UNIVERZA V LJUBLJANI
BIOTEHNIŠKA FAKULTETA
ODDELEK ZA AGRONOMIJO

Mateja COLARIČ

VSEBNOST IZBRANIH METABOLITOV V LISTIH IN PLODOVIH HRUŠKE (*Pyrus communis* L.) SORT 'WILLIAMS' IN 'CONFERENCE' GLEDE NA ARHITEKTONSKO ZGRADBO RODNE VEJE

DOKTORSKA DISERTACIJA

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THE CONTENTS OF SELECTED METABOLITES IN LEAVES AND FRUITS OF PEAR (*Pyrus communis* L.) CVS. ‘WILLIAMS’ AND ‘CONFERENCE’ REGARDING TO ARHITECTONIC STRUCTURE OF BEARING BRANCH

DOCTORAL DISSERTATION

Ljubljana, 2007

Doktorska disertacija je zaključek podiplomskega študija bioloških in biotehniških znanosti ter se nanaša na znanstveno področje agronomije. Praktični del poskusa je bil opravljen v sadovnjaku Sadjarstva Hudina v Zagaju pri Bistrici ob Sotli. Nadaljnje raziskave in analize so bile opravljene na Katedri za sadjarstvo, Oddelka za agronomijo, Biotehniške fakultete, Univerze v Ljubljani.

Tema in naslov doktorske disertacije sta bila sprejeta na podlagi Statuta Univerze v Ljubljani, po sklepu Senata Biotehniške fakultete in sklepu Komisije za podiplomski študij Univerze v Ljubljani (po pooblastilu senata Univerze v Ljubljani z dne, 14. 2. 2006) dne, 28. 2. 2006. Za mentorico je bila imenovana izr. prof. dr. Metka HUDINA in za somentorja prof. dr. Franci ŠTAMPAR.

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Mateja COLARIČ

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SA HUDINA, Metka (mentorica)/ŠTAMPAR, Franci (somentor)
KZ SI-1000 Ljubljana, Jamnikarjeva 101
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LI 2007
IN VSEBNOST IZBRANIH METABOLITOV V LISTIH IN PLODOVIH HRUŠKE (*Pyrus communis* L.) SORT 'WILLIAMS' IN 'CONFERENCE' GLEDE NA ARHITEKTONSKO ZGRADBO RODNE VEJE
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JI sl/en
AI V raziskavi smo ovrednotili vsebnosti metabolitov v plodovih in listih evropske žlahtne hruške (*Pyrus communis* L.) sort 'Williams' in 'Conference' kot odgovor na upogibanje rodne veje. Petletne rodne veje smo upognili v dveh terminih: pozno poleti 2003 in spomladi 2004. Kontrolno obravnavanje so predstavljale petletne rodne veje, ki jim nismo spremenili kota rasti. Na rodnih vejah smo v letih 2004 in 2005 vzorčili liste v rastni dobi in plodove v tehnološki zrelosti. S tekočinsko kromatografijo visoke ločljivosti (HPLC) smo izmerili vsebnosti določenih ogljikovih hidratov (saharoze, glukoze, fruktoze in sorbitola) in fenolnih snovi (klorogenske, vanilne in sinapinske kisline, epikatehina, katehina, rutina, kvercetin-3-D-galaktozida in kvercetin-3-β-D-glukozida) v listih in plodovih ter organskih kislin (citronske, jabolčne, šikimske in fumarne kisline) le v plodovih. Upogibanje rodnih vej je v obeh letih mnogo bolj vplivalo na vsebnosti fenolov kot na vsebnosti ogljikovih hidratov in organskih kislin. Proučevani sorti sta se različno odzvali na agrotehnični ukrep upogibanja. Zlasti poletno upogibanje je pri sorti 'Williams' zmanjšalo vsebnosti določanih fenolnih snovi v listih, toda pri sorti 'Conference' je zlasti spomladansko upogibanje povečalo vsebnosti fenolnih snovi. V letu 2004 so bile pri sorti 'Williams' največje vsebnosti posameznih fenolov v plodovih, ki smo jih obrali na rodnih vejah upognjenih spomladi 2004 in pri sorti 'Conference' v plodovih na vejah upognjenih poleti 2003. V naslednjem letu je prišlo do preobrata - morda tudi zaradi nižjih temperatur in obilnih padavin - in so bile največje vsebnosti posameznih fenolov pri sorti 'Williams' v plodovih s poletnega upogibanja ali kontrole in pri sorti 'Conference' s spomladanskega upogibanja ali kontrole. V kontrolnem obravnavanju so bile v drugem letu pri obeh sortah značilno večje vsebnosti kvercetin-3-D-galaktozida in kvercetin-3-β-D-glukozida ter sinapinske kisline in epikatehina le pri sorti 'Conference'. V poskusu smo ugotovili razlike v vsebnosti določenih metabolitov med proučevanima sortama, med obravnavanji ter med proučevanima letoma. Na rezultate je vplival tudi čas upogibanja (pozno poletno in spomladansko upogibanje).

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TI THE CONTENTS OF SELECTED METABOLITES IN LEAVES AND FRUITS OF PEAR (*Pyrus communis* L.) CVS. 'WILLIAMS' AND 'CONFERENCE' REGARDING TO ARHITECTONIC STRUCTURE OF BEARING BRANCH
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AB The study investigated the response of ‘Williams’ and ‘Conference’ European pear (*Pyrus communis* L.) subjected to branch bending, according to the contents of various metabolites in their leaves and fruit. Five-year-old branches were bent in the late summer of 2003 and in spring of 2004, and the third treatment with unbent branches was the control. During both growing seasons of 2004 and 2005, leaves from bent and control branches were sampled monthly from May to October and fruit at commercial maturity. The content levels of carbohydrates (sucrose, glucose, fructose and sorbitol) and phenolic compounds (chlorogenic, vanillic and sinapic acids, epicatechin, catechin, rutin, quercetin-3-D-galactoside and quercetin-3-β-D-glucoside) in the leaves and fruit, as well organic acids (citric, malic, shikimic and fumaric acid) in the fruit were measured by high-performance liquid chromatography (HPLC). Branch bending had a greater influence on phenolics than on carbohydrates and organic acids in ‘Williams’ and ‘Conference’ leaves and in the fruit from 2004 and 2005. Both cultivars displayed various responses to branch bending in their levels of metabolites. ‘Williams’ leaves from bent branches, especially those bent in the summer, had lower contents of some phenolic compounds; however, ‘Conference’ leaves from bent branches, especially those bent in the spring, had higher phenolics contents. In the first year, the highest content levels of most phenolics in ‘Williams’ fruit were found in the current spring treatment and in ‘Conference’ fruit in the summer treatment. However, in the next year, the opposite reaction occurred, perhaps because of lower temperatures and abundant rainy weather: the highest content levels of certain phenolics were found in ‘Williams’ fruit in the summer treatment and in the control, while in ‘Conference’ fruit the highest levels occurred in the current spring treatment and in the control. Nevertheless, in 2005 significantly higher content levels of quercetin-3-D-galactoside and quercetin-3-β-D-glucoside were measured in both cultivars, as well as levels of sinapic acid and epicatechin in ‘Conference’ fruit in the control. The differences in content levels of selected metabolites between cultivars and among treatments were confirmed in the research; moreover, the results were affected by the time of bending (late summer and spring) and by the year-to-year.

KAZALO VSEBINE

1 UVOD

Evropska žlahtna hruška (*Pyrus communis* L.) je zelo razširjena sadna vrsta v zmerno toplem podnebnem pasu. Sorti 'Williams' in 'Conference' predstavljata pomemben delež med sortami, ki jih pridelujemo v Sloveniji (Hudina in Štampar, 2000a). Obe sorti se poleg splošnih značilnosti razlikujeta tako v rasti in razraščanju rodnega lesa kot tudi v rodnosti. Sansavini (2002) razvršča sorte evropskih žlahtnih hrušk po rasti in rodnosti v pet modelov: tako spada sorta 'Williams' v model I in sorta 'Conference' v model III.

Med že uveljavljenimi agrotehničnimi ukrepi v nasadih sadnih dreves (gojitvena oblika, rez, prehrana, namakanje) se je upogibanje rodnih vej izkazalo za enega izmed najučinkovitejših ukrepov, s katerim uravnavamo rast in rodnost (Luckwill, 1970). Uravnavanje prekomerne vegetativne rasti in števila rodnih brstov ter s tem posledično tudi pridelka ima še toliko večji pomen zaradi gospodarskih razlogov – zmanjšanja stroškov, saj se je razmerje med stroški pridelave in tržnimi cenami pridelanega sadja v zadnjih nekaj letih povečalo. Upogibanje rodnih vej je dandanes pogost ukrep v intenzivnih nasadih in je vključen tudi v gojitveno obliko sončna os (Costes in sod., 2006).

Z upogibanjem zmanjšamo vegetativno rast, vplivamo na obilnejše cvetenje in večje pridelke ter imamo s tem večje donose v obdobju polne rodnosti (Lauri in Lespinasse, 2001). Podobno ugotavljajo na jablani (*Malus domestica* Borkh.) tudi Grochowska in sod. (2004). Goldschmidt-Reischel (1997) je primerjal dva agrotehnična ukrepa: upogibanje in rez. Drevesa jablan in evropskih hrušk so imela večje pridelke, če so jim uravnali rast in rodnost le z upogibanjem v primerjavi le z rezjo, prvi ukrep pa je vplival tudi na zgodnejšo pridelavo plodov. Upogibanje rodnih vej našija ali azijske hruške (*Pyrus pyrifolia* (Burm.) Nak. je vplivalo na večjo tvorbo cvetnih brstov (Ito in sod., 1999) ter na zgodnejši zaključek vegetativne rasti poganjkov (Banno in sod., 1985). Tudi evropska žlahtna hruška sorte 'Društvenka' se je odzvala na upogibanje s pospešeno tvorbo cvetnih brstov in z zmanjšano rastjo poganjkov (Lawes in sod., 1997).

Odziv sadnih dreves na upogibanje rodnih vej na fiziološkem in biokemičnem področju še ni dovolj raziskan. Do sedaj je bilo nekaj raziskav opravljenih na jablani in našiju. Upogibanje je ugodno vplivalo na fotosintezno aktivnost listov jablane zaradi spremembe kota osvetlitve listov (Pitushkan in Shtirbu, 1985). Ito in sod. (2004) so ugotovili, da upogibanje vej našija vpliva na vsebnost dveh pomembnih primarnih metabolitov - sorbitola in saharoze v lateralnih brstih in v internodijih poganjkov v primerjavi z neupognjenimi vejami. Na našiju povečanje kota poganjka upognjenega navzdol ni vplivalo le na razvoj lateralnih rodnih brstov, temveč tudi na zmanjšano vsebnost indol-3-ocetne kisline (oslabitev apikalne dominance) v poganjkih (Ito in sod., 2001) in v lateralnih brstih teh poganjkov (Ito in sod., 1999) v primerjavi z neupognjenimi poganjki. Z upogibanjem vej so se v le-teh vejah vsebnosti avksinov in giberelinov zmanjšale ter vsebnosti abscizinske kisline in citokininov tipa zeatin povečale. Ker je upogibanje poganjkov spremenilo hormonalno ravnovesje, so bili zato lateralni brsti veliko bolj sposobni, da tekmujejo za asimilate, zato tudi večja tvorba rodnih brstov. Podoben hormonalen odziv na upogibanje rodnih vej jablane sta ugotovila tudi Sanyal in Bangerth

(1998), saj je upogibanje povzročilo od 2 do 2,5-kratno zmanjšanje polarnega transporta avksinov v primerjavi s kontrolnimi - neupognjenimi vejami.

Odziv drevesa na upogibanje se razlikuje med sortami proučevane vrste, pomembno pa je tudi kdaj med letom in pod kolikšnim kotom so bile rodne veje upognjene, pa tudi koliko časa so bile te veje v upognjenem položaju (Lauri in Lespinasse, 2001).

Kar nekaj raziskav je bilo opravljenih s področja spremljanja vsebnosti metabolitov sadnih rastlin (tako primarnih kot tudi sekundarnih). V Sloveniji so bili do sedaj najbolj raziskani plodovi (Veberič in sod., 2005; Veberič in sod., 2007) in listi jablane (Veberič in sod., 2003; Šircelj in sod., 2005; Usenik in sod., 2004). Vsebnosti primarnih metabolitov - ogljikovih hidratov in organskih kislin v plodovih različnih sort evropske žlahtne hruške, pa tudi našija, so z različnih vidikov podrobno proučili Hudina in Štampar (1999; 2000a; 2000b; 2000c; 2004) ter Hudina in sod. (2003). Sekundarni metaboliti v hruškah do sedaj pri nas še niso bili raziskani, v tujini pa so jih proučili mnogi, zlasti v zrelih plodovih (Galvis-Sánchez in sod., 2003), manj pa v listih (Gunen in sod., 2005).

Plod hruške vsebuje največ vode (84 % sveže mase), sledijo ogljikovi hidrati: fruktoza (54 %), sorbitol (18 %), saharoza (15 %) in glukoza (13 %). Zaradi ugodnega razmerja med posameznimi ogljikovimi hidrati ter zaradi precejšnje vsebnosti surovih vlaken (1,5- 2,8 %), plodove priporočajo sladkornim bolnikom namesto slaščic (Blattný, 2003).

Med organskimi kislinami pri večini sort hrušk prevladuje jabolčna kislina, sledi ji citronska kislina; vsebnosti kininske, šikimske, fumarne in oksalne kisline pa so veliko manjše. Jabolčna in citronska kislina prispevata glavni delež k najbolj želeni stopnji kislosti plodov, njuno razmerje pa je povezano s senzoričnimi ocenami okusa (Colarič in sod., 2005). Sorte 'Williams','Red Williams' in 'Rosired' imajo citronske kisline več kot jabolčne kisline (Hudina in Štampar, 2000a). Seveda so vsebnosti metabolitov v plodovih odvisne tako od sorte (Hudina in Štampar, 2000a) kot tudi od zrelosti (Hudina in Štampar, 2000b; Herrmann, 2001). V različnih raziskavah Hudina in Štampar (2000c) ter Hudina in sod. (2003) ugotavljajo, da so vsebnosti ogljikovih hidratov in organskih kislin v plodovih hrušk velikokrat povezane tudi z različnimi agrotehničnimi ukrepi v nasadu.

V plodovih žlahtne hruške so med fenolnimi snovmi (sekundarni metaboliti) izmerili največje vsebnosti klorogenske kisline, hruška pa vsebuje tudi epikatehin, katehin, arbutin, flavonol glikozide (kvercetin in izoramnetin glikozide), procianidine, kavino kislino, *p*-kumarno kislino, ferulno kislino (Amiot in sod., 1995; Escarpa in González, 1999; Schieber in sod., 2001; Ferreira in sod., 2002; Leontowicz in sod., 2003; Galvis-Sánchez in sod., 2003). V plodu imajo fenolne snovi pomembno fiziološko vlogo, med drugim tudi v njegovi odpornosti na mehanski in biološki stres. Poleg tega fenolne snovi prispevajo k senzoričnim lastnostim ploda - aromi, trpkosti, grenkosti in obarvanosti (Macheix in sod., 1990). Še več, fenolne snovi skupaj s surovimi vlakni zmanjšujejo možnost pojava srčno-žilnih bolezni (Gorinstein in sod., 2002).

Evropska žlahtna hruška in tudi druge sadne vrste iz družine *Rosaceae* imajo v listih med ogljikovimi hidrati največje vsebnosti sorbitola in saharoze, manj pa glukoze in fruktoze (Loescher in Everard, 1996). Deguchi in sod. (2002) so ugotovili, da se vsebnosti zgoraj naštetih ogljikovih hidratov v listih japonske hruške povečajo, če so drevesa izpostavljena stresnim razmeram (npr. slana tla in mraz). V listih evropske žlahtne hruške med fenolnimi snovmi prevladujeta klorogenska kislina in arbutin (Gunen in sod., 2005). Slednjega je več pri sortah, ki so odpornejše na hrušev bakterijski ožig (*Erwinia amylovora* (Burill) Winslow et al.). Pomembne fenolne spojine najdene v listih različnih sort hrušk so še epikatehin, katehin, flavonol glikozidi (kvercetin glikozidi), procianidini, *p*-kumarna kislina, … (Andreotti in sod., 2006).

Ogljikovi hidrati so primarni produkt fotosinteze in osnovna organska snov, iz katere se sintetizirajo še druge organske snovi. Lesnate rastline iz ogljikovih hidratov, aminokislin in maščob proizvajajo različne sekundarne metabolite, kar omogoča obrambo pred okoljskimi dejavniki, med katerimi so fenolne snovi ene najpomembnejših. Mnoge raziskave so pokazale, da fenolne snovi vplivajo na rast rastline tako, da pospešujejo ali pa zavirajo delovanje hormonov (medsebojne interakcije) (Kozlowski in Pallardy, 1997; Taiz in Zeiger, 2006).

Kemična sestava različnih organov sadnih rastlin ni odvisna le od vrste, sorte in podlage (Amiot in sod., 1995; Hudina in Štampar, 2000a; Colarič in sod. 2005), temveč tudi od okoljskih dejavnikov (Hudina in Štampar, 1999; 2004) in različnih agrotehničnih ukrepov (gojitvena oblika, rez, prehrana, škropljenje, namakanje….) opravljenih v nasadu (Hudina in Štampar, 2000c; Hudina in sod., 2003). Ker je upogibanje tudi agrotehnični ukrep, smo v našem poskusu želeli ugotoviti ali z njim vplivamo na vsebnosti primarnih (ogljikovi hidrati in organske kisline) in sekundarnih metabolitov (fenolne snovi) v plodovih in listih sort 'Williams' in Conference', ki sta najbolj razširjeni sorti hrušk pri nas.

V svetovnem merilu do sedaj še ni poznanih raziskav evropske žlahtne hruške z biokemičnega področja (vsebnosti metabolitov v njenih organih), kjer izvajamo upogibanje rodnih vej. Kot smo omenili že na začetku, je upogibanje rodnih vej v nasadih že ustaljena praksa sadjarjev, s katerim zmanjšamo rast poganjkov in pospešimo tvorbo cvetnim brstov. Z upogibanjem vplivamo na arhitektonsko zgradbo rodne veje, saj s spremembo kota spremenimo njen položaj v krošnji. Najbolj arhitektonsko raziskano sadno drevo je jablana; manj pa oreh, breskev, marelica, češnja, oljka in hruška (Štampar in sod., 2005).

Obe sorti, ki sta bili vključeni v poskus, sta cepljeni na podlago kutina MA in sta bili posajeni v letu 1987 v nasadu Sadjarstva Hudina v Zagaju pri Bistrici ob Sotli. Poskus je zajemal naslednja obravnavanja:

- rodne veje upognjene 1. septembra v letu 2003 (pozno poleti 2003) pod kotom 120° od navpične lege,
- rodne veje upognjene 15. maja leta 2004 (pomlad 2004) pod kotom 120° od navpične lege ter
- kontrola - rodne veje, ki niso upognjene (izraščajo pod kotom 45° od navpične lege.

V vsako obravnavanje je bilo vključenih 10 dreves s po 1 rodno vejo na drevo (primerljivih lastnosti), ki izraščajo iz provodnika in so imele pri meritvi prirasta v aprilu 2003 popolnoma razvit eno-, dve-, tri-, štiri- in petletni les. Rodne veje, ki so bile vključene v poskus, v letih 2003, 2004 in 2005 niso bile rezane. Rodne veje, ki smo jih upognili, so pred tem izraščale pod kotom 45º, tako kot kontrolne veje. V dveh zaporednih letih (2004 in 2005) smo pri obeh sortah vzorčili razvite liste med rastno dobo (mesečno od maja do oktobra) ter plodove v tehnološki zrelosti za kemične analize vsebnosti izbranih primarnih in sekundarnih metabolitov.

Postavili smo naslednje hipoteze:

- med proučevanima sortama hrušk 'Williams' in 'Conference' bodo razlike v vsebnosti izbranih primarnih in sekundarnih metabolitov (posebej značilna slika za vsako sorto) v listih in plodovih, ker se proučevani sorti hrušk 'Williams' in 'Conference' razlikujeta tako v morfoloških lastnostih (po rasti in nameščenosti rodnih brstov) kot tudi v pomoloških lastnostih ploda,
- med rastno dobo se bodo vsebnosti ogljikovih hidratov in fenolnih snovi v listih spreminjale,
- upogibanje vej bo vplivalo na vsebnost sladkorjev in fenolnih snovi v listih v primerjavi s kontrolo,
- upogibanje vej bo vplivalo na vsebnost sladkorjev, organskih kislin in fenolnih snovi v plodovih (notranjo kakovost plodov) v primerjavi s kontrolo,
- proučevani sorti hrušk 'Williams' in 'Conference' se bosta na upogibanje različno odzvali v vsebnosti izbranih primarnih in sekundarnih metabolitov, tako v listih kot tudi v plodovih,
- pričakujemo razlike v vsebnosti fenolnih snovi, saj so iz različnih podrazredov: fenolne kisline (hidroksicimetne in hidroksibenzojske kisline), flavonoidi (flavan-3-oli in flavonol glikozidi),
- da bodo vidne razlike med dvema zaporednima letoma, saj ne smemo zanemariti vpliva okoljskih dejavnikov.

Glede na to katero upogibanje bo dalo boljše rezultate (vsebnosti posameznih metabolitov v plodovih in listih v dveh zaporednih letih), pa bomo lahko predvideli za proučevani sorti 'Williams' in 'Conference' najprimernejši čas upogibanja.

2 ZNANSTVENI ČLANKI

2.1 SPREMEMBE VSEBNOSTI SLADKORJEV IN FENOLNIH SNOVI V LISTIH HRUŠKE SORTE ‘WILLIAMS’ MED RASTNO DOBO

COLARIČ Mateja, ŠTAMPAR Franci in HUDINA Metka
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Ogljikovi hidrati so neposreden produkt fotosinteze v listih, glavna skladiščna snov in osnovna organska snov, iz katerih nastaja večina ostalih snovi v rastlini. Fenolne snovi imajo pomembno vlogo pri obrambni funkciji rastlin proti boleznim in škodljivcem. Precej fenolnih snovi se tvori preko vmesnega produkta fenilalanina, ki povezuje primarni in sekundarni metabolizem. Kar nekaj raziskav je bilo narejenih v povezavi s spremljanjem sezonskega gibanja ogljikovih hidratov v listih sadnih dreves iz družine *Rosaceae* med rastno dobo, toda spremljanje gibanja fenolnih snovi je manj pogosto. Evropska žlahtna hruška (*Pyrus communis* L.) je iz tega vidika še precej malo raziskana sadna vrsta, zato smo izvedli raziskavo gibanja vsebnosti določenih sladkorjev in fenolnih snovi med majem in oktobrom 2004. Analize smo opravili s tekočinsko kromatografijo visoke ločljivosti (HPLC). Podobno kot pri drugih sadnih vrstah iz družine *Rosaceae* je bil sorbitol (alkoholni sladkor) po vsebnosti najpomembnejši določani sladkor v listih hruške sorte ‘Williams’. Znotraj določanih fenolov smo izmerili največjo vsebnost klorogenske kisline, po vsebnosti sta ji sledila rutin in epikatehin. Med rastno dobo (6 vzorčenj) smo ugotovili statistično značilne razlike v vsebnosti sorbitola, saharoze, glukoze in v vsebnosti vseh analiziranih fenolov. Najmanjše vsebnosti posameznih snovi so bile v listih z začetka rastne dobe. Z izjemo sorbitola, so bile največje vsebnosti sladkorjev v oktobru. Med rastno dobo so vsebnosti posameznih fenolov v listih sprva naraščale, nato pa se zmanjševale: vsebnosti klorogenske kisline, rutina in kavine kisline so naraščale do julija, vanilne in sinapinske kisline do avgusta ter vsebnosti katehina, epikatehina in siringinske kisline vse do septembra. Na koncu rastne dobe je bila dinamika gibanja primarnih in sekundarnih metabolitov v listih najbolj različna: vsota merjenih sladkorjev se je še povečala od septembra do oktobra, toda vsota vsebnosti merjenih fenolov se je v oktobru razpolovila v primerjavi z mesecem prej.

