Europ.J.Hort.Sci., **79** (5). S. 241–247, 2014, ISSN 1611-4426. © Verlag Eugen Ulmer KG, Stuttgart

Leaf Assimilation, Carbon Translocation and Root Respiration in 'Budagovski 9' Apple Cuttings Grown in Low Soil Moisture Condition

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Summary

The objective of the present study was to investigate the effect of moderately limited soil water supply on root C allocation and root respiration of non-bearing apple trees. Potted 1-year-old apple trees of 'Budagovski 9' grown in sand where water content was reduced (7 % w:w) were compared to plants grown in soil irrigated at field capacity (12 % w:w). Canopies were labelled with $^{13}CO_2$ and excess ^{13}C allocation within tree organs and evolution from the soil were monitored for 16 days after the labelling. The moderate reduction of soil water content induced a decrease in predawn leaf water potential and leaf gas exchange; however, root and above ground dry weight were not affected. Compared to well-watered control, trees growing in condition of moderately limited soil water supply fixed a lower amount of pulsed ^{13}C. From day 1 to 16, ^{13}C concentration decreased in leaf, increased in root and trunk, and was stable in herbaceous shoots, in both irrigation treatments, with no difference in the ^{13}C partitioning. Root respiration rate of new fixed ^{13}C was reduced by water limitation, was linearly related to the amount of excess ^{13}C allocated into the roots, and became equal to zero by day 16.

Key words. ^{13}C – carbon partitioning – *Malus domestica* – stable isotopes – rootstock – soil water deficit

Introduction

Carbon (C) assimilation and distribution of biomass have fundamental importance for the commercial yield of perennial fruit crops. A large amount of the photosynthates produced daily is translocated to the root system where it can be used for C skeletons (growth) or to provide energy and reducing power (respiration) (Buwalda 1993). Water deficit often affects fruit orchards (even when they are irrigated by microirrigation) as a consequence of unexpected period of high temperature and intense evapotranspiration rate (Barriopedro et al. 2011), that makes the tree water demand higher than irrigation rates. Conditions of water deficit affect stomatal apertures and reduce leaf gas exchange, with consequences in both the reproductive and vegetative stages. The structure and functions of root systems can be also affected (Bradford and Hsiao 1982), i.e. a decrease in root:shoot biomass ratio was found in *Quercus robur* seedling under drought stress (Vivin and Guehl 1997). In *Vitis labrusca*, respiration was little affected by soil drying at 10 °C, while at a soil temperature between 20 and 30 °C, root respiration decreased with decreasing soil moisture (Huang et al. 2005). Other studies on apple, showed no change of the fraction of new fixed C allocated to roots in potted plant subjected to mild water deficit (Lombardini et al. 2001), however with more severe stress, an increase in the proportion of photoassimilates allocated to the root system was reported (Buwalda and Lenz 1992). In oil palm (Lamade et al. 1996) and Swedish conifer (Högberg et al. 2002), the fraction of C allocated to the roots for respiration was much higher than that used for renewal (growth). Therefore, root respiration may represent a relevant portion of the C budget, especially in the case of trees with large root biomass. Root respiration is complex to investigate, because of the difficulties to separate root from other contributions of soil C losses, such as organic matter decomposition and rhizo-microbial respiration. The use of a stable C isotope, such as ^{13}C, supplied to the tree canopy, fixed by the leaves, and moved to the roots can be a helpful tool to monitor C movement within the tree.

The objective of the present study was to investigate the effect of moderately limited soil water supply on root C allocation and root respiration of potted apple trees.

