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Carbohydrate and nitrogen reserves in two cultivars of Japanese plum grown under organic and conventional management

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Abstract

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Nitrogen and carbohydrate (soluble sugars and starch) reserves were analysed in twig samples of 'Red Beaut' and 'Showtime' Japanese plum (*Prunus salicina* Lindl.) cultivars cultivated in two similar experimental orchards under organic and conventional management. 'Red Beaut' is a vigorous cultivar while 'Showtime' is a middle to low vigorous cultivar. Both cultivars exhibited delayed flowering and premature defoliation under organic management. In general, there were no differences in the concentration of reserves between the two types of management for either of the two plum cultivars, and, likewise, no differences were observed between cultivars, regardless of their vigor. Additionally, reserves were also analysed in plantlets of 'Mariana 2624' rootstock (*Prunus cerasifera* Ehrh × *Prunus munsoniana* W. Wight & Hedrick) grown in a growth chamber that simulated conditions of spring and autumn periods and subjected to organic or mineral fertilisation. Plants subjected to organic fertilisation were smaller and defoliated earlier, but the sizes of carbohydrate and nitrogen reserves were similar to that of plants grown with mineral fertilisation.

Keywords: *Prunus salicina*; organic farming; reserves; defoliation; tree vigor

According to different authors, there are between 19 and 40 different species of plums (Blazek 2007; Rehder 1940); however, only two of these species are agronomically important: the European plum (*Prunus domestica* L.) and the Japanese plum (*Prunus salicina* Lindl). Plums are an important source of fibre and antioxidants (Stacewicz-Sapuntzakis et al. 2001; Kim et al. 2003). Moreover, organic plums have been described to harbour higher anthocyanin and polyphenol concentrations and greater antioxidant capacities (Cuevas et al. 2015). Reserves in deciduous fruit trees consist mainly of carbohydrates, and to a lesser extent, nitrogen (Tromp 1983). Carbohydrate reserves are comprised mainly of starch and soluble sugars, while nitrogen reserves consist of proteins and amino acids (Titus, Kang 1982; Oliveira, Priestley 1988; Cheng et al. 2004). Reserves are described to play an important role in budbreak, floral development and shoot growth (Titus, Kang 1982; Oliveira, Priestly 1988; Loescher et al. 1990; Cheng, Fuchigami 2002). Carbohydrate reserves are stored in different tissues in plants such as the leaves, shoots, roots, seeds and fruits (Pallardi 2008), reaching max. levels at the end of the vegetative cycle when leaves fall and dormancy occurs; carbohydrate reserves decrease after budbreak and the initiation of vegetative growth (Tromp 1983; Loescher et al. 1990). The concentration of carbohydrate reserves is higher in roots than in other

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tissues (Loescher et al. 1990); however, the total amount of reserves is higher in canopy than in roots due to the higher dry weight of this portion of the plant (Pallardi 2008). The highest concentrations of nitrogen reserves are stored in the roots (Tagliavini et al. 1999; Policarpo et al. 2002). Tree reserves may be affected by multiple factors, such as temperature (Raese et al. 1978; Ichiki, Yamaya 1982), water stress (Bradford, Hsiao 1982), an excess of nitrogen fertilisers (Choi et al. 2012), fruit load (Ryugo, Davis 1959; Roper et al. 1988), accumulated cold hours (González-Rossia et al. 2008) and pruning (Chesney, Vasquez 2007). It has also been observed that premature defoliation can decrease reserve stores (Oliveira, Priestley 1988). In previous work with different Japanese plum cultivars, it was found that in organic farming results in significant premature defoliation (García-Galavís et al. 2009). Moreover, it has also been observed that trees grown under organic management exhibit reduced vigour, somewhat delayed flowering and lower fruit production than trees grown in conventional agriculture (Arroyo et al. 2013). The goal of this study was to analyse whether plum trees reserves are affected by the type of management, i.e., conventional or organic, which employs different fertilization and causes premature defoliation, and to identify whether there is a correlation between tree vigour and carbohydrate and nitrogen reserves.