Changes in sugars and phenolics concentrations of Williams pear leaves during the growing season

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Colaric, M., Stampar, F. and Hudina, M. 2006. **Changes in sugars and phenolics concentrations of Williams pear leaves during the growing season.** Can. J. Plant Sci. **86**: 1203–1208. Leaves of Williams pear were collected during the growing season from May to October and the contents of sugars and phenolic compounds were analyzed by high-performance liquid chromatography method. Sorbitol was the major sugar (up to 83.8 g kg^{-1} DW), followed by sucrose (up to 22.1 g kg^{-1} DW). Concentrations of glucose and fructose were as high as 12.9 and 9.0 g kg^{-1} DW, respectively. Leaves contained up to 29 471.9 mg kg^{-1} DW of chlorogenic acid, followed in concentration by rutin (up to 6789.2 mg kg^{-1} DW), epicatechin (up to 7378.0 mg kg^{-1} DW), catechin (up to 3846.5 mg kg^{-1} DW), vanillic acid (up to 1832.1 mg kg^{-1} DW), syringic acid (up to 1123.5 mg kg^{-1} DW), caffeic acid (up to 122.5 mg kg^{-1} DW) and sinapic acid (up to 94.1 mg kg^{-1} DW). The significant differences in concentration of sorbitol, sucrose, glucose, and in all analyzed phenolics were observed during the growing season (six sampling dates). The lowest concentrations in the leaf were found at the beginning of the growing season in May and June. The highest contents of sugars were in October, with the exception of sorbitol. During the growing season, total phenolic content first increased, then declined. Chlorogenic acid, rutin and caffeic acid contents increased until July, vanillic acid and sinapic acid until August, and catechin, epicatechin and syringic acid until September. However, total phenolic content dropped by 50% from September to October.

Key words: Pear leaves, sugars, phenolics, growing season

Colaric, M., Stampar, F. et Hudina, M. 2006. **Évolution de la concentration de sucres et de phénols dans les feuilles du poirier Williams pendant la saison de croissance.** Can. J. Plant Sci. **86**: 1203–1208. Les auteurs ont recueilli des feuilles de poirier Williams pendant la période végétative (de mai à octobre) puis ont analysé la concentration de sucres et de phénols par chromatographie liquide à haute performance. Le sorbitol est le sucre le plus abondant (jusqu'à 83,8 g par kg de poids sec) suivi du sucrose (jusqu'à 22,1 g par kg de poids sec). La concentration de glucose et celle de fructose peuvent atteindre respectivement jusqu'à 12,9 et 9,0 g par kg de poids sec. Les feuilles renfermaient jusqu'à 29 471,9 mg par kg de poids sec d'acide chlorogénique. Venaient ensuite la rutine (jusqu'à 6 789,2 mg par kg de poids sec), l'épicatéchine (jusqu'à 7 378,0 mg par kg de poids sec), la catéchine (jusqu'à 3 846,5 mg par kg de poids sec), l'acide vanillique (jusqu'à 1 832,1 mg par kg de poids sec), l'acide syringique (jusqu'à 1 123,5 mg par kg de poids sec), l'acide caféique (jusqu'à 122,5 mg par kg de poids sec) et l'acide sinapique (jusqu'à 94,1 mg par kg de poids sec). L'écart significatif entre les concentrations de sorbitol, de sucrose, de glucose et des phénols examinés a été observé durant la saison de croissance (six dates d'échantillonnage). Les concentrations les plus faibles dans les feuilles ont été relevées au début de la période végétative, en mai et en juin. La plus forte teneur en sucres est survenue en octobre, sauf pour le sorbitol. La concentration totale de phénols augmente avant de diminuer pendant la période végétative. La concentration d'acide chlorogénique, de rutine et d'acide caféique augmente jusqu'en juillet; celle de l'acide vanillique et de l'acide sinapique le fait jusqu'en août et celle de la catéchine, de l'épicatéchine et de l'acide syringique jusqu'en septembre. Néanmoins, la concentration totale de phénols diminue de moitié entre septembre et octobre.

Mots clés: Feuilles de poirier, sucres, phénols, saison de croissance

Carbohydrates are direct products of photosynthesis and consequently the primary energy storage compounds and principal organic substances from which most other organic compounds found in plants are synthesized. Among simple sugars, glucose and fructose are common and abundant in woody plants. Sucrose is considered the most important oligosaccharide in higher plants. It has a high concentration in cells and it is the main transport form of photoassimilates from source leaves (synthesis) to sink tissues (use), and it is also a significant reserve carbohydrate (Kozlowski and Pallardy 1997). In the woody *Rosaceae* family, to which pear (*Pyrus communis* L.) belongs, sugar alcohol sorbitol (D-glucitol), in parallel with sucrose, are the major photosynthetic products in the mature leaves, the main translocatable and basic reserve carbohydrates in nonphotosynthesizing cells. Sorbitol is also found in the *Plantaginaceae* and may be derived from glucose as well (Loescher and Everard 1996; Moing et al. 1997; Deguchi et al. 2002; Watari et al. 2004).

Leaves often have a high content of carbohydrates; nevertheless, they only contain a small proportion of the total amount present in the whole tree. The leaves play a decisive role in growth and development of woody plants because they are the basic photosynthetic organs, which consecutively influence growth of vegetative and reproductive tissues. Leaves also store carbohydrates and mineral nutrients for short periods. Leaf longevity is of much interest, because of its importance to plant growth and also to plant responses (Kozlowski and Pallardy 1997). Carbohydrates produced in the leaves are transported to the other parts, and then used for the growth of various organs. Sorbitol accounts for

Abbreviations: **HPLC**, high-performance liquid chromatograph; **DW**, dry weight

60–90% of the carbon exported from the leaf (Bieleski 1982).

In contrast to primary metabolites, secondary metabolites are widely distributed in plant tissues and play a prominent role in general defense strategies of plants. Several thousand phenolics have been described, and their contents have been reported to vary from 5 to 50 g kg^{-1} dry weight of plant tissues (Swanson 2003). However, Laitinen et al. (2000) reported that the high intraspecific and interspecific variations in metabolite composition found in plants are the consequence of evolution of plant species and may be an effective means of adaptation in variable abiotic and biotic environments.

Phenolic compounds are synthesized mostly by the shikimate and acetate pathways (Kozlowski and Pallardy 1997). Several simple phenylpropanoids (with the basic C6-C3 carbon skeleton of phenylalanine) are produced from cinnamic acid via a series of hydroxylation, methylation, and dehydration reactions; these include *p*-coumaric, caffeic, ferulic and sinapic acids, and simple coumarins. Cinnamic acid is formed from phenylalanine by the action of phenylalanine ammonia-lyase, the branch point enzyme between primary (shikimate pathway) and secondary (phenylpropanoid) metabolism (Dixon and Paiva 1995).

Phenolics comprise a large group of endogenous growth inhibitors and therefore can affect plants directly by their involvement in metabolism and their function on growth and development, and ecological functions (Kozlowski and Pallardy 1997). As well, phenolic compounds play a major role in the resistance to plant pathogens. These compounds exist in many different plant parts: root, shoot, flower, leaf buds, woody tissues, leaf, phloem, pollen, and stylus (Misirli et al. 1995). Moreover, chlorogenic acid is induced in response to wounding and can act as defense compound (Dixon and Paiva 1995).

Many researchers have conducted studies on the seasonal changes of carbohydrates in leaves of *Rosaceae* trees (Loescher et al. 1982; Bieleski and Redgwell 1985; Merlo and Passera 1991; Moing et al. 1997; Wang et al. 1999; Lo Bianco et al. 2000; Deguchi et al. 2002; Veberic et al. 2003; Sircelj et al. 2005) while seasonal patterns of leaf phenolics have been investigated less (Mayr et al. 1995; Misirli et al. 1995; Treutter 2001; Usenik et al. 2004). If we focus on the carbohydrate and phenolic dynamics of leaves of European pear (*Pyrus communis* L.) during the growing season, there exists even less information (Gunen et al. 2005), and data are mostly restricted to ripe fruits. Consequently, this study investigated the changes of carbohydrates and phenolics partitioning in leaves of Williams pear from May to October.

MATERIALS AND METHODS

Plant Material

Williams pear trees grafted on quince MA were planted in 1987 in the eastern part of Slovenia (Bistrica ob Sotli). To evaluate variations in the chemical composition of pear leaves during the growing season, the healthy leaves were collected six times from May to October (May 15, Jun. 11, Jul. 09, Aug. 06, Sep. 02, Oct. 01). For each sample date, 12 representative pear leaves (*Pyrus communis* L.) were picked from the same marked 5-yr-old branches taken from shoots of equal length from the eastern part of the tree. Eight repetitions were prepared for each of the six sampling dates. Leaves were placed in liquid nitrogen immediately after they were collected and then lyophilised. The samples were stored at –20°C until the chemical analyses were performed.

Extraction of Sugars and Phenolic Compounds

For extraction of sugars, 1 g of ground leaves was weighed and bidistilled water was added (up to final volume of 50 mL) and left for 45 min at 20°C. The extracted samples were centrifuged at 12 000 × *g* for 7 min at 10°C (Eppendorf Centrifuge 5810 R, Hamburg, Germany) and the supernatant was used for analyses of sugars after filtration through a 0.45 µm cellulose mixed esters filter (Macherey-Nagel, Düren, Germany).

To prepare samples for analyses of phenolics, 100 mg of the ground leaves were weighed into a test tube and extracted once with 10 mL of methanol containing 1% of butylated hydroxytoluene (used as an antioxidative agent) in an ultrasonic bath for 45 min. After that, the homogenate was centrifuged (12 000 × *g* for 7 min at 10°C) and the supernatant was filtered through a 0.45 µm Chromafil polyamide filter, transferred into a vial and used for analyses of phenolic compounds.

Standards of fructose, glucose, sucrose, sorbitol, (-)-epicatechin, caffeic acid, vanillic acid, syringic acid, (+)-catechin, chlorogenic acid, sinapic acid, and rutin were used. Bidistilled water was used for the preparation of standards of sugars, and standards of phenolics were dissolved and diluted in methanol.

Analyses of Sugars and Phenolic Compounds

A high-performance liquid chromatograph (HPLC) of Thermo Separation Products equipment (Riviera Beach, FL) was used for sugar analyses (fructose, glucose, sucrose and sorbitol). Separation of sugars was done using a Rezex RCM Monosaccharide column (300 × 7.8 mm) from Phenomenex (Torrance, CA). The column temperature was maintained at 65°C. Bidistilled water was used for mobile phase with a flow rate 0.6 mL min^{-1}. The total run time was 60 min and monitoring of eluted sugars was carried out using refractive index detector.

A Surveyor HPLC system (Thermo Finnigan, San Jose, CA) equipped with a photo diode array detector was performed for the analyses of phenolic compounds (chlorogenic acid, rutin, epicatechin, catechin, vanillic acid, syringic acid, sinapic acid, and caffeic acid). Separation was carried out using a Chromsep HPLC column SS (250 × 4.6 mm, Hypersil 5 ODS) coupled with a Chromsep guard column SS (10 × 3 mm) from Chrompack (Middelburg, the Netherlands) and operated at 25°C. The chromatographic conditions were as previously described Schieber et al. (2001) with minor modifications. The mobile phases were 2% acetic acid in bidistilled water (A) and 0.5% acetic acid in bidistilled water and acetonitrile (ratio 1:1) (B). Initial gradient was 90% A, passing to 45% of A within 50 min, 45

Table 1. Sugar contents (sorbitol, sucrose, glucose and fructose) expressed as mean values ± SE during season

Sugar	May (g kg^{-1} DW)	June (g kg^{-1} DW)	September (g kg^{-1} DW)	October (g kg^{-1} DW)
Sorbitol	78.9 ± 7.2*ab*	59.3 ± 8.2*a*	83.8 ± 3.5*b*	76.1 ± 7.1*ab*
Sucrose	14.2 ± 2.8*a*	13.9 ± 0.9*a*	19.7 ± 1.7*ab*	22.1 ± 3.1*b*
Glucose	8.5 ± 2.1*ab*	5.3 ± 1.0*a*	7.5 ± 0.8*ab*	12.9 ± 2.4*b*
Fructose	7.1 ± 1.8	8.0 ± 1.3	6.6 ± 0.9	9.0 ± 2.2
Sum	108.7 ± 10.8	86.6 ± 11.0	117.6 ± 5.2	120.1 ± 14.1

a, b Values followed by different letters within each row are significantly different according to Duncan's multiple-range test at $P < 0.05$.

to 0% of A within 10 min and returning to the initial 90% of A within 5 min. The total run time was 65 min. The flow rate was 1 mL min^{-1}, and the injection volume was 20 µL. 15 min of equilibration treatment (90% of A) was performed between each analysis.

Identification and quantification of analyzed compounds were done as previously described (Colaric et al. 2005). Absorbance was monitored at 280 nm (catechin, rutin, vanillic acid and epicatechin), and at 320 nm (chlorogenic acid, syringic acid, caffeic acid and sinapic acid). Concentrations of analyzed compounds are expressed in g kg^{-1} (sugars) or mg kg^{-1} of dry weight (DW) (phenolics).

Data Analysis

Data were presented as mean ± standard error for each treatment. All data were tested by one-way analysis of variance (general linear model) using the Statgraphics Plus 4.0 program (Manugistics, Inc., Rockville, MD). Differences among mean values were determined by using Duncan's multiple-range test at the significant level 0.05.

RESULTS AND DISCUSSION

The results of high-performance liquid chromatography showed that identified sugars (sorbitol, sucrose, glucose and fructose) and phenolics (chlorogenic acid, rutin, epicatechin, catechin, vanillic acid, syringic acid, caffeic acid and sinapic acid) fluctuated in their contents in a seasonal pattern from May to October.

Sugar concentrations in leaf were almost invariable in the descending order: sorbitol, sucrose, glucose and fructose (Table 1). The total sugar concentration (sorbitol + sucrose + glucose + fructose) of analyzed leaves was between 108.7 and 120.1 g kg^{-1} DW, except in June (86.6 g kg^{-1} DW). The content of total sugars increased during the growing season, except in May, and the highest total sugars content was observed after harvest (at the last sampling date). However, Bieleski and Redgwell (1985) found that the total sugar concentration of apricot leaves was reasonably stable between 11 and 16 g kg^{-1} of fresh weight, except at the beginning of the growing season.

Sorbitol was the major translocating assimilate in all sampling dates (63.4-72.6% of total sugars) and a much lower proportion of sucrose (13.0-18.4%) was determined. With subsequent samplings (from May to October), the proportion of sorbitol to the total sugars decreased, and the proportion of sucrose to the total sugars increased. Glucose represented 6.2-10.7% and fructose 5.6-9.2% of total sugars.

Moreover, several authors reported that throughout the growing season sorbitol was also the major sugar found in leaves of many species of the woody *Rosaceae* family including the economically important genus *Malus* (apple). Leaves from Elstar apple trees had a slightly lower sorbitol to total sugars content (up to 48.7%) (Sircelj et al. 2005), while sucrose had a higher proportion to total sugars (up to 27.6%) than our findings in pear. Merlo and Passera (1991) described the levels of leaf sugars of peach [*Prunus persica* (L.) Batsch.] during the growing season, when their contents, especially of sorbitol, increased. In very young leaves only 20% of total sugars were present as sorbitol. However, at the last sampling (in October) sorbitol reached 40% of total sugars. Sucrose and glucose were predominated (40 and 30% of total sugars, respectively) in very young leaves and then decreased in mature leaves (up to 30 and 20% at the last sampling, respectively). The percentage of fructose was reasonably stable throughout the growing season (about 10% of total sugars). Similar results were also obtained by Lo Bianco et al. (2000). In their study, sorbitol and sucrose were the two main sugars found in mature peach leaves, although the sucrose content was from four- to eightfold lower than the sorbitol content. Bieleski and Redgwell (1985) have shown that throughout each stage of apricot leaf development, all leaves contained sorbitol as the major sugar (between 60 and 70% of total sugars), and a much lower proportion of sucrose (from 24 to 16%). Sorbitol was also found as a major phloem component in rosaceous fruit trees and can represent a major portion of the carbon molecules in the phloem sap. In a study with peach leaves (Moing et al. 1997), sorbitol accounted for 60 to 90% of the carbon exported from the source leaves.

In our study, the lowest sorbitol content in pear leaf was observed in June, and the peak of sorbitol content was reached in September. The above-mentioned samplings significantly differed in sorbitol contents (Duncan's test, $P < 0.05$). Sorbitol was found to allow continuation of photosynthetic activity and carbon metabolism under adverse environmental conditions such as water stress (advantage of sorbitol). Sorbitol accumulation in plants is considered an adaptive reaction to drought stress (Hudina and Stampar 1999). Moderate drought influenced higher sorbitol concentration in apple leaves, while severe drought caused a decrease in sorbitol concentration (Sircelj et al. 2005). Deguchi et al. (2002) reported sugar contents (sorbitol, sucrose, fructose and sucrose) increased in Japanese pear leaves responding to salinity and chilling stresses. Zhou and Quebedeaux (2003)

Table 2. Contents of phenolic compounds expressed as mean values ± SE during season

Phenolic compound	May (mg kg^{-1} DW)	June (mg kg^{-1} DW)	July (mg kg^{-1} DW)	August (mg kg^{-1} DW)	September (mg kg^{-1} DW)	October (mg kg^{-1} DW)
Chlorogenic acid	1 040.1 ± 537.2*a*	6 155.3 ± 1 023.0*ab*	29 471.9 ± 3 319.7*d*	20 759.2 ± 3 443.1*c*	22 712.3 ± 2 174.9*c*	12 422.7 ± 1 201.1*b*
Rutin	1 645.9 ± 510.1*a*	3 220.1 ± 388.5*b*	6 789.2 ± 439.9*d*	5 797.2 ± 428.9*cd*	5 709.6 ± 347.7*cd*	5 033.3 ± 211.0*c*
Epicatechin	390.8 ± 124.5*a*	1 525.4 ± 373.5*ab*	3 027.7 ± 180.1*b*	2 920.4 ± 511.9*b*	7 378.0 ± 667.7*c*	2 885.7 ± 425.9*b*
Catechin	178.7 ± 96.7*a*	728.4 ± 309.3*ab*	2 068.0 ± 229.9*c*	2 144.3 ± 539.4*c*	3 846.5 ± 521.9*d*	1 742.8 ± 246.6*bc*
Vanillic acid	434.9 ± 84.0*a*	996.5 ± 150.3*b*	1 617.7 ± 132.8*c*	1 832.1 ± 116.9*c*	1 094.0 ± 131.8*b*	1 690.3 ± 106.7*c*
Syringic acid	61.9 ± 11.8*a*	465.4 ± 164.7*b*	872.8 ± 132.5*c*	990.0 ± 36.6*c*	1 123.5 ± 67.5*c*	371.9 ± 65.1*b*
Caffeic acid	10.4 ± 1.3*a*	31.0 ± 6.8*ab*	122.5 ± 26.6*c*	110.0 ± 17.2*c*	119.1 ± 7.8*c*	60.7 ± 4.2*b*
Sinapic acid	21.4 ± 4.0*a*	68.2 ± 8.4*b*	87.8 ± 4.6*c*	94.1 ± 5.6*c*	58.2 ± 3.6*b*	61.6 ± 4.2*b*
Sum	3 784.0 ± 1 097.6*a*	13 190.3 ± 2 283.8*b*	44 057.5 ± 3 864.1*d*	34 647.3 ± 4 798.0*d*	42 041.2 ± 3 466.4*d*	24 269.0 ± 1 703.3*c*

a–d Values followed by different letters within each row are significantly different according to Duncan's multiple range test at $P < 0.05$.

found that metabolism of three photosynthetic end products in mature apple leaves (sorbitol, sucrose and starch) was accelerated in the source leaves in response to increased source/sink ratio, which was reflected as a sort of stress.

Our study agrees with that of Veberic et al. (2003), that fructose, glucose and sucrose contents increased in the leaves but that sorbitol contents decreased from September to October. They studied seasonal dynamics of leaf sugars in apple (*Malus domestica* Borkh.) from mid-August till November, and increasing levels of fructose towards the end of the growing season were observed, just the opposite of sorbitol. Sorbitol concentration in leaves decreased in the second half of the growing season (up to 85 g kg^{-1} DW) and was accompanied by decreased photosynthetic activity. Further, no significant differences in sugar leaf content among fruiting and defruited trees were reported in that study. According to Zhou et al. (2002), sucrose phosphate synthase activity in apple leaf was inhibited by sorbitol-6-phosphate, so it is feasible that the products of sorbitol biosynthesis may have inhibited sucrose biosynthesis in our Williams pear leaves until September. Therefore, it is suggested that sucrose content could not increase until the sorbitol content began to decrease. Generally, the much higher values of sorbitol than sucrose in Williams pear leaves (until September) could also be explained by Suzue at al. (2006), who found that the activity of sorbitol-related enzyme in pear leaves was more than 10 times that of the sucrose-related enzyme-activity.

Loescher et al. (1982) reported that as apple leaves develop in the spring, leaf sugar contents increase, especially in sorbitol. In autumn, the senescing leaves also change with leaf sugars decreasing substantially. Their sorbitol content decreased the most, and sucrose essentially fell to zero. Wang et al. (1999) observed the highest sorbitol content throughout various ages of apple leaves. Sorbitol increased throughout leaf age (to 40 g kg^{-1} DW) and sucrose reached a peak (28 g kg^{-1} DW), then decreased (to 12 g kg^{-1} DW). Sorbitol is not only the most important sugar in leaves, but it was also found as the most abundant sugar in buds of Japanese pear [*Pyrus pyrifolia* (Burm.) Nak.] during flower bud formation (Ito et al. 2002).

The highest sucrose, glucose and fructose contents were observed at the last sampling date in October. Sucrose contents in May and June were significantly lower than in October. The lowest value of glucose was reached in June, and again was significantly lower than in October. There were no significant differences in fructose content throughout the sampling dates.

Leaf concentrations of phenolic compounds declined mostly in the following order: chlorogenic acid, rutin, epicatechin, catechin, vanillic acid, syringic acid, caffeic acid and sinapic acid (Table 2). Generally, the lowest contents of phenolics were in May, followed by a strong increase in phenolics contents during the growing season, reaching a peak and then slowly decreasing until October. Consequently, the lowest concentration of total phenolics (i.e., the sum of the eight quantified phenolics) appeared at the first sampling date in May (3784.0 mg kg^{-1} DW). Further, the highest concentration of total phenolics occurred in July (44 057.5 mg kg^{-1} DW), and remained high in August and September. Similar patterns were observed in apple leaves, with phenolics increasing to a peak in July or August and then decreasing (Usenik et al. 2004).

Many significant differences in individual phenolics content among the sampling dates were observed. Among the analyzed phenolic compounds in pear leaves, chlorogenic acid, which is very important for fire blight resistance (Gunen et al. 2005), predominated in all samplings, except in May. Dixon and Paiva (1995) mentioned chlorogenic acid is induced in response to wounding and can act as a defense compound. In the first sampling (May) rutin predominated; its proportion to the total phenolics was the highest in that sampling (43.5%), and the proportion of chlorogenic acid to the total phenolics was only 27.5%. Although the lowest rutin content among samplings was observed in May, its content was still higher than the chlorogenic acid content. The highest chlorogenic acid (29 471.9 mg kg^{-1} DW) and rutin contents (6789.2 mg kg^{-1} DW) among samplings were observed in July. The proportion of chlorogenic acid to the total phenolics was the highest (66.9%) in July; however, the proportion of rutin was among the lowest (15.4%). Mayr et al. (1995) observed that the contents of chlorogenic acid increased during the seasonal development in leaves of Golden Delicious apple, reached its peak in older leaves, and then the content slowly decreased. In apple leaves of Gold Rush similar increases in rutin content were observed, reaching a peak in August (1400 mg kg^{-1} DW), followed by a continual decline thereafter (Usenik et al. 2004).

Among sampling dates, the highest contents of epicatechin and catechin were reached in September (7378.0 and 3846.5 mg kg^{-1} DW, respectively), followed by a decrease in October. The highest proportions of both epicatechin (17.6%) and catechin (9.2%) to total phenolics were noticed in September. Similar seasonal changes were also confirmed for epicatechin and catechin in apple leaves (Mayr et al. 1995).

Although the contents of vanillic acid in pear leaves reached the minimum values in May, its proportion to total phenolics was the highest (11.5%). Vanillic acid reached a peak in August, declined in September and increased once again in October. The content of syringic acid constantly increased until September and then declined. Caffeic acid reached its peak in July, and did not significantly decrease until October. The content of sinapic acid gradually increased until August, and decreased significantly thereafter.

Arbutin is characteristic of pear leaves and was also identified, but difficulties in its separation at the beginning of each analysis occurred; therefore, the data are not shown here. Arbutin has also been found in strawberry, bearberry, wheat and in wheat products (Clifford 2000). However, others have also had difficulties with arbutin in phenolics extractions (Schieber et al. 2001).