Materials and Methods

The experiment was conducted in the greenhouses of the Department of Horticulture at Michigan State University, East Lansing, MI. Sixteen one-year-old rooted cuttings of apple 'Budagovski 9' ('B.9'), an hybrid of 'M8' × 'Red Standard' (Fideghelli and Loreti 2009), were potted in 2-L pots filled with pure sand. After potting, plants were grown in greenhouse, under the following environmental conditions: midday photosynthetic photon flux density between 550 and 650 μmol m^{-2} s^{-1}; average day/night temperature 32/27 °C and relative humidity between 88 and 94 %. The natural photoperiod was extended to 14 hours using 1000-W metal halide lamps (General Electric Lighting, Cleveland, OH). After bud break, plants were trained to two shoots and regularly watered and fertilized for four weeks, then two watering regimes were applied (to 8 plants each) to achieve soil water contents (on a dry weight basis) of 12 % (field capacity) and 7 % (low water supply), which corresponded, according to Ley et al. (1994) to water matrix potentials of −0.03 and −0.25 MPa, respectively. Soil water content was monitored daily using a time-domain reflectometer (TDR, Tektronix 1502, Tektronix Inc., Beaverton, Oregon, USA), that included two steel rods (length 170 mm, diameter 5 mm) inserted vertically 5-cm apart in the center of each pot, to measure soil moisture in the 150-mm soil profile of each pot individually. When the soil water contents were differentiated, then they were maintained by returning the daily evapotranspiration rate measured by weight.

Four weeks after irrigation treatments were initiated, plants were enclosed in a chamber made of sheets of 50-μm polyester film (Mylar® type D, Dupont, Wilmington, DE) assembled with clear packing tape to form a CO_2-exchange chamber. The volume of the chamber was approximately 3,000 L, which left enough room above and along the sides of the plants to increase turbulent air mixing. Canopy of plants were then exposed to $^{13}CO_2$ generated from the reaction between $Ba^{13}CO_3$ (98 atom %^{13}C, Isotec Inc., OH, USA) and lactic acid 85 % (Sigma-Aldrich, St. Luis Missouri, USA). 8 g $Ba^{13}CO_3$ were dissolved in a continuously agitated solution of lactic acid to liberate $^{13}CO_2$ that was then pumped into the chamber. $^{13}CO_2$ was supplied simultaneously to all plants. During the labelling, a double-layer plastic bag was wrapped and sealed around each pot, to avoid ^{13}C contamination to the soil. A cooling/dehumidifying radiator maintained constant temperature and humidity inside the chamber at 28 ± 2 °C and 82 ± 4 %, respectively. The concentration of CO_2 inside the chamber, measured with an infrared gas analyzer (CIRAS-1, PP-Systems Inc., Amesbury, Massachussets, USA) immediately before labelling was equal to 400 μL L^{-1}. Two fans were set at the opposite side of the chamber to make air circulation and avoid temperature and CO_2 concentration gradients. Chamber relative humidity was also monitored by the infrared gas analyzer. The maximum level of CO_2 was 652 μL L^{-1}, measured 5 min after salt dissolution in acid. After three hours, when air inside CO_2 concentration had dropped to 200 μL L^{-1}, the chamber was removed. At each sampling time, 2 plants per treatments were harvested, roots were rinsed of debris and with all the other plant material were oven-dried at 70 °C for 48 h, then weighed and ground into powder in a coffee mill. A 1.5 mg aliquot of each sample was used to determine the total C and ^{13}C concentration in the plant tissues. The amout of ^{13}C was calculated according to Wu et al. (2009) as follows:

$$\text{Atom \% } ^{13}C = {}^{13}C/({}^{13}C+{}^{12}C) \times 100 \quad \text{Eq. 1}$$

$$\text{Atom \% excess} = \text{Atom \% } ^{13}C \text{ labelled organ} - \text{Atom \% } ^{13}C \text{ unlabelled organ} \quad \text{Eq. 2}$$

$$\text{Excess } ^{13}C \text{ content} = (\text{Atom \% excess}/100) \times \text{dry mass} \times [C] \quad \text{Eq. 3}$$

$$\text{Relative partitioning (\%)} = (\text{New } {}^{13}C_{organ}/\text{New } {}^{13}C_{tree}) \times 100 \quad \text{Eq. 4}$$

Atom % excess is an index to determine the enrichment level of a sample (Eq. 2). The excess ^{13}C content was determined in the different organs according to dry matter and C concentration (Eq. 3). The relative partitioning of ^{13}C in the plants was expressed as a percentage of the excess ^{13}C in the organ divided by total excess ^{13}C in the plant (Eq. 4).