MATERIAL AND METHODS

Description of experimental plots and plant material. This study was carried out from 2011 to 2014 in two similar orchards, one conventional and one organic, planted in 2005 as one-year-old bare roots, grafted onto 'Mariana GF 8-1' (*Prunus cerasifera* × *Prunus munsoniana*) on the experimental farm of the IFAPA Las Torres (SW Spain) (37°30'48'N; 5°57'46'W). The two orchards were separated by a distance of 200 m to avoid interference between pest and disease treatments. The chemical treatments and types of fertilisers used in both orchards were as described previously (García-Galavís et al. 2009). Both plots received furrow irrigation along two rows in parallel to the line of trees with the same volumes (350,000 l/ha) and frequencies. Irrigation was applied between six and nine times in the period from May to September depending on weather conditions. For both types of management, weed control was carried out using the reduced tillage method. Inter-row tree weed control was performed by using a tractor-mounted disc harrow. Control inside the tree rows was carried out in the organic orchard using a brushcutter and in the conventional orchard by the application of post-emergence herbicides. The plant material used in this work was two early season cultivars of Japanese plum, one of them, 'Showtime', with weak vigour and upright structure and the other, 'Red Beaut', which is highly vigorous and has an open structure. Trial design consisted of a randomised block with three replicates, with six trees per replicate and a total of 18 trees per management type and cultivar.

Tree vigour and phenological studies. The vigour of the trees was evaluated in November every year at the end of the growing season, by calculating the trunk cross-sectional area (TCSA) 20 cm above the graft (Layne 1994; Lepsis, Blanke 2006). The beginning of flowering, full flowering and end of flowering were registered according to the BBCH scale (Meier et al. 1994). Leaf fall was recorded weekly from October 1 until the end of December. Defoliation was evaluated using a 0–5 scale, where 0 is 0% leaf fall; 1, 20% leaf fall; 2, 40% leaf fall; 3, 60% leaf fall; 4, 80% leaf fall; and 5, total defoliation.

Sampling and analysis of plant reserves. Sampling was performed at two different time points of the crop cycle, dormancy: January 2012 (319 days after full bloom (DAFB)), November 2012 (240 DAFB), December 2013 (269 DAFB), and vegetative growth: May 2012 (58 DAFB), May 2013 (78 DAFB) and May 2014 (82 DAFB). For each analysis, 20 one- to two-year-old twigs with thicknesses of between 4 and 5 mm were randomly taken from each of the six trees of each replicate in both types of management. Wood samples were dried in a laboratory oven for 48 h at 65°C and subsequently cut into small pieces and ground in a Thomas Model 4 Wiley® Mill (Thomas Scientific, USA). Dry matter samples weighed about 60 grams. The carbohydrates analysed were soluble sugars and starch. The nitrogen fractions analysed were proteinaceous nitrogen, ammonium nitrogen and nitrate nitrogen. Soluble sugars were determined using dinitrosalicylic acid reagent as described by Miller (1959). Starch was determined using the method for soluble sugar, including a

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previous step for acid digestion to hydrolyse starch. Units of carbohydrates are given in mg/g. Proteinaceous nitrogen was determined using the Kjeldahl method as described by Hesse (1971); ammonium nitrogen was determined as described by Bremner (1965) and nitrate nitrogen was analysed using the same method (Bremner 1965), including the following modifications: Devarda alloy (50% copper, 45% aluminium and 5% Zinc) was used instead of MgO. Results are given as micrograms per gram and as the sum of the three fractions expressed as percentages.

Trial in growth chamber under controlled conditions. The trial was carried out in 2013 using 68 plantlets of 'Mariana 2624' rootstock with heights of between 6 and 8 cm. Thirty-four plantlets were placed in a plastic cell sheet filled with a 50:50 mix of perlite plus vermiculite, and were irrigated with Hoagland nutrient solution (Taiz, Zeiger 1998) in order to simulate conditions of conventional fertilisation in the field; the remaining 34 plantlets were placed in a plastic cell sheet filled with a 50:50 mix of peat and coconut fibre and were irrigated with distilled water in order to simulate the conditions of organic fertilization. Plastic cell sheets were put in a MLR-351H Sanyo Versatile Environmental Test Chamber (Sanyo Electric Co., Ltd., Japan) for 154 days. Initially, spring weather conditions were simulated: 96 days with 12 h light at 26°C and 12 h dark at 20°C, followed by a simulated transition to autumn and finally autumn conditions, which were 14 days with 8 h light at 22°C and 16 h dark at 14°C, followed by 44 days with 7 h light at 15°C and 17 h dark at 6°C, followed by 39 days with 6 h light at 15°C and 18 h dark at 5°C. At the end of the experiment, the grade of defoliation was determined and plantlets were removed from containers; the length and width of the trunk was measured and the entire plants, including roots, were dried and ground for the analysis of reserves.