Phenolic compounds (secondary metabolites of plants) are one of the biochemical factors that affect resistance to plant pathogens, such as *Erwinia amylovora*, which can cause fire blight on pears, apples, quinces, etc. Gunen et al. (2005) found that resistant cultivars had a higher arbutin content, and the chlorogenic acid content was also significantly higher in resistant varieties (120 mg kg^{-1}) than in susceptible varieties (70 mg kg^{-1} partially dried material). Cui et al. (2005) found chlorogenic acid and arbutin to be the main phenolic compounds in leaf bud, floral bud, flower and young fruit of Oriental pear (*Pyrus bretschnrideri*). They reported that the content of chlorogenic acid in leaf bud was 2260 mg kg^{-1} FW and of arbutin was 11 900 mg kg^{-1} FW.

Significant changes in primary and secondary metabolites over the growing season were confirmed for pear leaves in this study and found to have different distribution patterns. Further studies are needed to clarify how changes in carbohydrate and phenolic concentrations during the growing season are related to various stress situations or carbon assimilation patterns. Kelm et al. (2005) studied apple leaf chemical composition, with the aim of relating these primary and secondary metabolites to elevated levels of CO_2. However, their carbon nutrient balance hypothesis, which predicted that an increase in photosynthates, demonstrated by elevated CO_2 concentrations, would have an influence on increasing primary and secondary leaf metabolites, was refuted.

CONCLUSION

The changes in sugar and phenolic contents in leaves of Williams pear were confirmed during the growing season. Leaves from the early sample dates in spring had lower levels of carbohydrates, direct products of photosynthesis, and secondary metabolites, which play a prominent role in the general defense strategies of plants. The highest contents of sugars were observed in October, except for sorbitol. The lowest contents of phenolics were in May, then increased quickly, reaching a peak sometime in summer and decreasing thereafter until October. The patterns of primary and secondary metabolites from September to October were quite different, the content of total sugars continued to increase, while the total phenolics contents dropped by 50%. Similar to other rosaceous trees, sorbitol was always the major sugar followed by sucrose. Chlorogenic acid was identified as a major phenolic in pear leaf, followed by rutin, epicatechin, catechin, vanillic acid and syringic acid.

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2.2 VPLIV UPOGIBANJA RODNE VEJE NA VSEBNOSTI SLADKORJEV, ORGANSKIH KISLIN IN FENOLNIH SNOVI V PLODOVIH HRUŠKE SORTE ‘WILLIAMS’ (*Pyrus communis* L.)

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Kemična sestava plodov je pokazatelj notranje kakovosti ter je različna med sadnimi vrstami in sortami. Na vsebnosti metabolitov vplivajo tudi zrelost plodov, okoljski dejavniki in agrotehnični ukrepi, ki jih izvajamo v sadovnjaku. Med slednje uvrščamo tudi ukrep upogibanje vej, za katerega domnevamo, da bo vplival na vsebnosti sladkorjev, organskih kislin in fenolnih snovi v plodovih hrušk sorte ‘Williams’. V poskus smo vključili tri obravnavanja: petletne rodne veje smo upognili pozno poleti 2003 (i) in spomladi 2004 (ii) ter rodne veje, ki niso bile upognjene (iii) – kontrola. V tehnološki zrelosti smo obrali plodove in jih analizirali s tekočinsko kromatografijo visoke ločljivosti (HPLC). Med sladkorji smo izmerili največjo vsebnost fruktoze, med organskimi kislinami citronske kisline in med fenolnimi snovmi klorogenske kisline. Večina določanih metabolitov je imela najmanjše vsebnosti v plodovih na vejah upognjenih poleti 2003 in največje na vejah upognjenih spomladi 2004. V plodovih na vejah upognjenih poleti 2003 smo izmerili statistično značilno manjše vsebnosti fruktoze, sorbitola, epikatehina, katehina in sinapinske in siringinske kisline. Značilno največje vsebnosti fruktoze, sorbitola, epikatehina, katehina ter siringinske kisline so bile v plodovih na vejah upognjenih spomladi 2004 ter sinapinske kisline v kontrolnih plodovih. Razlike med obravnavanje so bile posledica upogibanja rodnih vej v poletnem in pomladnem obdobju.

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Influence of branch bending on sugar, organic acid and phenolic content in fruits of 'Williams' pears (*Pyrus communis* L.)

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Abstract: Selected sugars, organic acids and phenolic compounds were analysed in mature fruits of 'Williams' pears using high-performance liquid chromatography. Fruits were harvested from the branches of trees tested in three treatments: branches were bent in summer 2003 (1 September), in spring 2004 (15 May) and control (branches were not bent). Pears contained up to 73.54 g kg^{-1} fresh weight (FW) of fructose, 9.42 g kg^{-1} FW of glucose, 7.94 g kg^{-1} FW of sucrose and 24.59 g kg^{-1} FW of sorbitol. Major organic acids were (in order of descending quantity) citric, malic, shikimic and fumaric acid (up to 3.05 g kg^{-1} FW, 2.24 g kg^{-1} FW, 71.79 mg kg^{-1} FW and 0.49 mg kg^{-1} FW, respectively). Chlorogenic acid (280.86–357.34 mg kg^{-1} FW) was the predominant phenolic acid, followed in concentration (mg kg^{-1} FW) by syringic acid (95.46–131.32), epicatechin (46.55–83.09), catechin (25.67–44.81), vanillic acid (1.87–3.48), sinapic acid (0.83–1.72) and caffeic acid (0.72–1.04). Significant differences in content of fructose, sorbitol, total sugars, catechin, epicatechin, sinapic acid, syringic acid, and a sum of determined phenolic compounds were observed among the treatments. Fruits from summer bending branches had the lowest content of individual sugars, citric acid and phenolic compounds and the highest content of malic, shikimic and fumaric acid. The highest content of fructose, sorbitol, sucrose, total sugars, caffeic acid, catechin, epicatechin and syringic acid were determined in the fruits from the spring treatment. In the control treatment the highest content of glucose, citric acid, chlorogenic acid, sinapic acid, vanillic acid, as well a sum of determined phenolics, were observed. The lowest content of fumaric acid was in the spring treatment and of malic and shikimic acid in the control.

Keywords: pear fruit; sugars; organic acids; phenolics; bending

INTRODUCTION

Chemical composition of pear fruit, which determines its quality, has been investigated to a lesser extent in comparison with apples or peaches. However, for its determination various chromatographic procedures exist, among which high-performance liquid chromatography (HPLC) has been used most frequently. In this way many researchers[1–6] have reported that pear fruits of the cultivar (cv) 'Williams' contain fructose, glucose, sorbitol and sucrose. Among organic acids, 'Williams' pear fruits contain citric, malic, shikimic, fumaric and also quinic acid.[2–5]

Chlorogenic acid was the major phenolic in French pears cv 'Williams', followed by epicatechin and then catechin.[7] Separately from pear pulp of cv 'Decana' only chlorogenic acid and epicatechin were isolated by Escarpa and González,[8] whereas in the pear peel arbutin, catechin, caffeic acid, rutin and some glycosides, as well as chlorogenic acid and catechin were identified.[8] In Portuguese pear fruits the highest content of chlorogenic acid and flavonoids was also found in the peel, whereas in the pear pulp only chlorogenic acid was determined.[9] Leontowicz *et al.*[10] detected significantly higher content of phenolic acids in Israeli pear peels than in pear pulps, and Spanish pear peels and pulps of cv 'Blanquilla' were an even better source of phenolic acids.[11] Similar findings have been obtained in other studies, emphasising the importance of consuming the whole fruit (with peel), because phenolics are a natural source of antioxidants. However, the fear of pesticide residues often leads people to peel a fruit before eating it. Consequently, organic fruit is more desired for consumption.[12,13]

Sugars and organic acid composition of pear fruits grown in Slovenia have already been investigated.[3–5] Nevertheless, there is no known information available on the individual phenolic content of pears grown in Slovenia. Each species[14] and each cultivar[7,15] of fruit has its own significant chemical composition; however, differences in compound amounts may occur depending on the maturity stage,[7,16] environmental factors and technological measures (i.e., training system, pruning, tree nutrition, irrigation) applied in an orchard.[17] Consequently, bending treatment may affect chemical composition. Ito *et al.*[18] reported the influence of bending shoots on flowering and sugar metabolism[19] of lateral buds in Japanese pear (*Pyrus pyrifolia* (Burm.) Nak.). Bending is

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a long-established and widely used conventional agricultural technique to reduce vegetative growth (shoot extension) and promote flowering and fruiting in high-density orchards.[20] The aim of this study was to examine how bending affects sugars, organic acids and phenolic content in pear (*Pyrus communis* L.) fruits of cv 'Williams'.

EXPERIMENTAL

Chemicals

Malic acid and syringic acid were obtained from Merck KgaA (Darmstadt, Germany) and (+)-catechin was purchased from Carl Roth (Karlsruhe, Germany). Fructose, glucose, sucrose, sorbitol, citric acid, shikimic acid, fumaric acid, (−)-epicatechin, caffeic acid and vanillic acid were from Fluka Chemie GmbH (Buchs, Switzerland). Chlorogenic acid, sinapic acid and butylated hydroxytoluene (BHT; 2,6-di-*tert*-butyl-4-methylphenol), used as an antioxidative agent in the extraction solution, were obtained from Sigma Chemical Co. (St Louis, MO, USA). Methanol (an extraction solvent for phenolics) was from Riedel-de-Haën (Seelze, Germany) and acetonitrile (an elutant) was from Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

In all cases water used was bidistilled, and purified in a Milli-Q water purification system by Millipore (Bedford, MA, USA). Sugars and organic acids standards were prepared in water and phenolics standards were dissolved and diluted in methanol.

Plant material

Pear trees cv 'Williams' grafted on to quince MA were researched in the experiment. Trees were planted in 1987 in the eastern part of Slovenia (Bistrica ob Sotli). To study variations in the chemical composition of fruits three different treatments (ten trees per treatment) were given: (1) one five-year-old branch per tree was bent at an angle of 60° from the vertical to horizontal position in late summer 2003 (1 September) or (2) in late spring 2004 (15 May) or (3) was not bent – control tree. From those branches the undamaged pear fruits were harvested at commercial maturity on 1 September 2004.

Extraction of sugars, organic acids and phenolic compounds

Immediately after harvest, fruits were cut into thin slices and stored at −20 °C until preparation of the samples. Eight samples per treatment, consisting of three representative pear fruits each and picked from the same branch, were individually prepared for analyses of sugars, organic acids and phenolics. The pear slices were homogenized to a purée with a homogeniser (T25 basic Ultra Turrax, IKA Labortechnik, Janke and Kunkel GmbH, Staufen, Germany).

Bidistilled water (up to a final volume of 50 mL) was added to 10 g of homogenized tissue and left for 45 min at room temperature for extraction of sugars and organic acids. After the extraction the homogenate was centrifuged at 12 000 × *g* (Eppendorf Centrifuge 5810 R, Hamburg, Germany) for 7 min at 10 °C. The supernatant was filtered through a cellulose mixed esters filter (Macherey-Nagel, Düren, Germany), transferred to a vial and used for HPLC analyses of sugars and organic acids.

To obtain phenolic compounds, 1 g of the homogenized sample was weighed into a test tube and extracted once with 10 mL of methanol containing 1% BHT in an ultrasonic bath cooled with ice for 45 min. BHT prevented oxidation of samples, but did not influence the extraction process and did not interfere with the extracted phenolics in HPLC analysis. The extracted samples were centrifuged (12 000 × *g*, 7 min, 10 °C) and the supernatant was used for HPLC analyses of phenolic compounds after filtration through a 0.45 µm Chromafil polyamide filter.

Analyses of sugars, organic acids and phenolic compounds

Sugar (fructose, glucose, sucrose, sorbitol) and organic acid (malic, citric, shikimic and fumaric acid) content was analysed using HPLC equipment (Thermo Separation Products, Riviera Beach, FL, USA).

Separation of sugars and sorbitol was carried out using a Rezex RCM-monosaccharide column (300 × 7.8 mm) operated at 65 °C (Phenomenex, Torrance, CA, USA). The mobile phase was bidistilled water and flow rate was 0.6 mL min^{-1}; total run time was 60 min and a refractive index (RI) detector was used for monitoring eluted carbohydrates according to the method of Dolenc and Stampar.[21]

Organic acids were analysed with HPLC using an Aminex HPX-87H column (300 × 7.8 mm) (Bio-Rad, Hercules, CA, USA) associated with a UV detector set at 210 nm as described by Dolenc and Stampar.[21] The column temperature was set at 65 °C. The elution solvent was 4 mmol L^{-1} sulfuric acid in bidistilled water at a flow rate of 0.6 mL min^{-1}. The duration of the analysis was 30 min.

The analysis of phenolic compounds (chlorogenic acid, syringic acid, epicatechin, catechin, vanillic acid, sinapic acid and caffeic acid) was carried out using a Surveyor HPLC system (Thermo Finnigan, San Jose, CA, USA), equipped with a photo diode array (PDA) detector and controlled by the ChromQuest 4.0 Chromatography workstation software system. Separations were carried out using a Chromsep HPLC column SS (250 × 4.6 mm, Hypersil 5 ODS) coupled with a Chromsep guard column SS (10 × 3 mm) from Chrompack (Middelburg, The Netherlands). Column temperature was maintained at 25 °C.

The chromatographic conditions were similar to those previously described by Schieber *et al.*,[22] with minor modifications. The mobile phase A was 2% acetic acid prepared in bidistilled water; solvent B was 0.5% acetic acid in bidistilled water and acetonitrile (ratio 1:1). The gradient was as follows: 90 to 45% of

A within 50 min, 45 to 0% of A within 10 min, and returning to the initial 90% of A within 5 min. Between each analysis 15 min of equilibration treatment (90% of A) was performed. The flow rate was 1 mL min^{-1} and the injection volume was 20 μL. The total run time was 65 min.

Analysed compounds were identified by addition of standard solutions in combination with retention times as well as by comparing their spectra with those of corresponding standards. Absorbance monitoring of eluted phenolic compounds was done at 280 nm for catechin, vanillic acid and epicatechin, and at 320 nm for chlorogenic acid, syringic acid, caffeic acid and sinapic acid. Quantification was achieved according to the concentrations of a corresponding external standard. Concentrations of analysed compounds are expressed in g per kg or mg per kg of fruit fresh weight (FW).

Data analysis

Data are presented as mean ± standard error (SE) for each treatment. All data were tested by ANOVA (general linear model – one-way analysis of variance) using the Statgraphics Plus 4.0 program (Manugistics, Inc., Rockville, MD, USA). Means were separated by Tukey's least significance difference (LSD) test at a significant level of 0.05.

RESULTS AND DISCUSSION

Content of sugars and organic acids

Fructose was the most abundant compound of all those quantified in pear fruits (Table 1), followed by sorbitol. Glucose and sucrose content showed a similar range. These results are in accord with other investigations on 'Williams' fruits reported in the literature.[1–3,6]

Fruits from summer bending branches had the lowest content of individual sugars. The highest fructose, sucrose, sorbitol and total sugar content was observed in the fruits from spring bending branches; an exception was glucose, the levels of which were highest in control fruits. Significant differences in fructose, sorbitol and total sugars content were observed even among treatments. In Japanese pear, branch reorientation to the horizontal position had an influence on increase in sorbitol and sucrose concentration of the central internode in comparison with that of control vertical branches.[19] Sorbitol and sucrose are the main translocated sugars found in the family of rosaceous plants, to which the pear plant belongs.[23] Spring bending branches must have had more photosynthates, which were exported to the fruit, and therefore higher carbohydrate content in those fruits. Stored carbohydrates are essential for pear fruit growth and development.[24]

Among organic acids, malic acid especially is formed in most pear cvs. However, the results of our study showed that pear fruits of cv 'Williams' contained more citric acid than malic acid in all treatments (Table 2). The content of citric acid is mostly dependent on a ripening stage and cultivar.[6] Hudina and Stampar[3] confirmed our results. Besides cv 'Williams', cvs 'Red Williams' and 'Rosired' were also reported to contain higher content of citric acid. However, citric acid content in cvs 'Concorde' and 'Conference' were below detection.

Fumaric acid concentration was the lowest among the quantified organic acids; nonetheless, the taste effect of fumaric acid on the flavour of fruits is, despite its scarcity, stronger than the effect of citric acid.[25] Drake and Eisele[2] reported higher content of fumaric acid for 'Williams' pear juice (up to 1.6 mg L^{-1}), and the content of citric, malic and shikimic acids was in a similar range (up to 3.6 g L^{-1}, 2.0 g L^{-1} and 67.0 mg L^{-1}, respectively) to those measured in our study.

The highest content of citric acid and total organic acids was in fruits from control trees, while the same fruits had the lowest malic and shikimic acid content. Fruits from branches bent in spring 2004 attained the lowest fumaric acid content and also the total organic acids were the lowest. Malic, shikimic and fumaric acid content was the highest in fruits from summer bending branches, while those fruits were the poorest in citric acid. Interestingly, with the same treatment the fruits were poor in sugars. Nonethe less, there were no statistically significant differences in individual organic acid content among the treatments.

Table 1. Average content of sugars in 'Williams' pears after different treatments

Sugar	Bending treatment		
	Summer 2003	Spring 2004	Control
Fructose	64.48 ± 2.23a	73.54 ± 2.68b	67.61 ± 3.04ab
Glucose	8.46 ± 1.14	9.01 ± 1.26	9.42 ± 1.11
Sorbitol	19.29 ± 1.80a	24.59 ± 1.56b	21.34 ± 1.93ab
Sucrose	6.25 ± 0.75	7.94 ± 0.94	7.28 ± 1.06
Sum	98.48 ± 3.22a	115.07 ± 3.93b	105.66 ± 4.76ab

Values are presented as the mean ± standard error (SE) in g kg^{-1} fresh weight (FW). Values followed by different letters within each row are significantly different according to the LSD multiple-range test at $P < 0.05$.

Table 2. Average content of organic acids in 'Williams' pears after different treatments

Organic acid	Bending treatment		
	Summer 2003	Spring 2004	Control
Citric acid	2.87 ± 0.28	2.89 ± 0.07	3.05 ± 0.16
Malic acid	2.24 ± 0.13	2.15 ± 0.09	2.13 ± 0.13
Shikimic acid	71.79 ± 3.45	58.14 ± 6.94	57.67 ± 5.80
Fumaric acid	0.49 ± 0.16	0.41 ± 0.05	0.44 ± 0.14
Sum	5.19 ± 0.26	5.10 ± 0.15	5.24 ± 0.17

Values are presented as the mean ± SE in g kg^{-1} FW; exceptions are shikimic and fumaric acid values, which are in mg kg^{-1}. No values within each row are significantly different (ANOVA, $P > 0.05$).

Table 3. Average content of phenolic compounds in 'Williams' pears after different treatments

Phenolic compound	Bending treatment		
	Summer 2003	Spring 2004	Control
Caffeic acid	0.72 ± 0.11	1.04 ± 0.12	0.86 ± 0.12
Catechin	25.67 ± 1.74a	44.81 ± 5.67b	36.50 ± 4.50ab
Chlorogenic acid	280.86 ± 28.45	318.17 ± 22.82	357.34 ± 29.66
Epicatechin	46.55 ± 2.17a	83.09 ± 10.56b	69.82 ± 10.38ab
Sinapic acid	0.83 ± 0.08a	1.18 ± 0.11ab	1.72 ± 0.26b
Syringic acid	95.46 ± 4.80a	131.32 ± 10.33b	120.66 ± 9.97ab
Vanillic acid	1.87 ± 0.08	2.52 ± 0.65	3.48 ± 0.73
Sum	451.95 ± 31.50a	582.132 ± 46.48b	590.38 ± 48.58b

Values are presented as the mean ± SE in mg kg^{-1} FW. Values followed by different letters within each row are significantly different according to the LSD multiple-range test at $P < 0.05$.

Content of phenolic compounds

As shown in Table 3, chlorogenic acid was the major phenolic compound found in pear fruits. That is in accordance with Clifford,[26] who reported quite similar chlorogenic acid content in pear fruits (up to 280 mg kg^{-1} FW). Lower content of chlorogenic acid was reported for 'Williams' pears (up to 100 mg kg^{-1} FW) by Herrmann[6] and Amiot *et al.*[7] and even lower content for cv 'Red Williams' (21 mg kg^{-1} FW).[22]

Comparing our European pear fruits (*Pyrus communis* L.) Cui *et al.*[14] observed that European pears were a much better source of chlorogenic acid (309 mg kg^{-1}) than were Oriental pears (*Pyrus bretschnrideri*) (163 mg kg^{-1}). Juice made from 'Williams' pears contained 174 mg L^{-1} of chlorogenic acid, as shown by the findings by Tanrıöven and Ekşi.[27]

Syringic acid showed nearly one third the concentration of chlorogenic acid content in this study. Epicatechin content was very close to that shown by Tsanova-Savova *et al.*,[28] who reported that pear fruits contained 20.9–81.2 mg kg^{-1} FW. Herrmann[6] found 'Williams' pears had up to 30 mg kg^{-1} FW of epicatechin, Amiot *et al.*[7] cited 21 mg kg^{-1} FW of epicatechin and Tanrıöven and Ekşi[27] determined that 'Williams' juice contained 34.9 mg L^{-1} of epicatechin. However, Schieber *et al.*[22] reported that 'Red Williams' pears contained only up to 6 mg kg^{-1} FW of epicatechin.

Catechin values were about one half of epicatechin in all treatments (up to 44.81 mg kg^{-1} FW in pears from spring treatment). Herrmann[6] reported that 'Williams' fruits exhibited up to 10 mg kg^{-1} FW of catechin. Tsanova-Savova *et al.*[28] determined lower mean values (3.7 mg kg^{-1} FW) and Amiot *et al.*[7] also cited similar values of catechin (5 mg kg^{-1} FW).

Vanillic, sinapic and caffeic acids were minor phenolic compounds found in pear fruits. According to the findings of Tanrıöven and Ekşi,[27] 'Williams' pear juice contained 11.0 mg L^{-1} of caffeic acid. Much higher content of caffeic acid was found in Israeli pear pulps (198 mg kg^{-1} FW),[10] and Spanish pear pulps of cv 'Blanquilla' were an even better source of caffeic acid (701 mg kg^{-1} FW).[11]

Arbutin was also identified, but owing to difficulties in its separation at the beginning of each HPLC analysis the data are not shown here. Schieber *et al.*[22] also had problems with arbutin interpretation; its content in cv 'Red Williams' was only 0.4 mg kg^{-1} FW. Galvis-Sánchez *et al.*[9] analysed pear pulp and peel separately, and arbutin was found only in the pear peel (1158 mg kg^{-1} FW).

Fruits from summer bending branches (1 September 2003) had the lowest content of all individually analysed phenolic compounds. Content of catechin, epicatechin, sinapic acid, syringic acid and a sum of analysed phenolics differed significantly among the treatments (ANOVA, LSD test at $P < 0.05$). The highest concentrations of caffeic acid, catechin, epicatechin and syringic acid were detected in the fruits from spring bending branches. Chlorogenic acid, sinapic acid and vanillic acid, as well as total analysed phenolics, exhibited the highest values in control treatments.

We propose that the bending of branches altered the branch metabolism and consequently influences on fruit composition. Due to lower content of most analysed compounds in the fruits from the summer treatment and higher content from the spring treatment, it is supposed that spring bent branches saved the assimilates for fruits, but summer bent branches spent assimilates for other tissues before the fruit required them for development and growth.

CONCLUSION

This investigation demonstrated the sugar, organic acid and phenolic composition of 'Williams' pears. Fruits were harvested from branches on trees involved in three different treatments (branch was bent in summer 2003; branch was bent in spring 2004; and no bent branch – control). Statistical analysis showed significantly lower content of fructose, sorbitol, total sugars, catechin, epicatechin, sinapic acid, syringic acid, and sum of determined phenolic compounds in fruits from the summer bending branches. Significantly, the highest content of fructose, sorbitol, total sugars, catechin, epicatechin and syringic acid were in fruits from the spring bending branches, and the highest content of sinapic acid from the control treatment. It has been shown that differences

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among treatments regarding content of sugars, organic acids and phenolics could be the result of branch reorientation. Further studies are needed to confirm and clarify how those differences occur.

