5

O Gisela 5 é um híbrido triplóide (*Prunus cerasus* Schattenmorelle x *Prunus canescens*), resultante de ensaios realizados na Alemanha (Kappel, 1993). É classificado como ananicante, adequado para fornecer árvores com apenas 60–70% do volume da copa de árvores enxertadas no franco (Parente, 2004). A entrada em produção é rápida, ao 2º–3º ano, e a plena produção atinge-se ao 6º ano (Salvador *et al.*, 1993). A eficiência produtiva é elevada (Bujdosó *et al.*, 2004). Saunier *et al.* (1998) referem que é tolerante a vírus, não retouça e permite às cultivares enxertadas formar ângulos de inserção das pernadas e ramos bastante abertos.

Edabriz

O Edabriz é um clone de *Prunus cerasus* seleccionado em França (Edin, 1993c; Kappel, 1993). É classificado como muito ananicante, levando a uma redução de vigor de 60 a 80% do tamanho das árvores (Edin, 1993a; Sansavini *et al.*, 2001), sendo o diâmetro do tronco de 40 a 70% do F 12.1 (Edin, 1989ab). Quanto ao tipo de solos, é muito exigente, embora se adapte a texturas muito variadas (Edin, 1993b; Parente, 2004). Martí *et al.* (1998) obtiveram percentagens elevadas de mortalidade deste cavalo em solos muito calcários e com bastantes elementos grosseiros. Permite a entrada em produção logo ao 2º ano (300 a 1300 kg ha^{-1}), mas é geralmente ao 3º ano que ela tem início (3 a 4 t ha^{-1} –

Edin, 1993a). Ao 4º ano a produção já se situa entre 7 e 16 t ha^{-1}, com frutos de bom calibre. O Edabriz confere assim um forte potencial de produção às cultivares enxertadas, em consequência de maior quantidade de "ramalhetes de Maio" e de flores por metro de ramo, principalmente em madeira de 2 e 3 anos (Edin, 1993a). A produção acumulada por árvore é superior à do F 12.1 (Edin, 1989b) e a colheita possível a partir do chão contribuem para a alta competitividade deste porta-enxerto. No entanto, devem evitar-se as cultivares muito férteis e optar por formas de condução que facilitem a penetração da luz. Requer poda de frutificação no fim do Inverno a partir do 4º ano, para que o calibre dos frutos se mantenha bom, devendo-se privilegiar a produção na base dos ramos de um ano e nos ramalhetes de Maio bem expostos à luz (Edin, 1993a). Permite a cultura protegida, quer seja a produção de frutos em abrigo, a protecção dos frutos contra os pássaros ou de condições climáticas adversas.

ANEXO II

CARACTERÍSTICAS GERAIS DAS CULTIVARES ESTUDADAS

Quadro II
Características gerais das cultivares de cerejeira enxertadas.

Características	Burlat	Summit	Van	Saco
Origem	França[8]	Canadá[1-4]	Canadá[8]	Portugal[7]
Porte	Erecto[9]	Erecto[9]	Semi-prostrado[9]	
Vigor	Bom[8]	Bom[1,2,4,9]	Bom[1]	
Produção	Elevada e regular[6]	Média[8]	Elevada[8]	
Fruto				
Forma	Reniforme[5,6,9]	Cordiforme[1,9]	Reniforme[8,9]	
Peso	7–9 g[5,6] (25–27 mm)[9]	9–15 g[1] (25–28 mm)[9]	7–9 g[8] (25–27 mm)[9]	26–28 mm[10]
Polpa	Média firmeza, sumarenta, com boa qualidade gustativa[6,9]	Rija, com boa qualidade gustativa[1]	Rija, com boa qualidade gustativa[8]	Rija, muito doce e carnuda[7]

Fonte: [1]Saunier *et al.* (1982); [2]Saunier *et al.* (1987); [3]Edin e Tronel (1988); [4]Saunier *et al.* (1989); [5]Plotto (1990); [6]Godini (1993); [7]Nascimento e Luís (1993); [8]Bargioni (1996); [9]Edin *et al.* (1997); [10]Parente (2004).

Nota: As referências bibliográficas do Anexo II encontram-se no final da Introdução Geral.

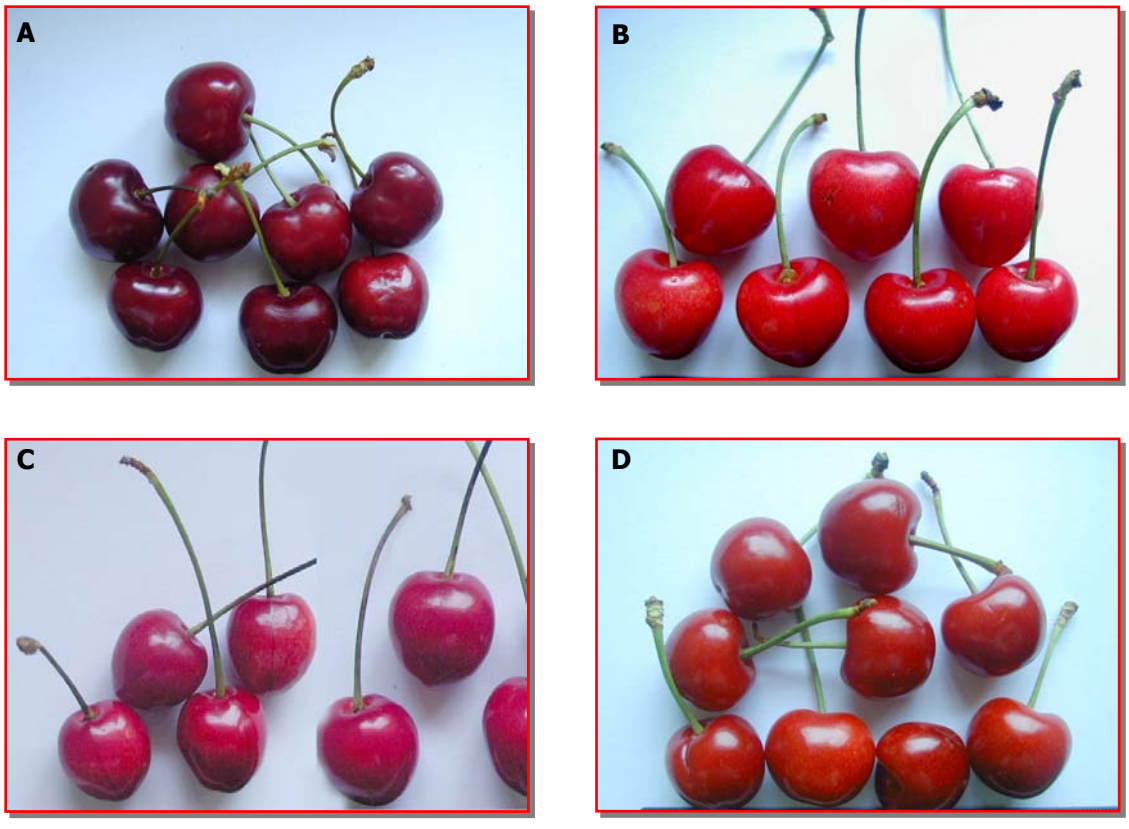

Figura I
Cultivares de cerejeira. A – Burlat; B – Summit; C – Saco; D – Van.