Statistical analysis. Data were analysed using Statistix software (version 9.0; NH Analytical Software, USA). Single analysis of variance (ANOVA) was used to analyse the different fractions of reserves. The Least Significant Different Test ($p \leq 0.05$) was used to discriminate among the mean values. Correlations were determined using Pearson's coefficients.

RESULTS

Vigour, flowering and defoliation in plum cultivars

Data on tree trunk cross-sectional areas (TCSA) and defoliation grade at the end of October for the two cultivars under the two types of management from 2011 to 2014 are detailed in Table 1, and flowering is detailed in Table 2. TCSA was significantly higher for 'Red Beaut' in the conventional orchard compared with the organically managed orchard; these values were also higher for 'Showtime' but the differences were only significant in 2012. As expected, according to the TCSA values, 'Red Beaut' was the most vigorous under both types of management. Conventional management resulted in plants that reached the beginning of flowering, full flowering and end of flowering significantly earlier during the four-year follow-up period, i.e., one and four, zero and six and zero and nine days earlier, respectively, in the two cultivars. On the other hand, defoliation was on average premature in the two cultivars when managed organically in all four years. There were also differences in the number of days that the trees in conventional management needed to reach com-

Table 1. Trunk cross-sectional areas (TCSA) and defoliation grades in two cultivars of Japanese plum grown under organic (O) and conventional (C) management from 2011 to 2014

Year	TCSA (cm^2)				Grade of defoliation in late October			
	'Red Beaut'		'Showtime'		'Red Beaut'		'Showtime'	
	O	C	O	C	O	C	O	C
2011	293.61[a]	330.19[a]	181.44[a]	235.38[a]	4.33[a]	3.17[b]	4.93[a]	3.83[b]
2012	301.16[b]	363.55[a]	201.81[b]	239.40[a]	2.16[a]	2.11[a]	2.00[a]	1.61[a]
2013	343.56[b]	396.79[a]	230.24[a]	287.54[a]	3.78[a]	1.39[b]	4.34[a]	1.26[b]
2014	375.26[b]	441.10[a]	234.70[a]	321.62[a]	4.60[a]	2.33[b]	5.00[a]	2.83[b]

for each parameter, year and cultivar, different letters in row indicate differences between the two types of management ($p \leq 0.05$)

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Table 2. Beginning (B), full (F) and end (E) of flowering (day of year) of two cultivars of Japanese plum grown under organic (O) and conventional (C) management and the differences in the number of days that the trees under conventional management needed to reach complete defoliation compared with the trees under organic management in the period 2011–2014

Year	'Red Beaut'						'Showtime'						'Red Beaut'	'Showtime'
	O			C			O			C				
	B	F	E	B	F	E	B	F	E	B	F	E	difference in the No. of days	
2011	46	56	74	45	56	74	61	70	87	60	67	85	14	18
2012	62	67	83	60	67	81	70	74	90	69	74	89	14	19
2013	59	65	76	55	63	76	68	72	100	65	69	91	28	35
2014	51	63	80	50	57	79	66	69	96	63	67	91	27	29

plete defoliation compared with the trees in organic management. For 'Red Beaut' the smallest difference was 14 days for the years 2011 and 2012 and the largest difference was 28 days for the year 2013, while for 'Showtime' the smallest difference was 18 days for the year 2011 and the largest difference was 35 days for the year 2013 (Table 2).

Reserves in twigs of adult plum trees

Starch. Overall starch concentrations in the twigs of the two cultivars were similar under the two types of management; however, some significant differences were observed, such as higher concentrations under conventional management for 'Red Beaut' in November 2012 and December 2013, and for 'Showtime' in December 2013 (Table 3). An increase in the starch concentration was observed from 2012 to 2014, under the two types of management and for the two cultivars.

Soluble sugars. Soluble sugar concentrations in twigs were similar for both treatments at almost all sampling times, although some significant differences were observed: in May 2013, a higher concentration in 'Showtime' under conventional management, and in December 2013 a statistically significant higher concentration in 'Red Beaut' under the conventional management (Table 3).