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2.3 UPOGIBANJE VPLIVA NA VSEBNOST DOLOČENIH FENOLNIH SNOVI V LISTIH HRUŠKE SORTE ‘WILLIAMS’

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Bending affects phenolic content of William pear leaves.
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Upogibanje vej je pri sadnih drevesih uveljavljen tehnološki ukrep in zelo razširjen v intenzivnih nasadih. Učinek upogibanja rodnih vej se odraža tako na zgodnejšem in večjem pridelku kot tudi na zmanjšani vegetativni rasti. Na biokemičnem nivoju je bilo ugotovljeno, da vpliva upogibanje na vsebnost hormonov in njihovo premeščanje ter na metabolizem sladkorjev. Učinek upogibanja rodnih vej hruške na fenolno sestavo listov do sedaj še ni bil poznan. Fenolne snovi so pomembna skupina endogenih rastnih inhibitorjev, ki imajo v rastlini pomembno vlogo v odpornosti na bolezni in škodljivce. V poskusu smo naključno razporedili 30 proučevanih dreves hruške sorte ‘Williams’ v tri obravnavanja, kjer smo upognili po eno petletno rodno vejo na drevo, in sicer 1. septembra 2003 in 15. maja 2004. Tretjo obravnavanje so predstavljale označene petletne rodne veje, ki niso bile nikoli upognjene - kontrolne veje. Z upognjenih in kontrolnih vej smo vzorčili liste od maja do oktobra 2004. Določili smo naslednje fenolne snovi: rutin, epikatehin in katehin, kavino, klorogensko, sinapinsko, siringinsko in vanilno kislino, katerih vsebnost je bila najmanjša v maju, nato so vsebnosti posameznih fenolov naraščale, dosegle največjo vrednosti v juliju, avgustu ali septembru ter se zatem do oktobra zmanjševale. Med posameznimi obravnavanji smo najmanjše vsebnosti pri večini določanih fenolnih snovi izmerili v listih na upognjenih rodnih vejah, zlasti na vejah upognjenih pozno poleti 2003. Značilno največje vsebnosti posameznih fenolov so bile v listih iz kontrolnega obravnavanja. Domnevamo, da je sprememba kota rodne veje hruške sorte ‘Williams’ sprožila fiziološki odgovor z različnimi vsebnostmi fenolnih snovi v listih z upognjenih in neupognjenih vej.

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ORIGINAL ARTICLE

Bending affects phenolic content of William pear leaves

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Abstract

The effect of branch bending on the phenolic content in pear leaves was investigated. Leaves were sampled from the tree branches which were bent in summer 2003 (1 September), in spring 2004 (15 May), and from unbent trees. Samplings were made during the growing season May to October 2004, on the following dates: 1st sampling, 15 May; 2nd sampling, 11 June; 3rd sampling, 9 July; 4th sampling, 6 August; 5th sampling, 2 September; and 6th sampling, 1 October. The leaves contained caffeic acid, chlorogenic acid, sinapic acid, syringic acid, vanillic acid, rutin, epicatechin and catechin. The lowest contents of phenolics were found on the first occasion. After that, in the sequence of sampling dates, an increase was noticed at first, but after reaching their highest point, the contents of phenolics decreased. In most cases fewer contents of phenolic compounds were found in leaves from bent branches (especially from branches bent in summer), and the highest ones in leaves from the control group. For all phenolics, apart from the caffeic and vanillic acids, significant differences were evident among treatments, with the highest contents in the control group and almost the lowest in the summer treatment. It is suggested that the change in branch angle caused the physiological response of pear tree, with different contents of phenolic compounds in its leaves from bent and non-bent branches.

Keywords: *Bending branches, HPLC analysis, leaves, phenolic compounds, pear, Pyrus communis L.*

Introduction

Bending is a long-established and widely used technological measure applied in high-density orchards to reduce vegetative growth and promote flowering and fruiting of fruit trees (Luckwill, 1970). According to Goldschmidt-Reischel (1997), by bending branches from a vertical to a horizontal position, mechanically induced stress is imposed. This has a wide range of influences on the growth and development of plants, varying from species to species and even within species. Lauri and Lespinasse (2001) reported that branch bending of two apple genotypes influenced the development and growth of lateral branches differently according to genotype. Bending branches instead of pruning proved to be an effective means of producing apple and pear fruit earlier and yielding more fruit as well as reducing vegetative growth (Goldschmidt-Reischel, 1997). Likewise, Grochowska, Hodun and Mika (2004) showed that branch bending increased fruiting of apple trees, but it had no effect on plum trees. In the lateral buds of Japanese pear, branch bending stimulated flowering as well as influencing hormone levels, their distribution and transport (Ito, Yaegaki, Hayama, Kusaba, Yamaguchi & Yoshioka, 1999), and sugar metabolism (Ito, Yoshioka, Hayama & Kashimura, 2004). Sanyal and Bangerth (1998) found that branch bending had an influence on the increase in flower buds number, higher internal ethylene production, and on reduction in polar auxin transport and cytokinin levels in the branches.

Since no known literature provides information about the effect of branch bending on the phenolic content of fruit tree leaves, its effect was examined in this study. In general, only few studies on the content of phenolic compounds in pear leaves are available; nevertheless, phenolic compounds are widely distributed in plant tissues where they play a significant role in general defence strategies of plants (Swanson, 2003). Kozlowski and Pallardy (1997) explained that phenolic compounds belong to a large group of endogenous growth inhibitors and can therefore

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affect plants directly by influencing their metabolism, growth and development.

The aim of our research was to evaluate the effect of the change in branch angle on content levels of phenolic compounds in its leaves. Therefore, William pear branches were bent in late summer and in late spring; and then phenolic variations among bending and control treatments were determined at different sampling dates.

Materials and methods

Plant material

The experiment was implemented on pear trees (*Pyrus communis* L.), cv. William, grafted on quince MA. The trees were planted in the eastern part of Slovenia (Bistrica ob Sotli) in 1987 and were divided into three different treatments (10 trees per treatment). One five-year-old branch per tree on the tree's eastern side was bent at an angle of 60° from a vertical to a horizontal position: 1) in late summer 2003 (1 September – the summer treatment), or 2) in late spring 2004 (15 May – the spring treatment), or 3) was not bent at all – the control group. From those branches, healthy leaves were sampled six times through the growing season from May to October 2004 to evaluate variations in the leaf phenolic composition among treatments. The samplings took place on the following dates: 1st sampling, 15 May; 2nd sampling, 11 June; 3rd sampling, 9 July; 4th sampling, 6 August; 5th sampling, 2 September; and 6th sampling, 1 October. On each sampling date, 12 representative pear leaves were picked from the same marked five-year-old branch and from the equally long shoots to make up one sample. For each treatment, eight replications were made. Leaves were immediately frozen in liquid nitrogen and then lyophilized.

Sample preparation

Each sample was ground in a mortar to achieve a fine texture. Precisely 100 mg of each sample was weighed into a test tube and extracted with 10 ml of methanol containing 1% of antioxidant agent BHT (butylated hydroxytoluene or 2,6-di-*tert*-butyl-4-methylphenol) for 45 min in an ultrasonic bath cooled with ice. The extracted sample was centrifuged at 12,000 $\times g$ for 7 min at 10°C (Eppendorf centrifuge 5810 R, Hamburg, Germany). The supernatant was filtered through a Chromafil polyamide filter with a 0.45 µm pore diameter (Macherey-Nagel, Düren, Germany), transferred into a vial and used for HPLC analyses of phenolic compounds.

Analyses of phenolic compounds

An HPLC analysis was performed using a Surveyor HPLC system and a diode array detector, controlled by a ChromQuest 4.0 chromatography workstation software system (Thermo Finnigan, San Jose, CA). Separation of phenolic compounds was carried out using a Chromsep HPLC column SS (250 × 4.6 mm, Hypersil 5 ODS) coupled with a Chromsep guard column SS (10 × 3 mm) from Chrompack (Middelburg, The Netherlands), and the column temperature maintained at 25°C. Two per cent acetic acid was prepared in bidistilled water as solvent A. Solvent B was 0.5% acetic acid in bidistilled water and acetonitrile (ratio 1:1). The gradient used for the phenolics analysis was as follows: from initial 90% A to 45% A (50 min), from 45% A to 0% A (10 min), from 0% A to 90% A (5 min) (Schieber, Keller & Carle, 2001). The analysis duration was 65 min, with 15 min of equilibriation treatment (90% A) between each analysis. The flow rate was 1 ml min^{-1}. The injection volume was 20 µl.

Identification and quantification of phenolic compounds

Identification was carried out by monitoring absorbance at 280 nm for catechin, rutin, vanillic acid and epicatechin, and at 320 nm for chlorogenic acid, syringic acid, caffeic acid and sinapic acid. Quantification of analysed compounds was carried out as previously described (Colaric, Veberic, Solar, Hudina & Stampar, 2005). Also, standards of phenolics, which were dissolved and diluted in methanol, were used for identification and quantification.

Data analysis

Data were analysed using the Statgraphics plus 4.0 programme (Manugistics Inc., Rockville, MD, USA). The bending effect on the content level of individual phenolic compounds was tested by one-way analysis of variance (ANOVA) for each sampling date. Means were separated by the test for least significant difference (LSD) at a significance level of 0.05.

Results and discussion

In William pear leaves, the levels of caffeic, chlorogenic, sinapic, syringic and vanillic acid, as well as rutin, catechin and epicatechin were defined. Generally, chlorogenic acid was found to have the highest content in all treatments compared to the other compounds analysed – the opposite of sinapic acid, the content of which was the lowest (Table I). Moreover, often the highest contents of phenolic compounds were determined in the control, and the lowest contents were found in leaves from the summer treatment (1 September 2003) (Figure 1).

Among the phenolic compounds studied, only caffeic and vanillic acid (Table I) showed no statistically significant differences among bending treatments on all sampling dates. However, on the third sampling date, the control differed most from both bending treatments.

Chlorogenic acid, which is also formed by the esterification of caffeic acid with quinic acid, reached its maximum in the third sampling, where it differed significantly in its content from the summer and the control (Table I). Also in all other samplings, the differences among treatments were observed, and the content of chlorogenic acid in the summer treatment was lower than in the control, but no more significant differences among treatments were found. Until now, no known study on the content levels of phenolic compounds in pear leaves contingent on bent and non-bent branches has been available. Moreover, only few studies on the content levels of phenolic compounds in pear leaves are available. Gunen, Misirli and Gulcan (2005) showed differences in the phenolic content in pear leaves which were a consequence of resistance to fire blight. Significantly higher leaf chlorogenic acid content was found in pear genotypes resistant to fire blight in the year 2001.

In our study, sinapic acid gave the most significant differences (Table I). The control showed the highest contents of sinapic acid on all sampling dates. On the second and the fourth sampling dates, the summer treatment differed significantly from the control. Furthermore, on the fifth sampling date, both bending treatments differed significantly from the control.

The syringic acid pattern was highly unusual (Figure 1). On the second, third and fourth sampling dates, the content of syringic acid was lowest in the control. However, statistically significant differences appeared only on the fifth sampling date, where the control showed the highest content of syringic acid among all treatments, and in both bending treatments quite similar values of syringic acid were found.

Although no significant differences were confirmed in vanillic acid content among treatments,

Table I. Phenolic contents (mean values, mg/100 g DW) according to different treatments and according to sampling dates. Different letters in each subcolumn indicate the statistically significant differences among treatments for individual phenolic compounds (LSD test, p-value <0.05).

Phenolic compound	Treatment	Sampling date 1st	2nd	3rd	4th	5th	6th
Caffeic acid	Summer 2003	0.9	3.7	9.1	9.8	10.4	5.0
	Spring 2004	1.0	5.1	8.2	12.9	12.7	4.9
	Control	1.0	3.1	12.2	11.0	11.9	6.1
Chlorogenic acid	Summer 2003	97.6	340.8	2009.0a	1742.1	1835.3	898.0
	Spring 2004	112.9	659.6	2508.6ab	2213.5	2335.5	875.0
	Control	104.0	615.5	2947.2b	2075.9	2271.2	1242.3
Sinapic acid	Summer 2003	1.6	4.5a	7.1	6.4a	4.3a	5.2
	Spring 2004	2.1	5.7ab	7.6	7.7ab	4.7a	4.7
	Control	2.1	6.8b	8.8	9.4b	5.8b	6.2
Syringic acid	Summer 2003	3.4	51.8	112.7	112.2	77.4a	33.1
	Spring 2004	6.6	58.8	109.7	112.3	76.2a	25.6
	Control	6.2	46.5	87.3	99.0	112.3b	37.2
Vanillic acid	Summer 2003	26.2	101.7	154.6	160.0	73.5	130.9
	Spring 2004	39.9	111.2	133.6	176.7	105.8	140.8
	Control	43.5	99.7	161.8	183.2	109.4	169.0
Rutin	Summer 2003	121.3	257.6	519.4a	493.1	468.0	387.7
	Spring 2004	153.3	310.9	663.0ab	544.9	469.4	395.0
	Control	164.6	322.0	678.9b	579.7	571.0	503.3
Catechin	Summer 2003	4.0	60.6	160.2	156.6	218.2a	109.1
	Spring 2004	9.3	81.3	154.5	168.7	303.6ab	112.1
	Control	17.9	72.8	206.8	214.4	384.7b	174.3
Epicatechin	Summer 2003	25.9	122.6	246.1a	246.9	475.3a	192.4
	Spring 2004	34.5	154.7	259.5ab	279.1	643.3ab	146.2
	Control	39.1	152.5	302.8b	292.0	737.8b	288.6

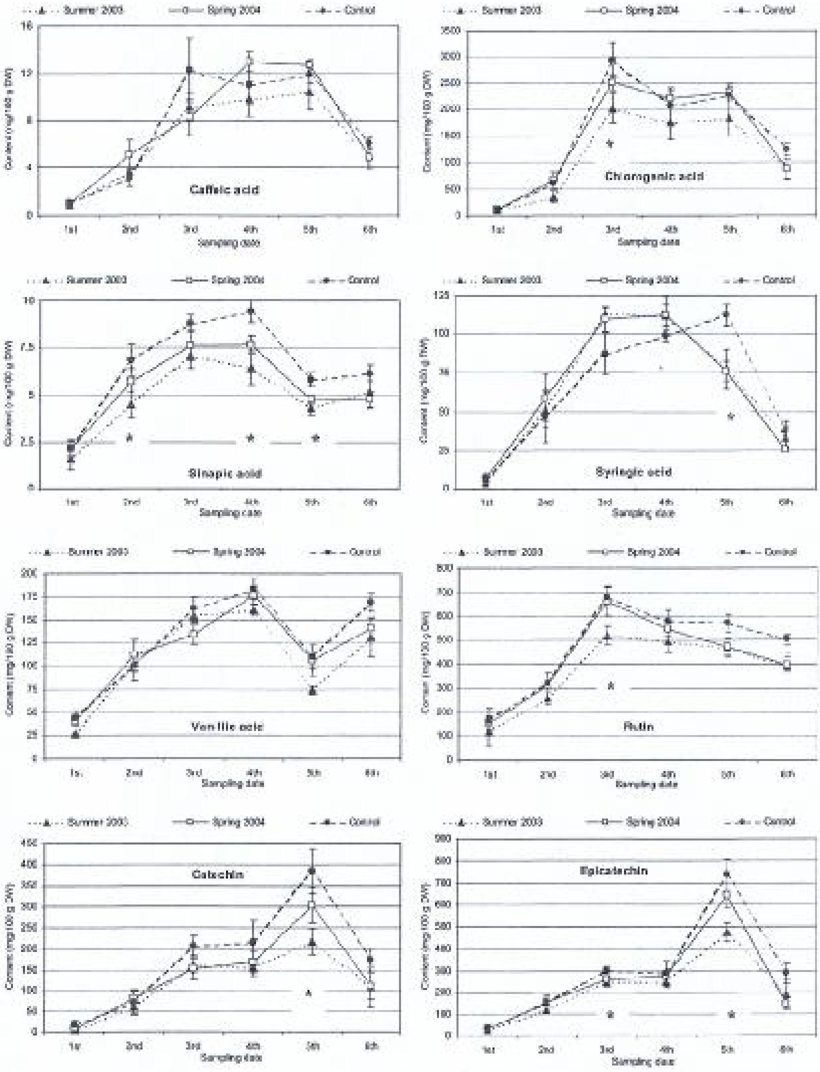

Figure 1. The progression of phenolic compounds in pear leaves sampled from branches bent in summer 2003, in spring 2004, and from non-bent branches (the control) through the sampling dates in year 2004 (the mean values and standard error bars). Marks: * statistically significant at p-value below 0.05 (one-way ANOVA, LSD test).

in most cases the lowest content of vanillic acid was found in the summer treatment, and the highest content was in the control (Table I). The most noticeable differences in vanillic acid content were observed on the last two sampling dates, but these were not significant.

The lowest rutin content was found in leaves from branches which were involved in the summer treatment, and the highest rutin content in leaves from non-bent branches on all six sampling dates (Table I). Nevertheless, only on the third sampling date did the summer treatment significantly differ from the control.

The highest catechin content was in the control, except on the second sampling date where the maximum content was reached in the spring treatment (Table I). The lowest catechin content was noted in the summer treatment, the only exception being on the third sampling date. On the fifth sampling date, the differences between the summer treatment and the control were statistically significant.

As with the other phenolics pattern, the highest epicatechin content was found in the control and the lowest content in the summer treatment, except for the sixth sampling, where the lowest epicatechin content was in the spring treatment. On the third and fifth sampling dates, the difference in epicatechin content between the summer and the control proved to be statistically significant. Both flavanols catechin and epicatechin had similar patterns from the point of treatment, but they differed in their contents. The third and fourth sampling dates had very similar contents, and after reaching a peak no earlier than the fifth sampling date (Figure 1), their contents declined. Mayr, Treutter, Santos-Buelga, Bauer and Feucht (1995) also reported epicatechin as exhibiting a maximum value in older apple leaves.

It is evident from the results above that branch bending and the time when it is carried out do influence the contents of phenolic compounds, which are important endogenous growth inhibitors that can affect plants directly through their involvement in metabolism, growth and development (Kozlowski & Pallardy, 1997), the reason being the existence of phenolic compounds in various parts of the plant, such as root, branch, flower and leaf bud, woody tissue, leaf, phloem, pollen, and stylus (Misirli, Gulcan & ve Tanrýsever, 1995).

Apart from technological growth regulation measures, such as bending, with the aim of reducing vegetative growth and improving flower bud quality, alternative solutions were sought. During the last few years, the chemical growth regulator prohexadione-calcium (P-Ca) has proven successful as one of these. Furthermore, this solution controls fire blight infections and *Psylla pyri* multiplication, and it thus considerably reduces the required application of fungicides (Deckers & Schoofs, 2004). It is being recommended because prohexadione-calcium alters flavonoid metabolism (Römmelt, Treutter, Speakman & Rademacher, 1999). The phenolic compounds content is not only under endogenous control but is also influenced by environmental conditions (Treutter, 2001). When nitrogen in plants becomes limited, the formation of phenolic compounds is accelerated (Lundegardh & Martensson, 2002).

According to Treutter (2001), the biosynthesis of phenolic compounds is induced by stress situations. It is suggested that by branch bending mechanically induced stress could not be induced in our study, for just the opposite reasons of phenolics: from bent branches (especially from the summer treatment) contents of phenolic compounds in leaves were almost lower than in the control, where most of the highest phenolics contents were observed. These data suggest that bent branches could receive less light, a situation that induces lower rates of photosynthesis. This could have resulted in lower activity of phenylalanine ammonia-lyase (PAL), the key enzyme between primary and secondary metabolism which catalyses the conversion phenylalanine to form cinnamic acid with the loss of an ammonia molecule (Taiz & Ziegler, 2002) and, consequently, in lower content levels of phenolic compounds. However, branch bending appears to be a good tool for further studying the involvement of plant phenolics in the plant growth and development processes. Because it is hard to reach clear conclusions from these results alone, it would be useful to include research into phenolic compounds and PAL accumulation in pear leaves at histological and subcellular levels as well as the vascular structure of a branch. Nevertheless, the present research provides the first known data on phenolic composition of pear leaves dependent on branch bending and showing differences between bent and non-bent branches. One possible reason for these differences could involve the physiological mode of action on the change in branch angle.

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2.4 VPLIV UPOGIBANJA RODNE VEJE NA VSEBNOSTI DOLOČENIH OGLJIKOVIH HIDRATOV IN FENOLNIH SNOVI V LISTIH HRUŠKE SORTE 'CONFERENCE'

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V rastnih dobah 2004 in 2005 smo proučevali učinek upogibanja rodne veje na vsebnost ogljikovih hidratov in fenolnih snovi v listih hruške sorte 'Conference'. Liste smo vzorčili na petletnih rodnih vejah, ki smo jih upognili pozno poleti 2003 in spomladi 2004 ter iz petletnih neupognjenih vej. S tekočinsko kromatografijo visoke ločljivosti (HPLC) smo določili vsebnosti saharoze, glukoze, fruktoze in sorbitola ter klorogenske, sinapinske in vanilne kisline, katehina, epikatehina, rutina, kvercetin-3-D-galaktozida in kvercetin-3-β-D-glukozida. V listih na rodnih vejah upognjenih spomladi 2003 smo v rastni dobi 2004 izmerili značilno večje vsebnosti klorogenske, vanilne in sinapinske kisline ter rutina in v letu 2005 značilno večje vsebnosti klorogenske in vanilne kisline (v dveh vzorčenjih) ter katehina in kvercetin-3-D-galaktozida (v enem vzorčenju). V kontrolnih listih smo v enem vzorčenju izmerili značilno večje vsebnosti katehina in epikatehina (v letu 2004) ter sinapinske kisline in kvercetin-3-D-galaktozida (v letu 2005) in kar v štirih vzorčenjih značilno večje vsebnosti kvercetin-3-β-D-glukozida (v letu 2005). Vsebnost epikatehina je bila značilno večja v listih na vejah upognjenih pozno poleti 2003 le v enem vzorčenju v letu 2004. Med obravnavanju nismo ugotovili razlik v vsebnosti posameznih ogljikovih hidratov, niti v letu 2004 niti ne v letu 2005. V poskusu smo spremljali tudi sezonsko spreminjanje vsebnosti posameznih ogljikovih hidratov in fenolnih snovi. V prvem letu so vsebnosti posameznih fenolnih snovi večinoma naraščale do julija in se nato do oktobra zmanjševale. V drugem letu so vsebnosti fenolnih snovi naraščale do junija, se zmanjševale do avgusta in se nato ponovno nekoliko povečale do oktobra. Med določanimi oglikovimi hidrati je po vsebnosti prevladoval sorbitol in med fenolnimi snovmi klorogenska kislina. V raziskavi so podani prvi rezultati dvoletnega poskusa spreminjanja vsebnosti ogljikovih hidratov in fenolnih snovi v listih med rastno dobo, kjer smo izvedli agrotehnični ukrep upogibanje rodnih vej, saj so razlike med obravnavanji rezultat fiziološkega odgovora drevesa in listov na ta ukrep.

Effects of branch bending on the levels of carbohydrates and phenolic compounds in 'Conference' pear leaves

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SUMMARY

The effects of bending on carbohydrates and phenolic compounds was investigated in 'Conference' pear leaves during the growing seasons 2004 and 2005. The leaves were sampled from tree branches bent in the late summer of 2003, in spring 2004 and from unbent branches. Levels of carbohydrates (sucrose, glucose, fructose and sorbitol) and phenolic compounds (chlorogenic acid, sinapic acid, vanillic acid, (+)-catechin, (-)-epicatechin, rutin, quercetin-3-D-galactoside and quercetin-3-β-D-glucoside) were determined in the pear leaves, using high performance liquid chromatography. In the first sampling year, significantly higher contents of some of the measured phenolics (chlorogenic acid, vanillic acid, sinapic acid, and rutin) were observed in leaves from the spring treatment. In the second year, a similar tendency among treatments was significant at two sampling times for chlorogenic acid and vanillic acid and at one for catechin and quercetin-3-D-galactoside. At one sampling, both measured flavan-3-ols (in 2004), as well sinapic acid and quercetin-3-D-galactoside (in 2005) exhibited significantly higher values in the control. The quercetin-3-β-D-glucoside was highest in the control leaves, at four samplings. At one sampling, significantly higher content of epicatechin was found in leaves from the summer treatment (in 2004). Carbohydrates showed no clear tendency among treatments as did some phenolics. During our research, the carbohydrate and phenolic dynamic over the growing season was also ascertained. Generally, in the first year, the levels of phenolics increased and then decreased over the growing season. In the second year, however, the maximum was reached earlier, and after decreasing, another increase occurred from August to October. Moreover, it was found that sorbitol predominated among carbohydrates and chlorogenic acid predominated among phenolics in both growing seasons. The first known data about carbohydrate and phenolic variations in pear leaves influenced by branch bending are given in two successive years. These variations appear to be the result of the physiological response of the pear tree and its leaves to this cultural practice.

Carbohydrates are the primary energy storage compounds and the basic organic substances from which most other organic compounds are synthesized. A variety of secondary compounds is produced by woody plants from carbohydrates, as well as from amino acids and lipids. The most important among these are phenolic compounds, which also influence plant growth and development, and many of them provide protection against herbivores and pathogens (Kozlowski and Pallardy, 1997). In this way, most of the phenolics in rosaccous fruit trees are synthesised from products of the shikimic acid pathway, such as from phenylalanine, which is one of the pathway intermediates between primary and secondary metabolism (Taiz and Zeiger, 2006). The accumulation of phenolics in leaves is also a consequence of various stresses, e.g. water stress (Cohen *et al.*, 1994). Higher content levels of leaf phenolics were detected in pear cultivars resistant to plant pathogens compared to susceptible cultivars (Gunen *et al.*, 2005).

The chemical composition of leaves and other organs of a fruit tree is dependent not only on species, cultivar and rootstock but also on environmental factors, orchard management and the various cultural practices (pruning, bending, spraying, etc.) applied in an orchard. To date, on the chemical level, Ito *et al.* (1999, 2004) ascertained that branch bending affected hormone levels and carbohydrate content and their related enzymes in the lateral buds of Japanese pear. Colaric *et al.* (2007) have also studied the influence of bending on phenolic content in 'Williams' leaves.

As an alternative to pruning, branch bending of pome fruit trees is a long-established and widely used method in high-density orchards, especially in the solaxe training system. It is well-known to reduce vegetative growth and induce flowering and then fruiting to maintain high annual economic yields as a mature tree (Lauri and Lespinasse, 2001). Lawes *et al.* (1997) reported that tree management by bending the leader resulted in greater floral precocity and reduced shoot vigour. Branch bending increased fruiting in apple trees (Grochowska *et al.*, 2004); moreover, apple and pear trees yielded more fruit if regulated only by bending instead of by pruning alone, and bending proved an effective means of producing fruit earlier (Goldschmidt-Reischel, 1997).

The aim of our study was to understand the effects of bending of five-year-old fruiting branches (in late summer and in spring) of 'Conference' pear compared to unbent branches (the control) on the contents of carbohydrates and phenolic compounds in leaves over the following two growing seasons. In addition, the seasonal dynamics of carbohydrates and phenolic compounds were studied for both growing seasons.

MATERIALS AND METHODS

Chemicals

Fructose, glucose, sucrose, sorbitol, citric acid, shikimic acid, fumaric acid, as well as (-)-epicatechin, vanillic acid, quercetin-3-D-galactoside and quercetin-3-β-D-glucoside were purchased from Fluka Chemie GmbH (Buchs, Switzerland). Malic acid was obtained from Merck KgaA (Darmstadt, Germany) and (+)-catechin from Carl Roth (Karlsruhe, Germany). Chlorogenic acid, sinapic acid, rutin (quercetin-3-rutinoside) and butylated hydroxytoluene (BHT) [2,6-di-*tert*-butyl-4-methylphenol], used as an antioxidative agent in the extraction solution, were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Methanol, acetonitrile and bidistilled water were of HPLC grade. Methanol was obtained from Riedel-de-Haën (Seelze, Germany) and acetonitrile from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Bidistilled water purified in a Milli-Q water purification system by Millipore (Bedford, MA, USA) was used. Sugar standards were prepared in bidistilled water and phenolic standards were dissolved and diluted in methanol.

Plant material and treatment

The experiment was performed on pear trees – 'Conference'/Quince MA, planted in 1987 in the eastern part of Slovenia. The pear orchard was maintained according to standard commercial practice for integrated fruit production (orchard management and cultural practice, i.e. spraying, irrigation, etc). Three different management treatments were applied to the trees, which were chosen randomly, and each single treatment was repeated on ten trees. One 5-year-old branch per tree (on the tree's eastern side and of comparable properties) was bent down to an angle 120° from the vertical position in late summer 2003 (summer treatment - 1 September) or in late spring 2004 (spring treatment - 15 May), and the third treatment was a control (branches were not bent). All bent and control branches prior to the experiment were grown at an angle 45° from the vertical position. The bent branches were tied down, using a cord and thus remained at the stated angle throughout the study. All bent and control branches were allowed to develop without pruning from 2003 through 2005. Branches fruited and were harvested on 9 September 2004 and 1 September 2005. The weather conditions (temperature, sunshine duration and precipitation) during the growing season 2004 were comparable to the long-term averages (1961 to 1990), but in 2005, 174 and 183% more rainfall was measured in July and August, respectively (Table I), than the long-term averages for these two months. In 2004 and 2005, healthy and mature spur leaves were sampled monthly from May to October (on a sunny day, at midday, in the middle of each month), immediately frozen in liquid nitrogen, then lyophilised and stored at –20°C, awaiting carbohydrate and phenolic extraction.

Extraction from leaves

The dried leaves were ground in a mortar with pestle. For carbohydrate extraction, bidistilled water up to a final volume of 50 ml was added to 1 g of leaf tissue and left for 45 min at 20°C, with occasional stirring. Afterwards, samples were centrifuged at 12000 *g* for 7 min, at 10°C (Eppendorf Centrifuge 5810 R, Hamburg, Germany). Then the supernatant was filtered through a 0.45 μm cellulose mixed Esters filter (Macherey-Nagel, Düren, Germany) and used for the carbohydrate analyses. For phenolic analysis, 100 mg of the weighed sample was transferred to a test tube and extracted once with 10 ml methanol in an ultrasonic bath for 45 min. The methanol contained 1% of the antioxidative agent BHT. Afterwards, the extracted sample was centrifuged at 12000 *g* for 7 min, at 10°C. The supernatant was then filtered through a 0.45 μm Chromafil polyamide filter, transferred into a vial and used for analyses of phenolic compounds.

Carbohydrates and phenolics analyses

Carbohydrates (sucrose, glucose, fructose and sorbitol) were determined with high-performance liquid chromatography (HPLC) from Thermo Separation Products (Riviera Beach, FL, USA), equipped with a Rezex RCM Monosaccharide column (300 × 7.8 mm; Phenomenex, Torrance, CA, USA) operated at 65°C and a refractive index (RI) detector for monitoring eluted carbohydrates. Bidistilled water was used as the mobile phase for the column at a flow rate of 0.6 ml min^{-1}. Total run time was 60 min.

Chromatographic separation of phenolic compounds was performed using a Surveyor HPLC system (Thermo Finnigan, San Jose, CA, USA) consisting of a vacuum degasser, a quaternary pump, a thermostatted autosampler (10°C) and a photo diode array detector. A Chromsep HPLC column (250 × 4.6 mm, Hypersil 5 ODS) operated at 25°C, coupled with a Chromsep guard column SS (10 × 3 mm) from Chrompack (Middelburg, The Netherlands) was used. The HPLC system was controlled with ChromQuest 4.0 Chromatography workstation software (Thermo Finnigan, San Jose, CA, USA). The chromatographic conditions were according to Schieber *et al.* (2001). Acetic acid solution in bidistilled water (2%, v/v) was used as the mobile phase A, and 0.5% acetic acid in bidistilled water and acetonitrile (ratio 1:1, v/v) was B. The elution gradient was: 0-50 min, from 10% to 55% B; 50-60 min, from 55% to 100% B; and 60-65 min, from 100% to 10% B. The total run time was 65 min, then washing and reconditioning of the column was performed for 15 min between each analysis (10% B). The flow rate was 1 ml min^{-1}, and the injection volume was 20 µl. Phenolics were monitored at 280 and 320 nm. Identification and quantification of phenolics was done according to Colaric *et al.* (2005).

Data analyses

Data were analyzed using the Statgraphics Plus 4.0 program (Manugistics, Inc., Rockville, MD, USA), and the effects of bending on the content of individual carbohydrates and phenolic compounds was tested by one-way analysis of variance (ANOVA; general linear model) for each sampling date and year, separately. Means between treatments were separated by Tukey's test ($P = 0.05$). Data are presented as mean ± standard error. Contents of carbohydrates in 'Conference' leaves are given in g 100 g^{-1} and contents of phenolics in mg 100 g^{-1} of dry weight (DW) through each growing season and separately for each compound analysed (Figures 1 and 2).

RESULTS AND DISCUSSION

In both growing seasons, sorbitol was the primary carbohydrate, and chlorogenic acid was the primary phenolic (Figures 1 and 2). According to Ito *et al.* (2004), sorbitol was the most abundant sugar in both the bud and internode segment of Japanese pear.

Our results also showed a seasonal dynamic involving individual carbohydrates and phenolics; moreover, it was found that the pattern among phenolics was rather similar. Generally, in the first year, the lowest values of analysed phenolics were observed in May, and then the levels increased to a maximum in July; afterwhich their contents decreased slowly, but not below the May values. An exception was epicatechin, whose contents increased till September but afterwards decreased. However, in the second year, the phenolics dynamics were not as explicit as in the year before: the maximum was reached earlier – mostly in June, and after decreasing until August, another small increase appeared from August to October. It is suspected that phenolic dynamics could be influenced by weather conditions (Table I). Warmer and sunny weather with less rain in May and June 2005 could have accelerated the phenolics peak. Afterwards, very rainy and less sunny conditions could have caused a decline in phenolics until August, and then a little warmer and sunny September and October may have resulted in another small increase of phenolics. In 2004, large differences emerged between the lowest and the highest value of each phenolic among sampling dates. The phenolic dynamic was similar to what has been reported for 'Williams' leaves (Colaric *et al.*, 2007), but the contents of some phenolics were lower in that study. Moreover, phenolics in apple leaves showed a similar seasonal pattern: their contents increased until a peak in July or August, and after that decreased (Usenik *et al.*, 2004).

Carbohydrates did not change through the growing season in an understandable progression, as did phenolics. In the first year, the highest contents of sucrose, glucose and fructose were noticed in October and of sorbitol in May. The lowest contents of sucrose, glucose and fructose occurred in June and of sorbitol in September. In the next year the lowest values of sucrose and glucose were again attained in June and of fructose and sorbitol in August. Maximum values of analysed sugars occurred in October, except for sucrose, where higher values occurred in July. Generally, some higher values of carbohydrates were noted in the second year in comparison with the levels in the first year. These carbohydrate levels, especially in the first year, are comparable to levels found in 'Williams' leaves (Colaric *et al.*, 2006). Additionally, the highest carbohydrate content in 'Williams' leaves was noticed in October, except for sorbitol, which peaked in September.

Many studies have tracked the seasonal changes of carbohydrates in the leaves of rosaceous trees, mostly in apples. In the study of Berüter and Studer Feusi (1997), glucose and sorbitol decreased, while fructose and sucrose increased during the growing season. Our results, however, are more in accordance with Veberic *et al.* (2003), who found fructose, glucose and sucrose contents slightly increased from August to the end of the growing season.

Branch bending is a conventional cultural practice that improves the balance between vegetative growth and fruiting in apple trees (Lauri and Lespinasse, 2001); however, on the physiological level, the response to bending has not been well understood. Ito *et al.* (1999; 2004) reported that changes in carbohydrates in buds are affected by bending treatments. Sorbitol and sucrose contents decreased significantly in the lateral buds 3 days after bending, but increased in the shoot internode 30 days after bending (Ito *et al.*, 2004). In our experiment, carbohydrate changes in 'Conference' leaves after the bending treatment showed no clear tendency in 2004 or 2005 (Figures 1 and 2).

In 2004, significantly higher sucrose content was observed once in leaves from the control treatment (in June), but then no significant differences were observed for sucrose the next year. For glucose and fructose, no significant changes among treatments were observed in the first year. However, in the second year, significant but unclear differences were indicated in fructose content in August and in glucose content in July, September and October. In the first year, the highest sorbitol content occurred in October, in leaves from the summer treatment, a phenomenon which was repeated next June; however, in the next October, sorbitol significantly increased in the control as well. According to Sanyal and Bangerth (1998), mechanically induced stress is created by branch bending, and sorbitol, which is, besides sucrose, the major photosynthetic product in the mature rosaceous leaves, increased under various stress conditions (Hudina and Stampar, 1999; Sircelj *et al.*, 2005). However, according to our results above, there seemed to be no apparent stress induced by branch bending.

Phenolic compounds are important endogenous growth inhibitors that can affect plants directly by influencing their metabolism, growth and development (Kozlowski and Pallardy, 1997). Branch bending and the time of bending had a greater influence on phenolics than on carbohydrates. In the first sampling year, significantly higher contents of some phenolics were measured in leaves from the spring treatment. Specifically, higher values of chlorogenic, vanillic, and sinapic acid and rutin were found in September. However, in August a significantly higher content of epicatechin occurred in leaves from the summer treatment and a higher content of catechin in leaves from the summer treatment and the control. Moreover, in June the epicatechin content was significantly higher in the control. The phenolic content in 'Williams' pear leaves was investigated in the first year after branch bending by Colaric *et al.* (2007), but this revealed responses different from those for 'Conference' leaves. 'Williams' leaves from branches bent in the summer had significantly lower contents (where control leaves had the highest contents at the same time) of the following phenolics: chlorogenic acid and rutin in July, sinapic acid in June, August and September, catechin in September and epicatechin in July and September (Colaric *et al.*, 2007).

In the second year, 'Conference' leaves from the spring treatment exhibited significantly higher levels of chlorogenic and vanillic acid in September and October, as well as significantly higher catechin levels in October. No significant differences in rutin content among treatments were evident. However, in June, the lowest content of sinapic acid was observed in leaves from the spring treatment, and in October in leaves from the summer treatment, whereas the highest contents were in the control leaves. In addition, the dynamic of the two quercetins, quercetin-3-D-galactoside and quercetin-3-β-D-glucoside, was observed in the second growing year (but not measured in 2004). In May, the content of quercetin-3-D-galactoside was significantly higher in leaves from the control, but in September it was higher in leaves from the spring treatment. The content of quercetin-3-β-D-glucoside was significantly higher in leaves from the control at four samplings. In the rainy summer 2005 (Table I) it was seen in leaves from the control some lower (but not significantly lower) contents of measured phenolic acids and rutin and some higher contents of both flavan-3-ols and quercetin-3-D-galactoside and quercetin-3-β-D-glucoside (Figure 2).

Moreover, the biosynthesis of phenolic compounds is induced by various stress situations (cultural practise, wounding, pathogen attack, environmental conditions, etc.). According to Sanyal and Bangerth (1998), branch bending inflicts mild stress. It was shown by Cohen *et al.* (1994) that water stress induced the accumulation of phenolics and suppressed vegetative growth. According to Rühmann *et al.* (2002), phenolics suppressed shoot growth, but nitrogen negatively affected phenolic content in apple leaves and then increased the susceptibility of trees to apple scab infections. Lux-Endrich *et al.* (2000) reported that the quantitative and qualitative composition of hydroxy-cinnamic acids and flavan-3-ols, and the quantitative composition of quercetins-glycosides were enhanced by sucrose content. Higher leaf chlorogenic acid content was detected in pear genotypes resistant to fire blight (Gunen *et al.*, 2005). According to Treutter (2006), flavonoids have a significant role in plant resistance, e.g. flavan-3-ols affected resistance of apple to scab, catechin acted as an allelochemical and quercetin-3-rutinoside acted as a defensive compound. Leaf wounding affected the accumulation of flavonol glycosides and flavan-3-ols in pear leaves (Andreotti *et al.*, 2006). Colarec *et al.* (2007) suggested that no stress occurred in bent branches since the reaction of most measured phenolics was the lowest in leaves from bent branches and the highest in the control leaves, although the study of 'Williams' leaves lasted for one year only. Surprisingly, 'Conference' leaves do not show so clear a tendency, but when significant differences were noticed for chlorogenic and vanillic acid, their contents were highest in leaves from the spring treatment in both growing seasons. As well, at one sampling, the highest contents of sinapic acid and rutin were in leaves from the spring treatment in 2004, and of catechin and quercetin-3-D-galactoside in 2005. At one sampling, both measured flavan-3-ols (in 2004), as well as sinapic acid and quercetin-3-D-galactoside (in 2005) exhibited significantly higher values in the control. Moreover, the flavonol glycoside, quercetin-3-β-D-glucoside, was highest in the control leaves, significantly greater than the bending treatments at four samplings. At just one sampling, significantly higher content of epicatechin was found in leaves from the summer treatment (in the first year after bending).

Bending is a cultural practice to balance vegetative growth and fruiting. However, it is also difficult to conclude if those abovementioned and inconsistent variations in 'Conference' leaves among bending treatments are solely the result of bending or whether they are indirectly affected by more general physiological processes at the level of the tree as a whole, and by the year to year variations, genotype properties, time of bending, etc.

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TABLE I

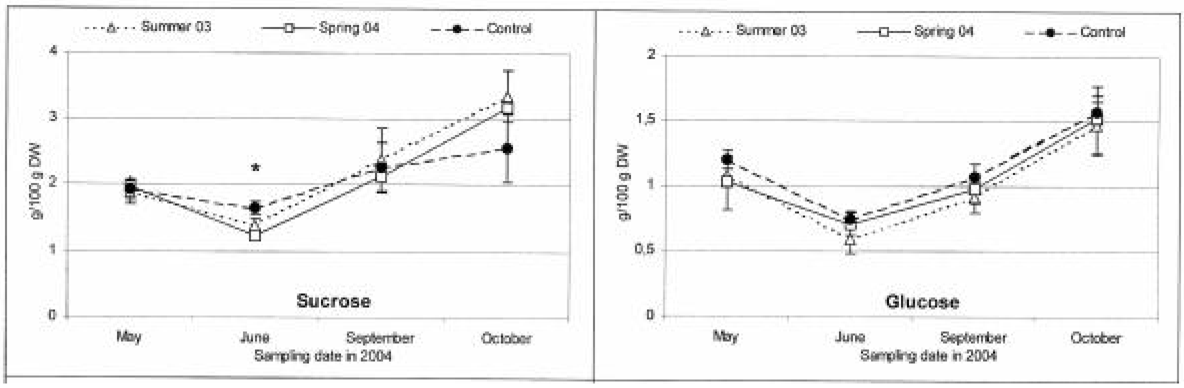
Monthly meteorological data from May to October 2004 and 2005 for meteorological station Celje[z].

Month	T_x (°C)	T_{dev} (°C)	OBS (h)	RO (%)	RR (mm)	RP (%)
May 2004	13.3	-0.8	209	107	84	87
June 2004	18.1	0.6	175	86	156	114
July 2004	19.9	0.7	246	102	102	76
August 2004	19.9	1.8	258	121	99	76
September 2004	14.7	0.1	180	110	82	79
October 2004	12.3	2.8	117	97	204	213
May 2005	15.6	1.5	236	120	92	95
June 2005	19.0	1.5	238	117	111	81
July 2005	20.3	1.1	228	95	233	174
August 2005	17.9	-0.2	172	81	239	183
September 2005	15.6	1.0	181	111	149	146
October 2005	10.9	1.3	130	107	68	71

[z]Monthly meteorological reports of the Environmental Agency of the Republic of Slovenia (2007).
T_x - average temperature (°C); T_{dev} - temperature deviation from 1961-1990 average (°C); OBS - bright sunshine duration (h); RO - % of average bright sunshine duration; RR - precipitations (mm); RP - % of average precipitations.

FIG. 1

Figure 1. The progression of carbohydrates and phenolic compounds in 'Conference' pear leaves sampled from branches bent in summer 2003, in spring 2004, and from unbent branches (the control) through the sampling dates in the year 2004 (the mean values and standard error bars). Marks: * statistically significant at *P* -value below 0.05 and ** below 0.01 (one-way ANOVA).