Nitrogen. In general, the concentration of nitrogen was similar between the two types of management; in fact, the average values were practically identical between the two types of management for the two cultivars (Table 4). However, some small but significant differences were observed; in May 2013, 'Red Beaut' exhibited a higher concentration under organic management and in May 2014 'Showtime' showed a higher concentration under

Table 3. Starch (mg/g) and soluble sugars (mg/g) in the twigs of two Japanese plum cultivars grown under organic (O) and conventional (C) management

Date	Starch				Soluble sugars			
	'Red Beaut'		'Showtime'		'Red Beaut'		'Showtime'	
	O	C	O	C	O	C	O	C
2012 January	16.71^{a}	12.98^{a}	15.88^{a}	11.98^{a}	9.89^{a}	6.23^{a}	7.36^{a}	7.63^{a}
2012 May	11.22^{a}	12.06^{a}	13.62^{a}	11.50^{a}	3.75^{a}	2.44^{a}	2.05^{a}	2.99^{a}
2012 November	12.91b	18.86^{a}	18.08^{a}	13.69^{a}	8.33^{a}	7.59^{a}	8.21^{a}	7.31^{a}
2013 May	19.96^{a}	20.65^{a}	20.50^{a}	21.06^{a}	8.46^{a}	9.09^{a}	8.63b	9.60^{a}
2013 December	27.72b	33.18^{a}	32.92b	35.26^{a}	10.29^{b}	10.68^{a}	10.99^{a}	11.87^{a}
2014 May	28.39^{a}	29.77^{a}	29.99^{a}	29.79^{a}	11.64^{a}	11.53^{a}	10.47^{a}	11.28^{a}
Average	19.48^{b}	21.25^{a}	21.83^{a}	20.55^{a}	8.73^{a}	7.93^{b}	7.95^{a}	8.45^{a}

for each parameter, year and cultivar, different letters in row indicate differences between the two types of management ($p \leq 0.05$)

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Table 4. Nitrogen (%) in the twigs of two Japanese plum cultivars grown under organic (O) and conventional (C) management

Date	'Red Beaut'		'Showtime'	
	O	C	O	C
2012 January	2.80[a]	2.78[a]	3.03[a]	3.03[a]
2012 May	2.12[a]	1.98[a]	2.39[a]	2.19[a]
2012 November	3.39[a]	3.69[a]	3.52[a]	3.59[a]
2013 May	3.14[a]	2.70[b]	3.03[a]	2.79[a]
2013 December	3.12[a]	3.11[a]	3.02[a]	3.00[a]
2014 May	3.04[a]	3.17[a]	3.18[a]	2.98[b]
Average	2.94[a]	2.91[a]	3.03[a]	2.93[a]

for each cultivar, different letters in row indicate differences between the two types of management ($p \leq 0.05$)

organic management. The concentrations were similar for the two cultivars. In general, nitrogen content was higher in dormancy for the two first years but not in 2013. Nitrogen concentration exhibited a positive correlation with soluble sugars (r = 0.79) but not with starch (r = –0.16).

Defoliation grade, size and reserves in plantlets of 'Mariana 2624' grown in controlled conditions. Data on defoliation, size (length and width), and reserves for plantlets of 'Mariana 2624' receiving the two types of fertilisation are detailed in Table 5. Defoliation was more intense in organically fertilised plantlets versus those receiving conventional treatment. Size was significantly higher for plantlets in the conventionally treated plantlets compared to those receiving organic fertiliser. Although starch concentrations were slightly higher in response to organic fertilisation, and soluble sugar concentrations were slightly higher in response to the mineral fertiliser treatment, no significant differences were observed. Nitrogen concentrations were similar in response to the two different types of fertilisation.

DISCUSSION

TCSA data from the two cultivars showed that trees under organic management had less vigour than those under conventional management. Also, as expected, the greater vigour of 'Red Beaut' compared to 'Showtime' was confirmed. On the other hand, both cultivars showed delayed flowering under; although the differences were not very large, blooming was always delayed under organic management during the period 2007–2014. Twelve other Japanese plum cultivars studied in this period of time showed the same trend (Arroyo et al. 2013). Under organic management, premature defoliation was observed in both cultivars. These results are in line with what was observed in previous crop years for these cultivars as well as for additional Japanese plum cultivars (Arroyo et al. 2013). These differences in time of defoliation are important, since the average number of days between the beginning and the end of defoliation for both managements and cultivars in the period studied was 65.25 days. The differences observed for tree vigour, time of defoliation and blooming could potentially be due to the more pronounced weed problem in the organic plot, which decreases the irrigation dose. This phenomenon has previously been described in organic farming by other authors (Bond, Grundy 1998). However, weeds were not a problem in the organic plots in this study. In fact, weed control was similar under both managements. Additionally, the irrigation dose was higher than necessary, since it was administered by furrow irrigation in the period of maximum need. We hypothesised that premature defoliation under organic management may decrease tree reserves. In previous studies, a correla-