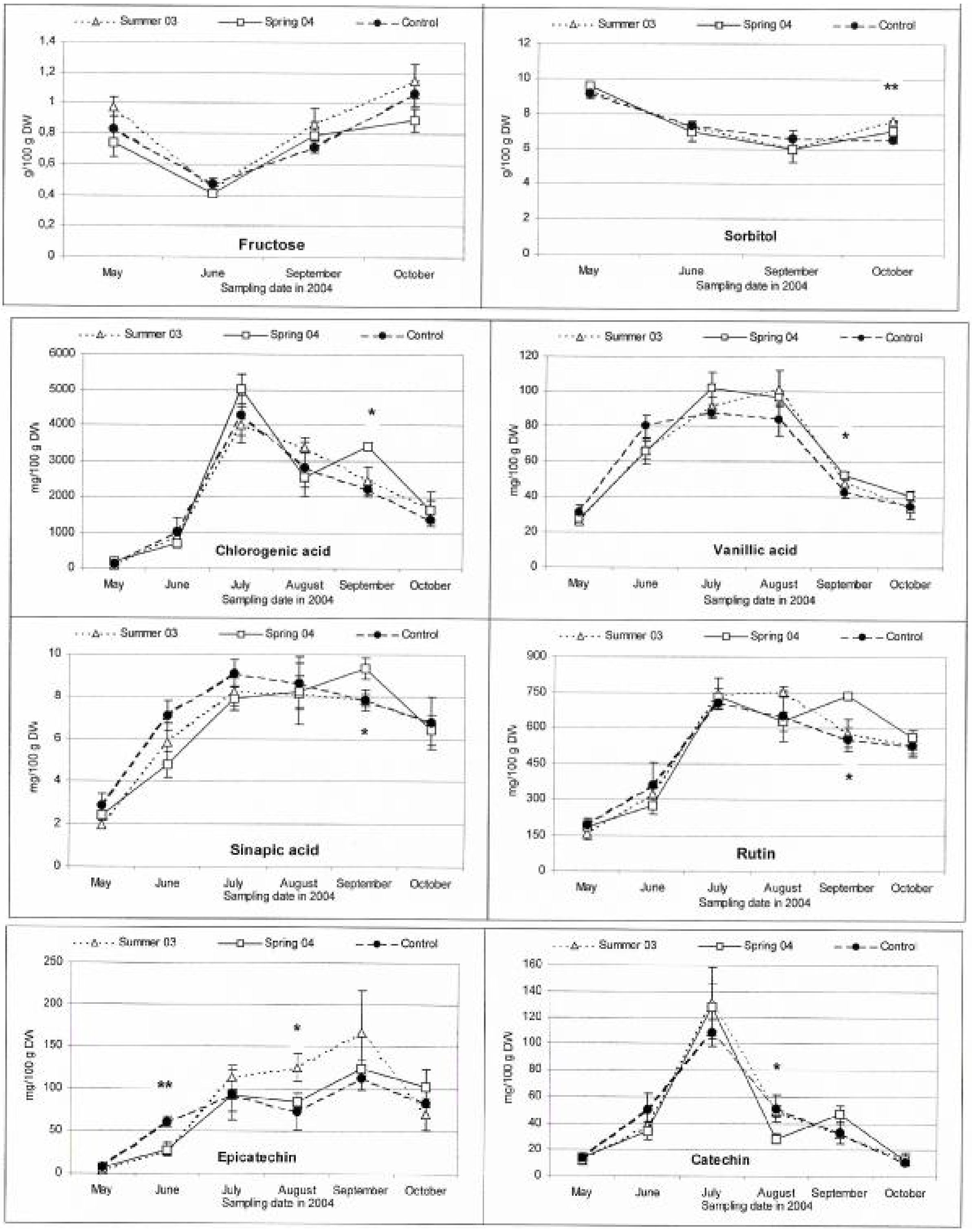
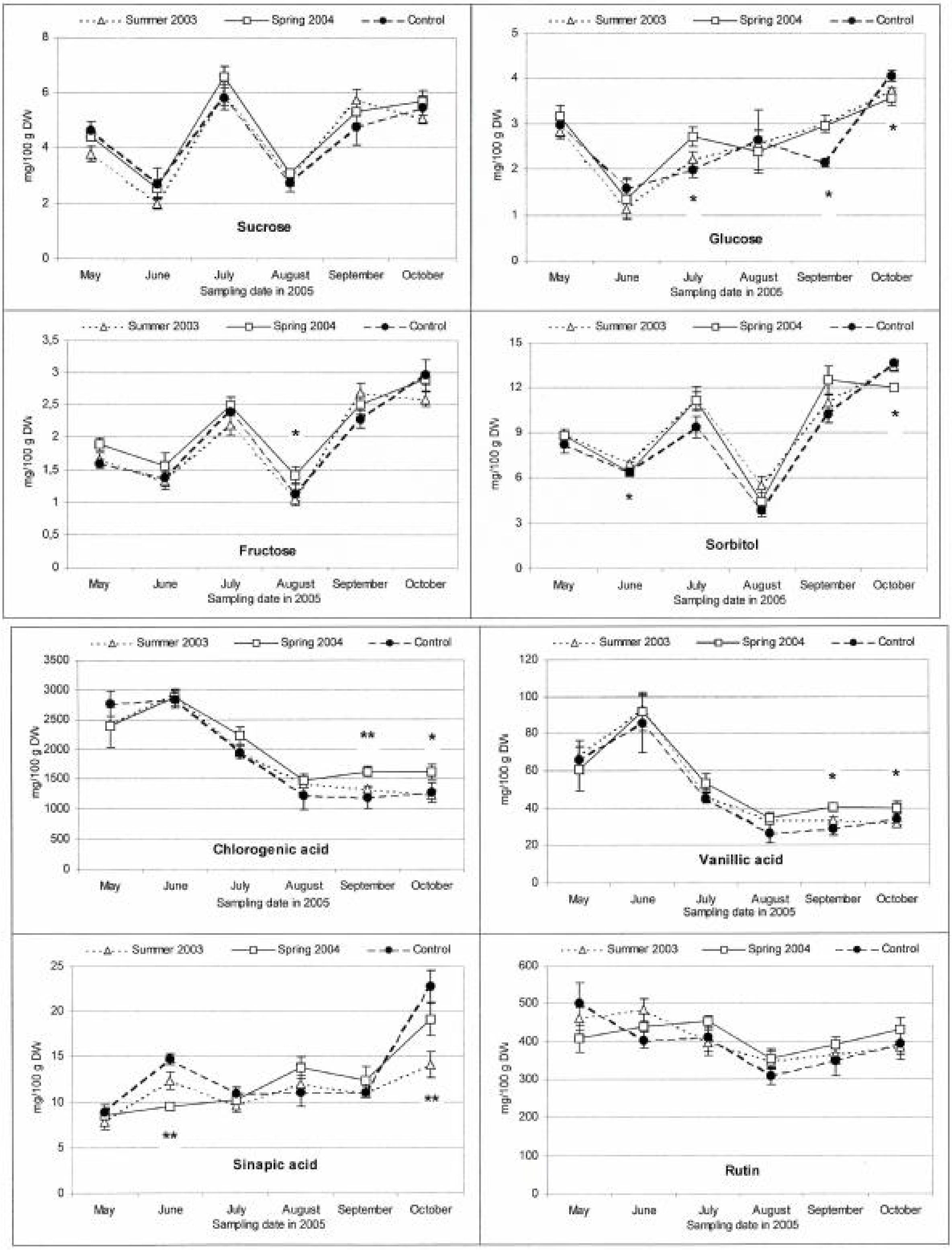
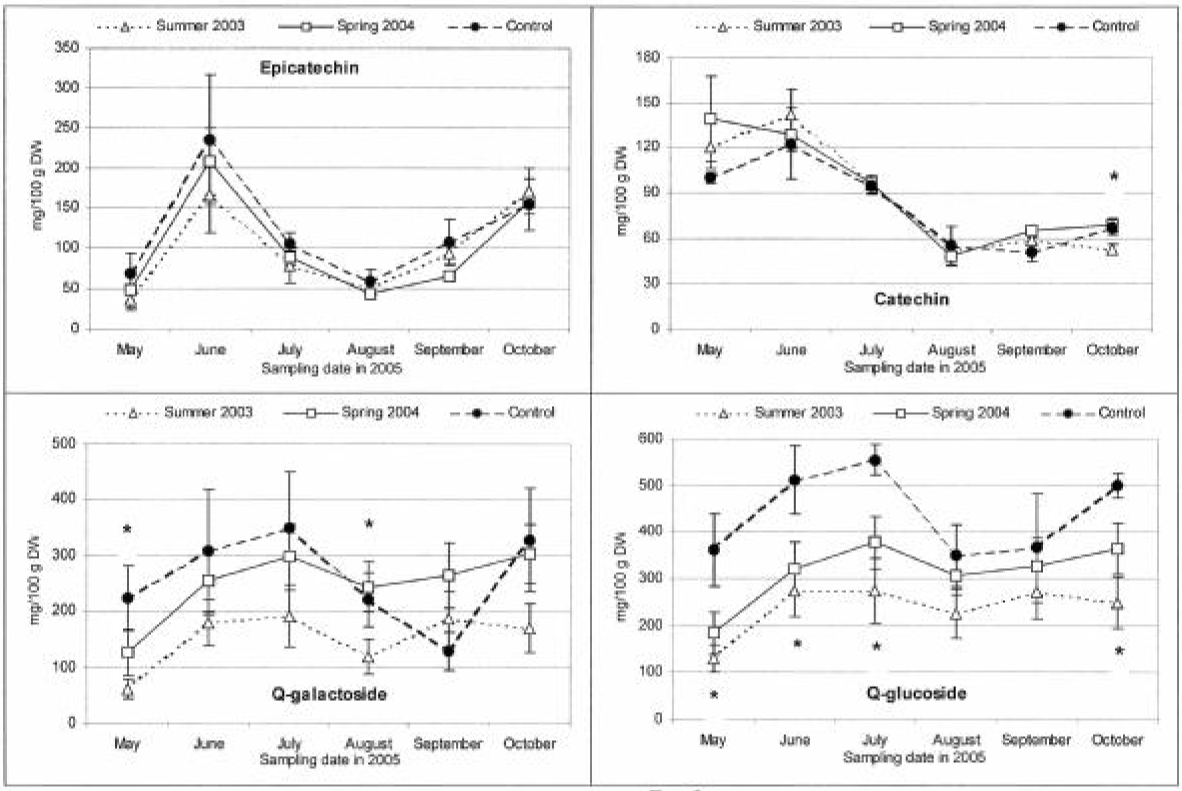

Summer 03
Spring 04
Control
Fructose
g/100 g DW
Sorbitol
Chlorogenic acid
mg/100 g DW
Vanillic acid
Sinapic acid
Rutin
Epicatechin
Catechin
May
June
July
August
September
October
Sampling date in 2004
*
**

FIG. 2

Figure 2. The progression of carbohydrates and phenolic compounds in 'Conference' pear leaves sampled from branches bent in summer 2003, in spring 2004, and from unbent branches (the control) through the sampling dates in the year 2005 (the mean values and standard error bars). Marks: * statistically significant at P -value below 0.05 and ** below 0.01 (one-way ANOVA).




Summer 2003
Spring 2004
Control
Epicatechin
Catechin
Q-galactoside
Q-glucoside
mg/100 g DW
May
June
July
August
September
October
Sampling date in 2005

2.5 VSEBNOSTI RAZLIČNIH METABOLITOV V PLODOVIH HRUŠKE SORTE 'CONFERENCE' GLEDE NA UPOGIBANJE RODNE VEJE

COLARIČ Mateja, ŠTAMPAR Franci in HUDINA Metka
Content levels of various fruit metabolites in the 'Conference' pear response to branch bending.
Scientia Horticulturae, 2007, x: 000-00.
Prejeto 14. 3. 2007, sprejeto 22. 3. 2007.

Sorta 'Conference' predstavlja pomemben delež med sortami hrušk, ki jih pridelujemo v Sloveniji. Rodi redno in obilno, in to kar 80 – 90 % iz brstičev na dveletnih ali triletnih vejah kot tudi na starejših. V poskusu smo upognili petletne rodne veje pozno poleti 2003 in spomladi 2004, kontrolno obravnavanje pa so predstavljale neupognjene rodne veje. V tehnološki zrelosti smo v letih 2004 in 2005 obrali plodove ter primerjali vsebnosti analiziranih ogljikovih hidratov, organskih kislin in fenolnih snovi med obravnavanji. V letu 2004 nismo ugotovili značilnih razlik v vsebnosti določanih ogljikovih hidratov, toda v letu 2005 sta bili v kontrolnih plodovih značilno večji vsebnosti glukoze in fruktoze in najmanjša vsebnost saharoze. V kontrolnih plodovih smo v prvem letu izmerili značilno večje vsebnosti jabolčne in fumarne kisline in manjše vsebnosti klorogenske in vanilne kisline ter v naslednjem letu najmanjše vsebnosti jabolčne kisline in največje vsebnosti nekaterih fenolnih snovi (epikatehina, kvercetin-3-D-galaktozida in kvercetin-3-β-D-glukozida). Med fenoli je bila v letu 2005 le vsebnost klorogenske kisline značilno večja v plodovih na vejah upognjenih spomladi 2004. Vsebnosti posameznih fenolnih snovi so bile v letu 2004 nekoliko večje v plodovih na vejah upognjenih poleti kot v plodovih na vejah upognjenih spomladi. V vsebnosti sorbitola, citronske kisline, katehina in sinapinske kisline v nobenem letu ni bilo razlik med obravnavanji. Razlike v vsebnosti posameznih metabolitov v plodovih sorte 'Conference' so bile posledica upogibanja rodnih vej, na le-te razlike je vplival tudi čas upogibanja (pozno poleti in spomladi).

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Content levels of various fruit metabolites in the 'Conference' pear response to branch bending

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Abstract

The response of 'Conference' pear subjected to branch bending in the content levels of various metabolites in its fruit was investigated. The fruits in commercial maturity were sampled in 2004 and 2005 from branches bent in the late summer of 2003 (the summer treatment), from branches bent in the late spring of 2004 (the spring treatment) and from control branches. The content levels of carbohydrates, organic acids and phenolic compounds were compared among treatments in two successive years. The fruit revealed various responses in content levels of metabolites. In the first year after bending, no significant differences were found in the content of each carbohydrate, but in 2005 by far the highest content level of glucose and fructose and the lowest content level of sucrose were found in fruits from the control. The control fruit showed significantly higher content levels of malic acid and lower content levels of some phenolics (chlorogenic and vanillic acid) in the first year after bending, but in the next year the opposite reaction occurred—the control fruit had the lowest content level of malic acid and the highest content level of epicatechin, quercetin-3-D-galactoside and quercetin-3-β-D-glucoside. The comparison of the two bending treatments alone in 2004 showed that the summer treatment often produced a slightly higher value of each phenolic in comparison to the spring treatment. However, in 2005 the significantly highest content of chlorogenic acid was in fruit from the spring treatment. Sorbitol, as well as citric acid, catechin and sinapic acid showed no clear tendency among treatments, neither in 2004 nor in 2005. It is suggested that these variations of 'Conference' fruit subjected to different bending treatments could not be the result of bending alone, but that they could be indirectly affected by other physiological responses of the fruit tree. However, it seemed that variations are affected by the time of bending and by the year-to-year, and such responses can be attributed to the 'Conference' genotype only.

Keywords: Pear; *Pyrus communis*; Branch bending; Fruits; Carbohydrates; Organic acids; Phenolic

1. Introduction

Pear (*Pyrus communis* L.) is a popular fruit in the temperate regions, and in addition to 'Williams', 'Conference' represents an important share of the cultivars grown in Slovenia (Hudina and Stampar, 2000). 'Conference' pear is a very fertile, high yielding cultivar that crops 80–90% on spurs from branches of 2- and 3-year of age as well as on older ones (Sansavini, 2002).

The necessity to regulate excessive vegetative (branch) growth and to increase flowering and fruiting becomes even more significant for economic reasons, i.e. cost reduction, since the ratio between production costs and market prices for fruit has increased in recent years. Among traditional methods of orchard management and cultural practices applied in an orchard to control growth and fruiting, branch bending has proved the most successful. Branch bending is a long established and widely used cultural practice in high-density orchards, and its concept has nowadays been integrated into the Solaxe training system (Costes et al., 2006). Lawes et al. (1997) report that bending resulted in higher floral precocity and in reduced shoot vigour of the 'Doyenne du Comice' pear. Apple and pear trees yielded more fruit and produced fruit earlier if regulated only by bending than those regulated by pruning alone (Goldschmidt-Reischel, 1997). However, Lauri and Lespinasse (2001) have shown that the tree's reaction to bending also varies with the genotype and the time of bending, as well as with the angle of bending, the duration of bending time, etc.

Fruit such as pears are an excellent source of carbohydrates for a diet. Despite the fact that pears contain a good quantity of

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sugars (on average 54% fructose, 18% sorbitol, and only 15% sucrose and 13% glucose) and dietary fiber (15–28 g/kg FW) (Blattný, 2003), they are a recommendable substitute for diabetics and the obese; moreover, dietary fiber together with phenolics helps to reduce the risk of developing cardiovascular disease (Gorinstein et al., 2002). In most pear genotypes, malic acid predominates among organic acids. However, Hudina and Stampar (2000) reported that 'Williams', 'Red Williams' and 'Rosired' pears contain a higher percentage of citric acid. Both acids mentioned are major contributors to the optimal degree of acidity, and their ratio (malic acid/citric acid ratio) correlates with the sensory evaluation of taste (Colaric et al., 2005).

Phenolic compounds have a great physiological role in fruit, as well in its resistance to mechanical and biological stress. Phenolic compounds in fruit are of great interest to the consumer, because they are an important factor in fruit quality: they contribute to their sensory qualities (aroma, astringency, bitterness and colour), some of them have pharmacological properties, too (anti-inflammatory, antitumor, antiallergic, etc.) (Macheix et al., 1990).

In light of the positive findings for bending, the aim of this study is to determine the response of 'Conference' pear to branch bending with respect to the content of various metabolites in its fruit. The content levels of carbohydrates, organic acids and phenolic compounds, which determine the nutritional value of fruit, are compared according to treatments in two successive years. Apart from sensory evaluation and physical measurements of the fruit, the parameters analysed represent the main indicator of fruit quality.

2. Material and methods

2.1. Chemicals

Carbohydrates (sucrose, glucose, fructose and sorbitol), citric acid, shikimic acid, fumaric acid, (−)-epicatechin, vanillic acid, quercetin-3-D-galactoside and quercetin-3-β-D-glucoside were obtained from Fluka Chemie GmbH (Buchs, Switzerland). The malic acid came from Merck KgaA (Darmstadt, Germany) and (+)-catechin from Carl Roth (Karlsruhe, Germany). Chlorogenic acid, sinapic acid, rutin (quercetin-3-rutinoside) and butylated hydroxytoluene (BHT) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Methanol was purchased from Riedel-de-Haën (Seelze, Germany) and acetonitrile from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). Bidistilled water purified in a Milli-Q water purification system by Millipore (Bedford, MA, USA) was used. Methanol, acetonitrile and bidistilled water were of the HPLC grade. Sugar and organic acid standards were prepared in bidistilled water and phenolic standards in methanol.

2.2. Plant material

The study involved pear trees 'Conference'/Quince MA planted in 1987, trained to a spindle system and grown in the eastern part of Slovenia on sandy loam soil. Trees were spaced 3.8 m × 1.4 m, and the row orientation was south-north. The pear orchard was maintained according to standard commercial practice for integrated fruit production (orchard management and cultural practice, i.e. spraying, irrigation, etc.). Three different management treatments were applied to the trees, and each single treatment was repeated randomly on ten trees. Treatments were the following: (i) the summer treatment, where one 5-year-old branch per tree (on the tree's eastern side and of comparable properties) was bent to an angle of 120° from the vertical position in late summer 2003 (1st September 2003); (ii) the spring treatment, where one 5-year-old branch per tree was bent in late spring 2004 (15th May 2004); (iii) the control treatment, where labeled branches were not bent (but remained 45° from the vertical). Before bending, the bent branches were grown like the control branches—at an angle of 45° from the vertical position. All bent branches, as well control branches, were allowed to develop without pruning from 2003 to 2005. The undamaged pear fruit were harvested from those branches (three representative fruit from each branch, repeated on ten trees per treatment) at commercial maturity on 9th September 2004 and 1st September 2005. Immediately after harvest, the fruits were stored at −20 °C.

2.3. Extraction procedure

The samples were homogenized to a puree with the T25 basic Ultra Turrax homogeniser (IKA Labortechnik, Janke and Kunkel GmbH, Staufen, Germany). Then the puree was prepared separately for the carbohydrate and organic acid analyses, and for the phenolic compound analyses.

For sugars and organic acids extraction, bidistilled water up to a final volume of 50 ml was added to 10 g of puree and left for 45 min at room temperature, with occasional stirring. After extraction, the puree was centrifuged at 12,000 × *g* (Eppendorf Centrifuge 5810 R, Hamburg, Germany) for 7 min at 10 °C. The supernatant was then filtered through a 0.45 μm cellulose mixed esters filter (Macherey-Nagel, Düren, Germany) into a vial and used for the carbohydrate and organic acid analysis.

For phenolic analyses, 1 g of the homogenized sample was transferred to a test tube and extracted once with 10 ml of methanol for 45 min in an ultrasonic bath cooled with ice. The methanol contained 1% of the antioxidative agent BHT, which had no influence on the extraction process and the HPLC-analysis. The extracted sample was centrifuged 12,000 × *g* for 7 min at 10 °C. Then the supernatant was filtered through a 0.45 μm Chromafil (Macherey-Nagel) polyamide filter and used for the phenolic compound analysis.

2.4. HPLC analyses

Chromatographic separation of the carbohydrates and organic acids was performed using a Thermo Separation Products HPLC system (Riviera Beach, FL, USA). The HPLC-analyses of carbohydrates were made using a Rezex RCM-monosaccharide column (300 mm × 7.8 mm) operated at 65 °C from Phenomenex (Torrance, CA, USA). The mobile phase was bidistilled water and the flow rate was 0.6 ml/min. The duration of the analysis was 60 min, and a refractive index (RI) detector (Shodex RI-71, Showa Denko K.K., Kawasaki, Japan) was used for monitoring eluted carbohydrates. The

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HPLC-analyses of organic acids were carried out using an Aminex HPX-87H column (300 mm × 7.8 mm) (Bio-Rad, Hercules, CA, USA) operated at 65 °C and associated with a Knauer K-2500 UV–vis detector (Knauer, Berlin, Germany) set at 210 nm. The mobile phase was 4 mM sulphuric acid in bidistilled water at a flow rate of 0.6 ml/min, and the total run time was 30 min (Colaric et al., 2006).

Separation of phenolic compounds was carried out using the Surveyor HPLC system (Thermo Finnigan, San Jose, CA, USA) with a photo diode array (PDA) detector, and it was controlled by the ChromQuest 4.0 Chromatography workstation software system. A Chromsep HPLC column SS (250 mm × 4.6 mm, Hypersil 5 ODS) coupled with a Chromsep guard column SS (10 mm × 3 mm) from Chrompack (Middelburg, the Netherlands) operated at 25 °C, was used. The chromatographic conditions were identical to those recommended by Schieber et al. (2001). The mobile phase consisted of solvent A: 2% acetic acid in bidistilled water (v/v), and of solvent B: 0.5% acetic acid in bidistilled water and acetonitrile (ratio 1:1, v/v), with a flow rate of 1 ml/min. The elution gradient was as follows: 0–50 min, 10–50% B; 50–60 min, 55–100% B; 60–65 min, 100–10% B. The total run time was 65 min, and between each analysis 15 min of equilibration treatment (10% B) was performed. Analysed compounds were identified and quantified in a manner similar in that previously described by Colaric et al. (2006). Identification of phenolics was done at 280 nm, except for chlorogenic and sinapic acid, which were identified at 320 nm.

2.5. Data analyses

All data were tested by one-way analysis of variance (ANOVA; general linear model) using the Statgraphics Plus 4.0 program (Manugistics, Inc., Rockville, MD, USA). Means among treatments were separated by the least significance difference (LSD) test ($P < 0.05$). Data in Tables 1–6 are presented as means with standard errors (S.E.) and LSD values. Contents of carbohydrates, as well as malic and citric acid in 'Conference' fruit are given in g/kg of fresh fruit weight (FW), while the contents of shikimic and fumaric acid and phenolics are given in mg/kg FW.

3. Results and discussion

3.1. The content of carbohydrates

Among carbohydrates, sucrose, glucose, fructose and sorbitol were determined in 'Conference' fruit, and their ratio was in accordance with Blattný (2003), who reported the average ratio in pears to be 15% sucrose, 13% glucose, 54%

Table 1
Average content of carbohydrates in 'Conference' fruit (the mean ± S.E., g/kg FW) harvested in 2004 regarding branch bending

Sugar	Bending treatment			LSD value
	Summer 2003	Spring 2004	Control	
Sucrose	15.20 ± 1.60a	17.03 ± 2.48a	18.53 ± 1.73a	6.22
Glucose	10.03 ± 1.67a	8.35 ± 1.88a	7.63 ± 1.20a	5.07
Fructose	51.38 ± 1.98a	50.24 ± 1.49a	48.47 ± 1.45a	5.03
Sorbitol	24.11 ± 1.62a	24.38 ± 2.19a	23.85 ± 2.30a	6.10

No values are significantly different (LSD test, $P < 0.05$).

Table 2
Average content of carbohydrates in 'Conference' fruit (the mean ± S.E., g/kg FW) harvested in 2005 regarding branch bending

Sugar	Bending treatment			LSD value
	Summer 2003	Spring 2004	Control	
Sucrose	25.62 ± 2.45b	21.78 ± 1.26b	6.60 ± 0.61a	6.53
Glucose	10.43 ± 0.35a	11.40 ± 0.24b	14.75 ± 0.13c	0.96
Fructose	73.65 ± 1.39ab	68.88 ± 1.46a	76.23 ± 4.69b	7.00
Sorbitol	34.90 ± 3.02a	37.04 ± 1.33a	33.58 ± 1.48a	7.33

Different letters within each row: significantly different (LSD test, $P < 0.05$).

Table 3
Average content of organic acids in 'Conference' fruit (the mean ± S.E., g/kg FW; except for shikimic and fumaric acids; their values are in mg/kg FW) harvested in 2004 regarding branch bending

Organic acid	Bending treatment			LSD value
	Summer 2003	Spring 2004	Control	
Citric acid	0.23 ± 0.01a	0.25 ± 0.01a	0.22 ± 0.02a	0.04
Malic acid	3.78 ± 0.08a	3.77 ± 0.09a	4.25 ± 0.18b	0.42
Shikimic acid	73.13 ± 7.75a	92.57 ± 8.11a	85.22 ± 5.58a	22.29
Fumaric acid	2.75 ± 0.13ab	2.59 ± 0.13a	3.09 ± 0.23b	0.50

Different letters within each row: significantly different (LSD test, $P < 0.05$).

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Table 4
Average content of organic acids in 'Conference' fruit (the mean ± S.E., g/kg FW; except for shikimic and fumaric acids; their values are in mg/kg FW) harvested in 2005 regarding branch bending

Organic acid	Bending treatment			LSD value
	Summer 2003	Spring 2004	Control	
Citric acid	0.21 ± 0.08a	0.17 ± 0.01a	0.21 ± 0.02a	0.13
Malic acid	2.76 ± 0.22b	2.70 ± 0.07b	1.97 ± 0.01a	0.41
Shikimic acid	9.56 ± 3.17a	10.71 ± 0.49a	47.09 ± 6.15b	6.32
Fumaric acid	2.83 ± 0.42a	2.54 ± 0.21a	2.42 ± 0.23a	1.06

Different letters within each row: significantly different (LSD test, $P < 0.05$).

Table 5
Average content of phenolic compounds in 'Conference' fruit (mean ± S.E., mg/kg FW) harvested in 2004 regarding branch bending

Phenolic compound	Bending treatment			LSD value
	Summer 2003	Spring 2004	Control	
Chlorogenic acid	240.32 ± 8.20b	212.58 ± 4.66ab	196.66 ± 21.86a	42.36
Catechin	1.75 ± 0.10a	1.31 ± 0.29a	1.68 ± 0.20a	0.62
Epicatechin	38.13 ± 4.43a	37.75 ± 5.57a	30.13 ± 3.35a	13.59
Sinapic acid	0.13 ± 0.02a	0.16 ± 0.03a	0.11 ± 0.02a	0.07
Vanillic acid	3.84 ± 0.41b	2.78 ± 0.18a	2.52 ± 0.35a	1.02

Different letters within each row: significantly different (LSD test, $P < 0.05$).

fructose, 18% sorbitol and the sum of those sugars about 124–158 g/kg fruit FW.

In both successive years, fructose predominated among carbohydrates, as well as among all analysed compounds; it was followed by sorbitol with about one half the fructose content (Tables 1 and 2). In the first year after bending, no significant differences were found in carbohydrate content among treatments (Table 1). However, the glucose and fructose contents were a little higher in fruit harvested from branches bent in the late summer of 2003 (the summer treatment), whereas the lowest sucrose content was noticed. Some lower values of other carbohydrates were found in the control fruit.

In the following year, the carbohydrate content – sucrose, glucose and fructose – significantly differed among treatments (Table 2). Sucrose content was lowest in fruit from the control treatment, and it differed significantly from both bending treatments. Just the opposite, the highest content levels of glucose and fructose were attained in fruit from the control.

Sanyal and Bangerth (1998) claimed that branch bending imposes mechanically induced stress. Moreover, Hudina and Stampar (1999) reported that sorbitol content increased in pear fruit from trees that were subjected to stress conditions. Nevertheless, on the basis of these statements and our results showing the opposite, it is suggested that no stress occurred in our study, since no significant differences between bending treatments and the control were observed for sorbitol in both years.

To date, Colaric et al. (2006) have researched the influence of branch bending on 'Williams' fruit at the chemical level, and they obtained different responses from those for 'Conference' fruit in the first year, although the study lasted for 1 year only. 'Williams' fruit picked from branches bent in the previous summer (2003) showed the lowest content levels of each carbohydrate, and the highest ones were found in the fruit from branches bent in the current spring (2004), except for glucose. Probably, branches bent in the spring had more photosynthates that were exported to the fruit. Ito et al. (2004) studied

Table 6
Average content of phenolic compounds in 'Conference' fruit (mean ± S.E., mg/kg FW) harvested in 2005 regarding branch bending

Phenolic compound	Bending treatment			LSD value
	Summer 2003	Spring 2004	Control	
Chlorogenic acid	64.45 ± 10.61a	174.14 ± 22.61b	116.51 ± 15.09ab	106.7
Catechin	10.41 ± 2.76a	8.57 ± 0.97a	9.69 ± 1.74a	5.73
Epicatechin	5.96 ± 0.84a	7.03 ± 0.81a	17.58 ± 3.63b	5.47
Sinapic acid	0.93 ± 0.12a	0.88 ± 0.08a	1.18 ± 0.11a	0.38
Vanillic acid	1.09 ± 0.39a	3.33 ± 0.48a	2.55 ± 1.01a	2.46
Rutin	3.61 ± 0.95a	2.65 ± 0.21a	3.68 ± 0.11a	1.52
Q-3-D-galactoside	0.92 ± 0.53a	0.94 ± 0.29a	4.51 ± 0.35b	1.43
Q-3-β-D-glucoside	2.73 ± 0.90a	2.88 ± 0.53a	6.09 ± 0.34b	2.62

Different letters within each row: significantly different (LSD test, $P < 0.05$).

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carbohydrate metabolism in the lateral buds and in the shoot internodes of Japanese pear. They observed that bending influenced higher contents of sorbitol and sucrose in the central internode of the bent branch in comparison to that of the control, i.e. the vertical branch. Sorbitol and sucrose are the main translocating carbohydrates found in rosaceous fruit trees, the group to which pear belongs.

3.2. *The content of organic acids*

'Conference' pear is classified into the group of pear genotypes where, among organic acids, malic acid usually predominates in the fruit (Hudina and Stampar, 2000), and this was also confirmed in our study. The content of malic acid in the fruit from the control was significantly higher than in the fruit from both bending treatments in 2004, where similar content levels were found (Table 3). In the following year, however, significantly lower values of malic acid were measured in the control compared to those from the bending treatments (Table 4). In both years, a similar trend was noticed: the highest malic acid content corresponded with the highest sucrose content and with the lowest glucose content and vice versa.

Fumaric and shikimic acids were present in very small amounts. In 2004, the highest values of fumaric acid were found in fruit from the control, whereas citric and shikimic acids showed no significant differences in content levels among treatments. In the following year, the highest values of shikimic acid were found in the control, whereas some lower values of fumaric acid were found. Citric acid showed no clear tendency among treatments, neither in 2004 nor in 2005.

However, 'Williams' pears contained more citric than malic acid (Hudina and Stampar, 2000). Moreover, the content levels of all organic acids except citric (its content level was the lowest in the same treatment) proved the highest in the 'Williams' fruit from the branches bent in summer, whereas the same fruit were the poorest in carbohydrates (Colaric et al., 2006), as mentioned before. Both major acids (malic and citric) are the most important contributors to the optimal degree of acidity, and it was found that their ratio correlated with sensory evaluation of taste (Colaric et al., 2005).

3.3. *The content of selected secondary compounds*

Three phenolic acids (chlorogenic, sinapic and vanillic acids) and two flavonoids from the flavan-3-ols subclass (catechin and epicatechin) were detected in the first year after bending; moreover, three additional flavonoids from the flavonol glycosides subclass (rutin, quercetin-3-D-galactoside and quercetin-3-β-D-glucoside) were detected in the next year. The separation of phenolics (chromatographic conditions, mobile phases) was done according to Schieber et al. (2001), who determined rather similar content levels of flavonol glycosides in pear fruit. However, chlorogenic acid was the major phenolic in the 'Conference' fruit, and its content level is more in accordance with Macheix et al. (1990), who reported values from 10 to 516 mg/kg FW.

Generally, in the first year, some lower values of each determined phenolic compound, except for catechin, were observed in fruit from the control (Table 5). Content levels of chlorogenic acid and vanillic acid in fruit from the control differed significantly from the fruit involved in the summer treatment. The comparison of both bending treatments resulted in an observation that the summer treatment often showed a slightly higher value of each phenolic in comparison to the spring treatment. In the second year, epicatechin, sinapic acid and flavonol glycosides appeared in higher levels in fruit from the control, and among them epicatechin, quercetin-3-D-galactoside and quercetin-3-β-D-glucoside differed significantly from both bending treatments (Table 6). Andreotti et al. (2006) reported that the accumulation of flavan-3-ols and flavonol glycosides is affected by wounding. However, the content levels of chlorogenic and vanillic acids in 2005 were the highest (significant for the chlorogenic acid) in the fruit from branches bent in the late spring of 2004 and lowest in the fruit from branches bent in the late summer of 2003, but the content levels of sinapic acid were the opposite.

It is supposed that some lower values of phenolics in fruit from the control (in 2004) could be connected to some lower values of carbohydrates (except for sucrose) in the same treatment, despite the fact that phenolics are mostly produced from carbohydrates (Macheix et al., 1990). The key enzyme that links primary and secondary metabolism is phenylalanine ammonialyase (PAL). It was reported that PAL activity reaches its maximum in very young fruit, and that corresponds to an increase in the accumulation of phenolic compounds; therefore, higher content levels of phenolics appear in young fruit. However, the phenolic metabolism is also greatly dependent on many external (light, temperature, stress) and internal (hormones, nutrients) factors (Macheix et al., 1990). Macheix et al. (1990) reported that fruit phenolics were also increased by lighting, which raises primary production (carbohydrates), which then supports higher PAL activity, and thus causes an increase in the accumulation of phenolic compounds, especially flavonoids.

Treutter (2001) stated that stress situations induce synthesis of phenolic compounds, and, according to Sanyal and Bangerth (1998), mild stress is imposed by branch bending. Considering only the phenolics content levels in 'Conference' fruit from the bending treatments (in 2004), we can confirm the statement of Sanyal and Bangerth (1998). Nevertheless, one can judge that branch bending was not functioning as a type of stress factor, since the content levels of most phenolics in the 'Williams' fruit involved in the summer treatment were among the lowest and in the fruit from the spring treatment among the highest (Colaric et al., 2006). What is more, 'Williams' leaves that responded to branch bending had almost the lowest phenolic content levels in the summer treatment (Colaric et al., 2007).

It seems that the response of fruit to bending varies most with genotype (since 'Williams' and 'Conference' pears responded differently), as well as with the time of bending, by the year-to-year variations, etc. Moreover, we should not forget that the variations observed in that study could also be a consequence of the physiological responses of the fruit tree and the fruit. Lauri and Lespinasse (2001) showed that the response in growth and fruiting of apples subjected to shoot bending also varied with genotype and time of bending. Furthermore, Sansavini (2002) reported that 'Conference' differed from 'Williams' in growth and fruiting habits. 'Conference' has the advantage that it is regarded as regular bearing cultivar, which bears 80–90% on spurs (model 3 of five fruiting models), mostly from branches of 2–3 years in age, but it never stops cropping on the spurs of the oldest branches, while 'Williams' crops less than 50% on the spur buds.

The decision about the timing of branch bending still remains open for 'Conference' pears, which showed an unclear tendency between both bending treatments, although some differences were found among the fruit involved in bending and those in the control treatments (Tables 1–6). But for the 'Williams' pears it can be inferred that branch bending in spring is more recommended (Colaric et al., 2006). However, it seems from the above results that branch bending had a positive effect on the level of phenolics in 'Conference' fruit in 2004.

4. Conclusion

The focus of the research was the effect of branch angle change on 'Conference' fruit in two successive years at the chemical level. The fruit revealed various responses in carbohydrates, organic acids and phenolics content levels. Nevertheless, it was shown that fruit from the control reached significantly higher content levels of malic acid and lower content levels of certain phenolics in the first year after bending, but in the next year the exactly opposite reaction occurred. Sorbitol, as well as citric acid, catechin and sinapic acid showed no clear tendency among treatments, neither in 2004 nor in 2005. It can be inferred that those variations of 'Conference' fruit among bending treatments could not be the result of bending alone, but that they could be indirectly affected by other physiological responses of the fruit tree. However, it seems that variations are affected by bending time and by the year-to-year variations, and such responses can be attributed to the 'Conference' genotype only.

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3 RAZPRAVA IN SKLEPI

3.1 RAZPRAVA

V dveh zaporednih letih 2004 in 2005 smo spremljali vsebnosti primarnih in sekundarnih metabolitov v plodovih in listih evropske žlahtne hruške sort 'Williams' in 'Conference', ki so bili vzorčeni na petletnih rodnih vejah. Rodne veje so bile vključene v različna obravnavanja: rodne veje, ki smo jim spremenili kot rasti iz 45° na 120° od navpične lege (i) pozno poleti 2003 in (ii) spomladi 2004 ter (iii) rodne veje, ki niso bile upognjene, temveč so vseskozi rasle pod kotom 45° od navpične lege.

V listih, kjer smo spremljali vsebnosti metabolitov od maja do oktobra, smo določili ogljikove hidrate - saharozo, glukozo, fruktozo in sorbitol ter fenolne snovi - klorogensko, vanilno in sinapinsko kislino ter (-)-epikatehin, (+)-katehin in rutin ter v naslednjem letu dodatno še dva flavonola: kvercetin-3-D-galaktozid in kvercetin-3-β-D-glukozid (Colarič in sod., 2006a; 2007a; 2007b).

Ogljikove hidrate so spremljali tudi v listih drugih sadnih rastlin iz družine rožnic (Loescher in Everard, 1996; Deguchi in sod., 2002; Veberič in sod. , 2003; Šircelj in sod., 2005). V našem poskusu je v obeh letih pri obeh sortah pa tudi v vseh vzorčenjih v listih prevladoval sorbitol nad ostalimi določanimi snovmi (Colarič in sod., 2006a; 2007b). Hudina in Štampar (1999) sta pri evropski žlahtni hruški ugotovila, da se je v sušnih razmerah vsebnost sorbitola povečala. Podobno so Šircelj in sod. (2005) ugotovili pri jablani: sorbitol se je tvoril kljub neugodnim razmeram kot je zmeren sušni stres, toda dolgotrajna suša je zmanjšala kopičenje sorbitola.

V letu 2004 smo izmerili največje vrednosti sorbitola v listih pri sorti 'Williams' v septembru in pri sorti 'Conference' v maju; največje vrednosti saharoze, glukoze in fruktoze pa so bile pri obeh sortah oktobra (Colarič in sod., 2006a; 2007b). Sezonsko gibanje ogljikovih hidratov v listih obeh proučevanih sort proti koncu prvega leta je bilo podobno kot navajajo Veberič in sod. (2003) za liste jablane: vsebnosti fruktoze, glukoze in saharoze so se od konca avgusta pa do oktobra povečale in vsebnosti sorbitola zmanjšale. Naslednje leto smo pri sorti 'Williams' v oktobru izmerili le največje vrednosti sorbitola, fruktoze je bilo največ v septembru ter saharoze in glukoze največ že v maju. Pri sorti 'Conference' smo v letu 2005 izmerili največje vsebnosti treh ogljikovih hidratov (fruktoze, sorbitola in glukoze) v oktobru, z izjemo saharoze, ki je dosegla največjo vrednost v juliju (Colarič in sod., 2007b).

Med vsemi proučevanimi fenolnimi snovmi, smo izmerili največje vsebnosti klorogenske kisline. Le-ta je zelo pomembna pri odpornosti na hrušev ožig (Gunen in sod., 2005). V prvem letu smo pri obeh sortah zasledili najmanjše vsebnosti posameznih fenolnih snovi v maju, nato so se pri sorti 'Conference' vsebnosti povečevale do julija, zatem pa počasi zmanjševale do oktobra; katehin pa je dosegel največjo vrednost šele v septembru (Colarič in sod., 2007b). Sezonska dinamika sorte 'Williams' je bila nekoliko drugačna: v juliju sta največje vrednosti dosegli le klorogenska kislina in rutin ter šele avgusta vanilna in sinapinska kislina in septembra katehin in epikatehin (Colarič in sod., 2006a). Podobno sezonsko gibanje fenolnih snovi so zabeležili tudi Usenik in sod. (2004) v listih jablane: vsebnosti posameznih fenolov so naraščale vse do julija ali avgusta, nato pa so se zmanjšale. V letu 2005 smo pri obeh sortah zabeležili največje vrednosti večine fenolov že v juniju, nato so se le-te zmanjšale do avgusta in nato ponovno povečale do konca sezone. Domnevamo, da so na tak potek vplivale tudi ekstremne vremenske razmere (deževje in nižje temperature), še posebej v juliju in avgustu 2005 (Colarič in sod., 2006a; 2007b).

V plodovih smo v tehnološki zrelosti poleg ogljikovih hidratov ter fenolnih snovi, ki smo jih določali tudi v listih, izmerili vsebnosti organskih kislin: citronske, jabolčne, šikimske in fumarne kisline. Sorta ‘Williams’ je po času zorenja še poletna hruška (Blattný, 2003), v letu 2004 smo jo obrali 1. septembra in v letu 2005 19. avgusta. Sorto ‘Conference’ uvrščamo med jesenske hruške (Blattný, 2003) in smo jo v letu 2004 obrali 9. septembra in v letu 2005 1. septembra. Razmerje med posameznimi ogljikovimi hidrati je bilo pri obeh sortah hrušk podobno kot navaja Blattný (2003) in vsebnost fruktoze je prevladala nad sorbitolom. Močno pa se sorti ‘Williams’ in ‘Conference’ razlikujeta po vsebnosti citronske in jabolčne kisline (Hudina in Štampar, 2000a), podobno smo ugotovili tudi v našem poskusu (Colarič in sod., 2006b; 2007c). Hudina in Štampar (2000a) sta poleg sorte ‘Williams’ izmerila večje vsebnosti citronske kisline tudi pri sortah ‘Red Williams’ in ‘Rosired’, ostale sorte pa so imele največje vsebnosti jabolčne kisline.

Tudi v plodovih obeh sort hrušk smo izmerili največje vsebnosti klorogenske kisline, sledila sta epikatehin in katehin. Vsebnosti omenjenih fenolov so nekoliko večje kot poročajo Amiot in sod. (1995) ter Herrmann (2001). V naslednjem letu nam je uspelo določiti tri flavonol glikozide (rutin, kvercetin-3-D-galaktozid in kvercetin-3-β-D-glukozid), katerih vsebnost je podobna kot navajajo Schieber in sod. (2001), po čigar metodi smo tudi ločili fenole v plodovih in listih.

Zgoraj smo opisali vsebnosti različnih metabolitov v listih (med rastno dobo) in v plodovih (v tehnološki zrelosti) proučevanih sort hrušk, saj do sedaj pri nas še niso bili tako raziskani, z izjemo plodov, kjer sta Hudina in Štampar (2000a; 2000b; 2004) podrobno proučila vsebnosti sladkorjev in organskih kislin. Glavni namen naših raziskav pa je bil ugotoviti ali upogibanje, kot eden izmed učinkovitih agrotehničnih ukrepov, s katerim v sadovnjakih uravnavamo rast in rodnost (Luckwill, 1970; Lauri in Lespinasse, 2001; Costes in sod., 2006), vpliva na vsebnost že omenjenih primarnih in sekundarnih metabolitov, saj do sedaj ta del še ni bil predmet raziskav. Vsebnost metabolitov je pomembna tudi z vidika kakovosti plodov, saj le-ti prispevajo k senzoričnim lastnostim (sladkost, kislost, vonj, okus, trpkost, grenkost, obarvanost), mnogi med njimi imajo zdravilne lastnosti kot npr. nekatere fenolne snovi, ki zavirajo razna vnetja, rast tumorjev, pojav alergij in srčno-žilnih bolezni (Macheix in sod., 1990; Gorinstein in sod., 2002). Ogljikovi hidrati so glavna skladiščna snov in osnovna organska snov, iz katere se tvorijo še druge organske snovi, tudi fenoli (Kozlowski in Pallardy, 1997). Fenolne snovi imajo v rastlini pomembno fiziološko vlogo, saj vplivajo tako na njeno rast kot tudi razvoj, in vlogo v odpornosti na mehanski in biološki stres (Macheix in sod., 1990).

V prvem letu našega poskusa smo pri sorti ‘Williams’ zasledili, da se listi, ki smo jih vzorčili na upognjenih in na neupognjenih vejah, niso značilno razlikovali med seboj v vsebnosti ogljikovih hidratov, razlikovali pa so se v vsebnosti fenolov. Kadar so bile statistično značilne razlike v vsebnosti posameznih fenolov, so bile vsebnosti le-teh največje v listih iz kontrolnega obravnavanja ter najmanjše v listih, ki so bili vzorčeni na rodnih vejah upognjenih poleti 2003 (Colarič in sod., 2007a). V vsebnosti klorogenske kisline in rutina so bile razlike v mesecu juliju, sinapinske kisline v avgustu in septembru, katehina le v septembru ter epikatehina v juliju in septembru.

V listih sorte 'Williams' smo v letu 2005 zabeležili razlike v vsebnosti fruktoze in sorbitola, oba ogljikova hidrata sta imela v avgustu najmanjšo vrednost v kontrolnem obravnavanju, sorbitol pa še v juniju. Toda v septembru je prišlo do preobrata in sorbitol je prav pri kontroli dosegel največjo vsebnost. Tudi v drugem letu smo izmerili večje vsebnosti nekaterih fenolov v listih s kontrolnega obravnavanja, še posebej vseh treh določanih flavonol glikozidov. Statistično značilne razlike v vsebnosti vanilne kisline, rutina in kvercetin-3-D-galaktozida smo zabeležili v mesecu juniju, katehina v juliju in kvercetin-3-β-D-glukozida v avgustu. Epikatehin je bil izjema, saj je bila njegova vsebnost v oktobru značilno najmanjša prav v listih z neupognjenih vej. Andreotti in sod. (2006) so izmerili večje vsebnosti flavonol glikozidov in flavan-3-olov v poškodovanih listih, za liste analizirane v našem poskusu, pa tega ne moremo trditi, saj smo v vseh obravnavanjih vzorčili zdrave liste. Sanyal in Bangerth (1998) trdita, da upogibanje rodnih vej povzroči v sadnih rastlinah zmeren stres, nastajanje fenolnih snovi pa se običajno poveča ob stresnih situacijah (Treutter, 2001), kar je v neskladju z našimi dvoletnimi rezultati, saj so bile vsebnosti fenolov sorte 'Williams' največkrat značilno večje v listih z neupognjenih vej - kontrole.

Upogibanje rodnih vej tudi v listih sorte 'Conference' ni pokazalo jasnih razlik v vsebnosti posameznih ogljikovih hidratov, niti v letu 2004 niti ne v letu 2005 (Colarič in sod., 2007b). V prvem letu je bila v kontrolnih listih vsebnost saharoze značilno večja v juniju in sorbitola značilno manjša v oktobru. Upogibanje je mnogo bolj vplivalo na vsebnosti fenolov, saj smo v prvem letu izmerili značilno večje vsebnosti klorogenske, vanilne in sinapinske kisline ter rutina v listih na rodnih vejah upognjenih spomladi 2004. Izjema sta bila oba določana flavan-3-ola, katerih vsebnost je bila vsakega po enkrat največja v kontrolnih listihin le epikatehina enkrat v listih na rodnih vejah upognjenih poleti 2003.

V naslednjem letu je bila slika sladkorjev v listih sorte 'Conference' še bolj nejasna: pri glukozi smo tako zasledili značilno največje vrednosti po enkrat v vsakem obravnavanju, sorbitol je junija dosegel največjo vsebnost v listih na rodnih vejah upognjenih poleti 2003 in oktobra v kontrolnih listih, največje vrednosti fruktoze pa smo izmerili avgusta v listih na rodnih vejah upognjenih spomladi 2004 (Colarič in sod., 2007b). Ito in sod. (2004), ki so proučevali odgovor lateralnih brstov in poganjkov našija na upogibanje vej, so ugotovili, da so se z upogibanjem vsebnosti sorbitola in saharoze v lateralnih brstih zmanjšale, toda v internodijih poganjkov povečale. Tudi v letu 2005 smo izmerili večje vsebnosti klorogenske in vanilne kisline ter rutina v listih na rodnih vejah upognjenih spomladi 2004, v tem obravnavanju sta imela največjo vsebnost tudi katehin v oktobru in kvercetin-3-D-galaktozida v avgustu. Sicer pa so bile največje vsebnosti sinapinske kisline (junija in oktobra), kvercetin-3-D-galaktozida (maja) in kvercetin-3-β-D-glukozida (maja, junija, julija in oktobra) v kontrolnih listih.

Če povzamemo dvoletne rezultate obeh sort, vidimo, da se vsebnosti ogljikovih hidratov v listih na upogibanje ne spremenijo v nekem sosledju, vsebnosti fenolnih snovi pa. Proučevani sorti sta se na upogibanje različno odzvali: upogibanje, še posebej poletno, je povzročilo pri sorti 'Williams' največkrat zmanjšanje vsebnosti posameznih fenolov. Pri sorti 'Conference' pa je upogibanje, še posebej spomladansko, povečalo vsebnosti nekaterih fenolov. Iz zgoraj opisanega lahko sklepamo, da se odziv drevesa na upogibanje razlikuje med sortami, pomembno pa je tudi kdaj so bile rodne veje upognjene, s tem pa lahko povežemo dognanja Lauri-ja in Lespinasse-ja (2001), ki sta ugotovila, da se jablane na upogibanje različno odzovejo v rasti in rodnosti, saj v veliki meri vplivata tako sorta kot tudi čas upogibanja. Proučevani sorti sta imeli podobne vrednosti posameznih fenolov, le obeh flavan-3-olov je bilo več v listih sorte 'Williams' ter kvercetin-3-D-galaktozida in kvercetin-3-β-D-glukozida manj. Gunen in sod. (2005) so izmerili večje vsebnosti fenolov v listih na hrušev bakterijski ožig odpornih sortah hrušk.

V plodovih sorte 'Williams', ki smo jih obrali na rodnih vejah upognjenih poleti 2003, so bile v prvem letu vsebnosti ogljikovih hidratov najmanjše, največje pa so bile v plodovih na vejah upognjenih spomladi 2004 (z izjemo glukoze), kar pa je v naslednjem letu obveljalo le za saharozo (Colarič in sod., 2006b). V vsebnosti organskih kislin v nobenem letu ni bilo značilnih razlik, čeprav so bile vsebnosti posameznih organskih kislin z izjemo citronske, v prvem letu nekoliko večje prav v obravnavanju, kjer so bile vsebnosti ogljikovih hidratov najmanjše. V prvem letu smo v vsebnosti posameznih fenolnih snovi zabeležili enako kot pri sladkorjih, z izjemo sinapinske kisline, katere vsebnost je bila sicer tudi najmanjša v plodovih na vejah upognjenih poleti 2003, toda največja vsebnost le-te pa je bila izmerjena v kontrolnih plodovih. V naslednjem letu sta bili vsebnosti glukoze in fruktoze najmanjši pri kontroli, vsebnost sorbitola pa je bila nekoliko večja v plodovih na vejah upognjenih poleti 2003. Fenoli so nam v drugem letu podali drugačno sliko v primerjavi s prejšnjim letom: največje vsebnosti smo izmerili v plodovih obranih na vejah, ki smo jih upognili poleti 2003 in najmanjše v kontrolnih plodovih. Izjema sta bila kvercetin-3-D-galaktozid in kvercetin-3-β-D-glukozid, katerih vsebnost je bila ponovno večja pri kontroli. Vsebnost sinapinske kisline je bila najmanjša v plodovih na vejah upognjenih spomladi 2004, ostali obravnavanji pa se v vsebnosti nista razlikovali med sabo.