Table 5. Grade of defoliation at the end of the cycle, plant size, starch, soluble sugars and nitrogen in plantlets of 'Mariana 2624' after growth under controlled conditions with organic (O) and mineral (M) fertilization. Samples for the analysis of reserves consisted of material from the whole plant (roots + canopy)

Type of fertilization	Defoliation (0 a 5)	Plant size		Reserves		
		length (cm)	width (mm)	starch (mg/g)	s. sugars (mg/g)	nitrogen (%)
O	4.62[a]	15.68[b]	2.92[b]	30.88[a]	10.50[a]	3.06[a]
M	3.56[b]	21.08[a]	3.49[a]	28.86[a]	11.39[a]	3.03[a]

for each parameter, different letters in column indicate differences between the two types of fertilization ($p \leq 0.05$)

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tion between defoliation and a decrease in reserves has been described (Hudgeons et al. 2007; Alves et al. 2008). Nevertheless, we found no such correlation since similar levels of carbohydrates and nitrogen reserves were found under both types of management, including for the cultivar 'Showtime', which exhibited markedly earlier defoliation under organic management due to its increased susceptibility to rust, a fungal disease not well controlled under organic management (García-Galavís et al. 2009). We also hypothesised that differences in fertilisation between the two types of management, which influences the vigour and size of the trees, may affect reserve concentrations. However, no differences in the levels of reserves between the two cultivars, regardless of the type of management or the vigour of the cultivar, were found. Nevertheless, some indirect data suggest that differences in vigour are not strictly correlated with the concentrations of reserves in the trees, as also indicated by the results of this work. For example, Gaudillere et al. (1992) found no differences in carbohydrate reserves in different prune cultivars that were grafted onto different rootstocks resulting in differing levels of vigour. In a similar study with prunes, Moing et al. (1994) studied carbohydrate and nitrogen reserves in trees under two different pruning and training systems that result in different levels of vigour, and observed no differences in the concentrations of reserves. Fruit trees store reserves in roots, the trunk and secondary branches (Tromp 1983). In our study, we only analysed reserves in a part of the canopy (twigs of one- to two-year-old trees), so potential differences in the reserve concentrations of the trunk and main roots cannot be ruled out. We also performed some analyses of reserves in thin roots were in November 2012 and found no differences between the two types of management (data not shown). Moreover, although reserve concentrations were similar under the two types of management, the total amount of reserves under conventional management would be expected to be higher since these trees were taller than those under organic management (Arroyo et al. 2013). To our knowledge, there are no previous studies analysing reserve levels in adult fruit trees grown under organic versus conventional managements. Many of the studies carried out to study reserves in trees have been carried out with young seedlings (Bi et al. 2004; Cheng et al. 2004). Our trial with the plantlets of 'Marianna 2624' was carried out as an attempt to simulate to some extent growth under organic versus conventional management, and to enable analysis of the reserve concentrations in whole plants. The results have shown that plantlets grown with organic fertilisation were smaller and defoliated earlier after autumn growth conditions were established, but no differences in carbohydrate and nitrogen reserves were observed between the two types of fertilization. However, based on our results we believe that it would be premature to conclude that the type of management (i.e., organic or conventional) does not affect the concentrations of reserves in the plant, and we suggest that in future studies levels of reserves should be determined in different parts of the tree, including in higher trunk biomass, main branches and thicker roots. Moreover, the study of reserves in trees is a complex issue due to the fluctuations observed throughout the annual cycle (Yamashita 1986; Keller, Loescher 1989), in addition to differences in carbohydrate and nitrogen concentrations in the different tissues of the tree (Hillcottingham, Lloydjones 1975; Loescher et al. 1990). Another aspect that increases the difficulty of the study of carbohydrate and nitrogen reserves is the relationship between these two fractions, since some authors have reported a negative correlation between the content of nitrogen and carbohydrates (Cheng, Fuchigami 2002). Our results showed a positive correlation between nitrogen and total soluble sugars and no correlation between nitrogen and starch. Overall, we found lower total soluble sugar levels than those described in other studies with apples and plums (Sivaci 2006; González-Rossia et al. 2008), but similar values to those described for pistachios (Nzima et al. 1997). In contrast, nitrogen levels were higher than those reported previously. Finally, our starch concentration data were similar to those described in some of the relevant works cited here.

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