V plodovih sorte 'Conference' v prvem letu nismo ugotovili nobenih značilnih razlik v vsebnosti ogljikovih hidratov med obravnavanji (Colarič in sod., 2007c). Vsebnosti jabolčne in fumarne kisline sta bili značilno največji pri kontroli. Vsebnosti citronske kisline so bile podobne med obravnavanji, a majhne, tako v letu 2004 kot tudi v letu 2005. V prvem letu so bile vsebnosti posameznih fenolov, z izjemo katehina, najmanjše v kontrolnih plodovih ter največje v plodovih z upognjenih rodnih vej, še posebej poleti 2003. V letu 2005 smo v kontrolnih plodovih izmerili značilno večje vsebnosti glukoze in fruktoze ter manjše vsebnosti saharoze. Tudi pri tej sorti so nam fenoli v drugem letu pokazali drugačno sliko. Največje vsebnosti epikatehina, sinapinske kisline ter določanih flavonol glikozidov smo izmerili v kontrolnih plodovih ter najmanjše vsebnosti vseh posameznih fenolov v plodovih na upognjenih vejah, še posebej upognjenih poleti 2003. Toda vsebnosti klorogenske in vanilne kisline so bile nekoliko večje v plodovih na vejah upognjenih spomladi 2004, kar je zanimivo, saj sta imela oba fenola največje vrednosti v enakem obravnavanju tudi v listih, in to kar obe leti (Colarič in sod., 2007b). Katehin je bil še večja izjema, saj je imel najmanjšo vsebnost v plodovih na vejah upognjenih spomladi 2004 ter največjo na vejah upognjenih poleti 2003.

S pomočjo zgoraj opisanih dognanj lahko povežemo rezultate dvoletnih meritev vsebnosti metabolitov v listih in plodovih posamezne sorte. Pri sorti 'Williams' so bile prvo leto najmanjše vsebnosti posameznih fenolov, tako v listih kot tudi v plodovih, ki smo jih vzorčili na rodnih vejah upognjenih poleti 2003, enako smo opazili le v listih tudi v naslednjem letu. V obeh letih smo izmerili največje vsebnosti fenolov v listih iz kontrolnega obravnavanja, kjer pa so bile v letu 2005 najmanjše vsebnosti v plodovih. V letu 2004 smo izmerili največje vsebnosti fenolov v plodovih na rodnih vejah upognjenih spomladi 2004. V naslednjem letu pa je prišlo do preobrata in smo v plodovih, ki smo jih vzorčili na vejah upognjenih poleti 2003, izmerili največje vsebnosti fenolov (izjema so bili flavonol glikozidi z največjo vsebnostjo pri kontroli). Pri sorti 'Conference' so se vsebnosti fenolnih snovi v listih in plodovih precej izključevale: v obeh letih so bile največje vsebnosti v listih na vejah upognjenih spomladi 2004 ter le v prvem letu v plodovih na vejah upognjenih poleti 2003, naslednje leto pa v kontrolnih plodovih. Izjeme so bile klorogenska in vanilna kislina, katerih vsebnost je bila največja v plodovih na vejah upognjenih spomladi 2004 in katehin z največjo vsebnostjo v plodovih na vejah upognjenih poleti 2003. V dvoletnem poskusu smo potrdili razlike med proučevanima sortama, ki smo jih tudi pričakovali, saj se sorti razlikujeta v rasti in rodnosti ter v pomoloških lastnostih ploda (Sansavini, 2002).

Glede na rezultate vsebnosti metabolitov v listih in plodovih, lahko domnevamo, da je za sorto 'Williams' primernejše upogibanje vej spomladi ter za sorto 'Conference' pozno poleti. Seveda pa bilo potrebno v prihodnje poskus zastaviti še na večjemu številu sort. Za leto 2005 velja omeniti, da je bilo poletje precej deževno in hladnejše od dolgoletnega povprečja (Colarič in sod., 2007b), kar bi lahko vplivalo na preobrat v vsebnosti metabolitov, saj je vsebnost fenolov močno odvisna tudi od mnogih zunanjih ali okoljskih (stres, temperatura, svetloba) in notranjih dejavnikov (hranila, hormoni) (Macheix in sod., 1990). Vsebnosti fenolnih snovi: (hidroksicimetne kisline, flavan-3-oli in flavonol glikozidi) lahko povečamo s hranili in ogljikovimi hidrati kot je npr. saharoza (Lux-Endrich in sod., 2000). Glede na večje vsebnosti saharoze in fenolov v plodovih na rodnih vejah upognjenih spomladi 2004 pri sorti 'Williams', lahko povežemo ugotovitve Lux-Endrich-a in sod. (2000) z našimi, vendar pa to ne moremo trditi za sorto 'Conference'.

3.2 SKLEPI

V dvoletnem poskusu smo podrobno proučili kemično sestavo listov in plodov v Sloveniji razširjenih sort hrušk 'Williams' in 'Conference'. S tekočinsko kromatografijo visoke ločljivosti smo v listih med rastno dobo in plodovih v tehnološki zrelosti izmerili vsebnosti ogljikovih hidratov (saharoze, glukoze, fruktoze in sorbitola) in fenolnih snovi (klorogenske, vanilne in sinapinske kisline, (-)-epikatehina, (+)-katehina, rutina, kvercetin-3-D-galaktozida in kvercetin-3-β-D-glukozida) ter v plodovih tudi vsebnosti organskih kislin (citronske, jabolčne, šikimske in fumarne kisline).

Med ogljikovimi hidrati smo v plodovih izmerili največje vsebnosti fruktoze in v listih sorbitola, med fenolnimi snovmi pa je bilo v plodovih in listih največ klorogenske kisline. V plodovih sorte 'Williams' smo izmerili večje vsebnosti citronske kisline in v plodovih sorte 'Conference' večje vsebnosti jabolčne kisline.

Vsebnosti metabolitov v listih, ki smo jih vzorčili med rastno dobo od maja do oktobra, so se med vzorčenji razlikovale, najbolj značilne razlike pa so bile v vsebnosti fenolov. Vsebnosti le-teh so bile običajno najmanjše na začetku rastne dobe, nato so naraščale do junija, julija ali vse do avgusta in se zmanjševale do oktobra. Opazili smo razlike tudi med obema letoma, saj so fenoli v drugem letu dosegli dva vrha, na kar so morda vplivale tudi vremenske razmere (okoljski dejavniki). Proučevani sorti sta se med rastno dobo razlikovali v vsebnosti metabolitov tudi med seboj, primer: v prvem letu smo pri sorti 'Williams' izmerili največje vsebnosti sorbitola in katehina septembra ter pri sorti 'Conference' največje vsebnosti sorbitola maja in katehina julija.

Upogibanje rodnih vej ni pokazalo jasnega vpliva na vsebnosti ogljikovih hidratov v listih. Upogibanje (še posebej poletno upogibanje) je pri sorti 'Williams' povzročilo v listih manjšo vsebnost fenolnih snovi, pri sorti 'Conference' pa je upogibanje (še posebej spomladansko) povečalo vsebnosti nekaterih fenolov, zato menimo, da imata vpliv na učinek upogibanja tudi sorta in čas, ko smo izvedli upogibanje.

Tudi v plodovih sta se proučevani sorti različno odzvali na agrotehnični ukrep upogibanje rodnih vej. Pri sorti 'Williams' so bile v prvem letu največje vsebnosti fenolnih snovi (pa tudi ogljikovih hidratov) v plodovih, ki smo jih obrali na rodnih vejah upognjenih spomladi 2004, zato domnevamo, da je upogibanje rodnih vej v pomladnem času za to sorto bolj primerno, tudi z vidika notranje kakovosti plodov. Pri sorti 'Conference' menimo, da je primernejši čas upogibanja pozno poleti, saj smo v prvem letu zabeležili največje vsebnosti fenolov v plodovih na upognjenih rodnih vejah poleti 2003.

Znotraj fenolnih podrazredov so se fenoli v plodovih in listih različno odzvali na upogibanje: vsebnosti flavonol glikozidov - kvercetin-3-D-galaktozida in kvercetin-3-β-D-glukozida so bile pogosto večje pri kontroli, fenolnih kislin - klorogenske in vanilne kisline pa v plodovih in listih, ki so bili vzorčeni na upognjenih vejah.

V raziskavi smo potrdili razlike v vsebnosti metabolitov v listih in plodovih, ki smo jih vzorčili na upognjenih (spremenjen kot rasti) in neupognjenih vejah, razlike v času upogibanja (poletno in spomladansko), med letoma ter seveda med proučevanima sortama.

4 POVZETEK (SUMMARY)

4.1 POVZETEK

Evropska žlahtna hruška (*Pyrus communis* L.) je zelo razširjena sadna vrsta v zmerno toplem podnebnem pasu. Proučevani sorti ‘Williams’ in ‘Conference’ predstavljata pomemben delež med sortami, ki jih pridelujemo v Sloveniji. Sorti se med sabo razlikujeta po splošnih značilnostih, v rasti in razraščanju rodnega lesa, rodnosti ter tudi v pomoloških lastnostih ploda.

Med uveljavljenimi agrotehničnimi ukrepi v intenzivnih nasadih sadnih dreves se je upogibanje rodnih vej izkazalo za enega izmed učinkovitih, s katerim uravnavamo rast in rodnost. Upogibanje je vključeno tudi v gojitveno obliko sončna os in je celo boljši ukrep kot sama rez. Upogibanje rodnih vej vpliva tudi na manjšo rast poganjkov in njihov zgodnejši zaključek vegetativne rasti ter na pospešeno tvorbo cvetnih brstov.

Odziv sadnih dreves na upogibanje rodnih vej na biokemičnem področju še ni dovolj raziskan, do sedaj pa tudi ni poznanih raziskav evropske žlahtne hruške z vidika vsebnosti metabolitov v njenih organih kot odziv na upogibanje rodnih vej, zato smo slednje poskušali ovrednotiti v našem dvoletnem poskusu. Obe sorti sta bili cepljeni na podlago kutina MA in bili posajeni v letu 1987 v nasadu Sadjarstva Hudina v Zagaju pri Bistrici ob Sotli. Poskus je vključeval tri obravnavanja: petletne rodne veje, ki smo jih spremenili kot rasti iz 45° na 120° od navpične lege (i) pozno poleti 2003 in (ii) spomladi 2004 ter (iii) petletne rodne veje, ki niso bile upognjene, temveč so vseskozi rasle pod kotom 45° od navpične lege. Na teh rodnih vejah smo v letih 2004 in 2005 v rastni dobi vzorčili liste in v tehnološki zrelosti plodove ter jim s tekočinsko kromatografijo visoke ločljivosti določili in izmerili vsebnosti ogljikovih hidratov (saharoze, glukoze, fruktoze in sorbitola), fenolnih snovi (klorogenske, vanilne in sinapinske kisline ter (-)-epikatehina, (+)-katehina, rutina, kvercetin-3-D-galaktozida in kvercetin-3-β-D-glukozida) ter v plodovih tudi vsebnosti organskih kislin (citronske, jabolčne, šikimske in fumarne kisline).

Med ogljikovimi hidrati smo v plodovih izmerili največje vsebnosti fruktoze in v listih sorbitola. V plodovih sorte ‘Williams’ smo izmerili večje vsebnosti citronske kisline in v plodovih sorte ‘Conference’ večje vsebnosti jabolčne kisline. Med fenolnimi snovmi je bilo v plodovih in listih največ klorogenske kisline.

V listih, kjer smo merili vsebnosti metabolitov med rastno dobo od maja do oktobra, so se vsebnosti le-teh med vzorčenji razlikovale, najbolj značilne razlike pa so bile v vsebnosti fenolov. Obe sorti sta imeli v prvem letu najmanjše vsebnosti posameznih fenolnih snovi v maju, nato so se vsebnosti fenolov pri sorti ‘Conference’ povečevale do julija (le vsebnosti katehina vse do septembra), pri sorti ‘Williams’ pa do julija (klorogenska kislina in rutin), avgusta (vanilna in sinapinska kislina) ali septembra (katehin in epikatehin), zatem pa počasi zmanjševale do oktobra. V letu 2005 smo pri obeh sortah zabeležili največje vrednosti večine fenolov že v juniju, nato so se le-te zmanjšale do avgusta in nato ponovno

povečale do konca sezone. Domnevamo, da so na potek fenolov v letu 2005 vplivali tudi okoljski dejavniki, predvsem ekstremne vremenske razmere v juliju in avgustu.

Upogibanje rodnih vej v listih ni pokazalo jasnega vpliva na vsebnosti ogljikovih hidratov pri nobeni sorti. Pri sorti 'Williams' v prvem letu našega poskusa v vsebnosti ogljikovih hidratov v listih sploh nismo zasledili značilnih razlik, v drugem letu pa sta imela fruktoza in sorbitol najmanjšo vsebnost v avgustu pri kontrolnem obravnavanju, sorbitol pa še v juniju. Toda v septembru je prišlo do preobrata in sorbitol je prav pri kontroli dosegel največjo vsebnost. V listih sorte 'Conference' je bila v prvem letu v kontrolnih listih vsebnost saharoze značilno večja v juniju in sorbitola značilno manjša v oktobru, v letu 2005 pa je bila slika sladkorjev še bolj nejasna, saj smo pri glukozi zasledili značilno največje vrednosti po enkrat v vsakem obravnavanju, sorbitol je junija dosegel največjo vsebnost v listih na vejah upognjenih poleti 2003 in oktobra v kontrolnih listih, največje vrednosti fruktoze pa smo izmerili avgusta v listih na vejah upognjenih spomladi 2004.

Upogibanje rodnih vej je v listih mnogo bolj vplivalo na vsebnosti fenolov, saj je še posebej poletno upogibanje pri sorti 'Williams' v prvem letu povzročilo manjše vsebnosti posameznih fenolnih snovi, največje vsebnosti le-teh pa smo izmerili v listih s kontrolnega obravnavanja. Tudi v naslednjem letu smo izmerili večje vsebnosti nekaterih fenolov v listih z neupognjenih rodnih vej, še posebej vseh treh določanih flavonol glikozidov. Pri sorti 'Conference' pa je v prvem letu upogibanje rodnih vej (še posebej spomladansko) povečalo vsebnosti nekaterih fenolov v listih, z izjemo obeh flavan-3-olov. Tudi v letu 2005 smo izmerili večje vsebnosti klorogenske in vanilne kisline ter rutina v listih na vejah upognjenih spomladi 2004, kjer sta imela največjo vsebnost tudi katehin v oktobru in kvercetin-3-D-galaktozida v avgustu. Sicer pa so bile največje vsebnosti sinapinske kisline (junija in oktobra), kvercetin-3-D-galaktozida (maja) in kvercetin-3-β-D-glukozida (maja, junija, julija in oktobra) v kontrolnih listih. Na podlagi navedenih rezultatov menimo, da sta imela vpliv na učinek upogibanja tudi sorta in čas, ko smo izvedli upogibanje.

Proučevani sorti sta se v plodovih različno odzvali na agrotehnični ukrep upogibanje rodnih vej. Pri sorti 'Williams' so bile v prvem letu najmanjše vsebnosti ogljikovih hidratov in fenolnih snovi v plodovih, ki smo jih obrali na vejah upognjenih poleti 2003 ter največje vsebnosti omenjenih metabolitov v plodovih na vejah upognjenih spomladi 2004, zato domnevamo, da je upogibanje rodnih vej v pomladnem času za to sorto bolj primerno, tudi z vidika notranje kakovosti plodov. V vsebnosti organskih kislin v nobenem letu ni bilo značilnih razlik. V naslednjem letu so bile vsebnosti glukoze, fruktoze in fenolnih snovi najmanjše pri kontroli; vsebnost sorbitola pa je bila nekoliko večja v plodovih na vejah upognjenih poleti 2003, kjer smo izmerili tudi značilno večje vsebnosti fenolov (z izjemo kvercetin-3-D-galaktozida in kvercetin-3-β-D-glukozida, katerih vsebnost je bila večja pri kontroli).

Pri sorti 'Conference' v prvem letu nismo ugotovili značilnih razlik v vsebnosti ogljikovih hidratov med obravnavanji, čeprav so bile vsebnosti glukoze in fruktoze nekoliko večje v plodovih na rodnih vejah upognjenih pozno poleti 2003. Toda v naslednjem letu so bile ravno vsebnosti glukoze in fruktoze značilno večje v kontrolnih plodovih, kjer pa je bila vsebnost saharoze najmanjša. V letu 2004 sta bili vsebnosti jabolčne in fumarne kisline značilno največji pri kontroli, kjer smo hkrati zabeležili najmanjše vsebnosti posameznih fenolov (z izjemo katehina). Največje vsebnosti fenolov smo v prvem letu izmerili v plodovih na upognjenih rodnih vejah, zlasti upognjenih pozno poleti 2003, zato menimo, da je primernejši čas upogibanja konec poletja. V naslednjem letu smo v kontrolnih plodovih izmerili najmanjšo vsebnost jabolčne kisline ter največje vsebnosti šikimske kisline, epikatehina, sinapinske kisline ter določanih flavonol glikozidov. Toda vsebnosti klorogenske in vanilne kisline so bile nekoliko večje v plodovih na vejah upognjenih spomladi 2004. Najmanjše vsebnosti posameznih fenolov so bile v drugem letu v plodovih na upognjenih vejah: bodisi upognjenih poleti 2003 ali spomladi 2004.

V dvoletnem poskusu smo potrdili razlike med proučevanima sortama, pa tudi razlike v času upogibanja - pozno poletno in spomladansko upogibanje - ki smo jih tudi predvidevali. Menimo, da so bistveni rezultati iz prvega poskusnega leta, saj so bile okoljske razmere v mejah dolgoletnih povprečij, v drugem poskusnem letu pa je bilo poletje precej deževno in hladnejše, kar bi lahko vplivalo na preobrat v vsebnosti metabolitov. Vsebnost fenolov je namreč močno odvisna tudi od mnogih okoljskih dejavnikov (stres, temperatura, svetloba), nikakor pa ne smemo zanemariti tudi možnega vpliva notranjih dejavnikov (hranila, hormoni).

4.2 SUMMARY

European pear (*Pyrus communis* L.) is a widespread fruit species in the temperate regions. The investigated cultivars, 'Williams' and 'Conference', represent an important share of the cultivars grown in Slovenia. They differ in general properties, in growth, branching and fruiting habit; and their fruit differ pomologically.

Among the traditional methods of orchard management and cultural practice applied in high-density orchards, branch bending has proven the most successful measure for regulating vegetative growth and fruiting. Its concept has now been integrated into the Solaxe training system, and bending has proven a better measure than pruning alone. Branch bending resulted in reduced shoot vigour, an earlier end to vegetative growth in autumn, and higher floral precocity.

The response of fruit trees to branch bending has not been sufficiently researched on the biochemical level. Moreover, no known investigation has been made to date into the content levels of metabolites in the tissues of the European pear as a response to branch bending. Consequently, the bending measure was evaluated in our two-year research. Pear trees 'Williams' and 'Conference' grafted onto the MA quince were planted in 1987 in the orchard owned by Sadjarstvo Hudina from Zagaj near Bistrica ob Sotli. The research encompassed three treatments: five-year-old branches were bent to an angle of 120° from the vertical position (i) in the late summer of 2003 (the summer treatment), (ii) in spring of 2004 (the spring treatment), and (iii) the control, where five-year-old branches were not bent but remained at a 45° angle from the vertical. Before bending, the bent branches were grown like control branches at an angle of 45°. During the growing seasons of 2004 and 2005, leaves were sampled monthly from May to October, and fruit from those branches were sampled at commercial maturity. The content levels of carbohydrates (sucrose, glucose, fructose and sorbitol), phenolic compounds (chlorogenic, vanillic and sinapic acid, as well as (-)-epicatechin, (+)-catechin, rutin, quercetin-3-D-galactoside and quercetin-3-β-D-glucoside) in leaves and fruit, as well as organic acids (citric, malic, shikimic and fumaric acid) in the fruit were measured using high-performance liquid chromatography.
Among carbohydrates, fructose predominated in the fruit and sorbitol in the leaves in both years. 'Williams' fruit had higher content levels of citric acid, and 'Conference' fruit higher levels of malic acid. Chlorogenic acid was the major phenolic found in pear leaves and fruit.

In the leaves, where the content levels of metabolites were measured during both growing seasons from May to October, these levels differed among sampling dates, mostly in terms of phenolic compounds. In the first year, both cultivars had the lowest content levels of analysed phenolics in May, and then in 'Conference' pear the content levels increased to a maximum in July (catechin until September) and in 'Williams' to a maximum in July (chlorogenic acid and rutin), August (vanillic and sinapic acid) or September (catechin and epicatechin); afterwards, their content levels decreased slowly until October. In the next year, the maximum in 'Williams' and 'Conference' leaves was reached earlier – mostly in June, and after decreasing until August, another small increase appeared until the end of

the growing season. It is supposed that the dynamics of phenolics in 2005 could have been influenced by environmental factors, especially by the extreme weather conditions in July and August.

Carbohydrate changes in pear leaves after the bending showed no clear tendency for either sampling date, in 2004 or 2005. For selected carbohydrates, no significant changes in their content levels among treatments were observed in 'Williams' leaves in the first year. However, in the second year, the control leaves had the lowest levels of fructose in August and of sorbitol in June and August. But in September, the highest sorbitol content was in the control leaves. In 2004, significantly higher sucrose content was observed in 'Conference' leaves from the control, where a lower sorbitol content was observed in October. In the second year, unclear differences in carbohydrate content levels emerged, as the glucose content was the highest once in each treatment; then the highest level of sorbitol occurred in June in leaves from the summer treatment and in October in the control leaves; the highest fructose content was found in leaves from the spring treatment.

Branch bending had a greater influence on leaf phenolics than on carbohydrates. In 2004, 'Williams' leaves from bent branches, especially those bent in summer, had lower content levels of some phenolics, and the highest ones were found in the control leaves. In the second year, significantly higher content levels of some phenolics were measured in the control leaves, particularly of all determined flavonol glycosides. In 'Conference' leaves, branch bending (particularly bending in the current spring) influenced higher content levels of some phenolics, with the exception of both flavan-3-ols. Also in the second year, 'Conference' leaves from the spring treatment exhibited higher content levels of rutin, chlorogenic and vanillic acid, as well as catechin (in October) and quercetin-3-D-galactoside (in August). Otherwise, the highest content levels of sinapic acid (in June and October), quercetin-3-D-galactoside (in May) and quercetin-3-β-D-glucoside (in May, June, July and October) were observed in the control leaves. Based on these results, it is suggested that the bending effect is also dependent on cultivar and time of bending (i. e. season).

'Williams' and 'Conference' fruit responded differently to the branch bending technique. In the year 2004, the content levels of most analysed carbohydrates and phenolics were in 'Williams' fruit among the lowest in the summer treatment and among the highest in the current spring treatments, it is therefore presumed that branch bending in spring is more recommended for 'Williams' pears, also from the point of view of internal fruit quality. There were no significant differences in content levels of individual organic acids among the treatments, neither in 2004 nor in 2005. In 2005, the content levels of glucose, fructose and phenolic compounds were among the lowest in the control fruit, and the sorbitol content was slightly higher in fruit involved in the summer treatment, where significantly higher content levels of phenolics were measured. The two exceptions were quercetin-3-D-galactoside and quercetin-3-β-D-glucoside, the content levels of which were highest in the control fruit.

In the first year after bending, no significant differences were found in carbohydrate content levels in 'Conference' fruit among treatments, although slightly higher glucose and fructose content levels occurred in fruit from the summer treatment. However, in the following year, only the glucose and fructose levels were significantly higher in the control fruit, where the lowest sucrose content was observed. In 2004, the control fruit showed significantly higher content levels of malic and fumaric acid and lower levels of certain phenolics, except for catechin. In 2004, the highest levels of individual phenolics were measured in 'Conference' fruit grown on bent branches, particularly those bent in the late summer of 2003; therefore, a more appropriate time for branch bending is late summer. In 2005, the lowest content of malic acid was in the control fruit. Shikimic acid, epicatechin, sinapic acid and flavonol glycosides appeared in higher content levels in the control fruits. However, chlorogenic and vanillic acid content levels were higher in the fruit involved in the spring treatment. In 2005, the lowest content levels of individual phenolics were found in the fruit from bent branches: either bent in the late summer of 2003 or in the spring of 2004.

In the two-year research, the expected differences in content levels of selected metabolites between cultivars and among bending treatments (bent in late summer and in spring) were confirmed. The most relevant results are those from the first year, since weather conditions were similar to an average over several years. However, in the second experimental year, lower temperatures and abundant rainy weather in summer could have influenced the opposite reaction in content levels of metabolites, since the content levels of phenolics tend to be dependent on a variety of environmental factors (stress, temperature, light). Moreover, the influence of internal factors (hormones, nutrients) cannot be ignored.

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