Total soil respiration was estimated, on 2 plants per treatment for each of the following sampling times: 7 hours 1, 2, 4 and 16 days after the application of $^{13}CO_2$, following the method described by Parkinson (1981). Each pot was placed and sealed inside a 5-L chamber. The absolute CO_2 concentration within each chamber was measured using the CIRAS-1, over a period of 10–15 min, during the measurement, CO_2 concentration was monitored (Buwalda et al. 1992). Measurements were always made between 1400 and 1500 hr at an ambient temperature of 26–28 °C.

Immediately after total soil respiration measurements, excess ^{13}C atom of total below ground CO_2 was determined, in order to evaluate the amount of ^{13}C assimilated and respired by root. Sheets of polyethylene were wrapped and sealed to cover the top of each pot and the headspace air was drawn for 30 min (1.1 L min^{-1}) via Tygon tubing (internal diameter 0.3 cm) through an alkali trap containing 20 mL of 2 M NaOH. Trapped CO_2 was then precipitated as $SrCO_3$ and analyzed in an automated N and C analyzer (ANCA) mass spectrometer (Europa Scientific, Crewe, UK). After the measurements, the free volume of the chamber was evaluated by weighing the amount of distilled water required to saturate the soil and fill the

chamber. Root evolution rate of excess ^{13}C as CO_2 was calculated as follows:

Excess ^{13}C-CO_2 μg = $[CO_2] \times PV/22.4 \times 12 \times [(^{13}C_s) - (^{13}C_{na})]/DW_{root}$,

where $[CO_2]$ is the concentration of carbon doxide evolved from the soil, PV is the free volume of the chamber, 22.4 is the gas constant, 12 is the C atomic weight, $^{13}C_s$ is the atom % ^{13}C of ^{13}C-CO_2 trapped in NaOH from pulsed plots and $^{13}C_{na}$ is the 'natural' atom % ^{13}C of below ground ^{13}C-CO_2 trapped in NaOH from not pulsed plants that was assumed to be equal to the untreated air (1.1024 % corresponding to a δ of −8) and DW_{root} is the root dry weight. After soil respiration measurements, two plants per treatment were harvested and partitioned into roots, trunk, shoots, and leaves. The amount of excess ^{13}C respired by roots between two successive sampling days, was calculated on the basis of the ^{13}C-CO_2 respiration rate measured at the two sampling times and the time between them. The amount between 4 and 16 days was not calculated because uncertain the moment when ^{13}C-CO_2 became 0.

Leaf net photosynthesis rate (Pn), stomatal conductance (g_s), and transpiration rate (E) were measured using the infrared gas analyzer unit on three fully expanded leaves per plant per treatment. Leaf gas exchange data were collected right before labelling and 1, 2, 4 and 16 days after labelling, between 1200 and 1300 hr. Predawn leaf water potential (ψ_w) was measured 1, 2, 4, and 16 days after labelling. Measurements were collected on the youngest fully expanded healthy leaves using a pressure bomb (Plant Moisture Stress Instrument Co., Corvallis, Oregon, USA) (Scholander et al. 1965), following the standard technique (Ritchie and Hinckley 1975).

A factorial experimental design was arranged with water supply (two levels: 12 and 7 % w:w) and sampling day (four levels: 1, 2, 4, 16 days after $^{13}CO_2$ pulse) as main factors. Two plants per water supply were harvested at each sampling day. When statistical analysis showed a significant effect of treatments, means were separated by Student Neumann Keuls test ($P \leq 0.05$). When interaction between main factors was significant, two standard error of mean (SEM) was used as minimum significant difference between two means (Saville and Rowarth 2008).

Results

Leaf ψ_w, Pn, E, and g_s were lower in moderately limited soil water supply, compared to untreated controls, but no effect of sampling day was observed (Table 1). Water availability and sampling day did not affect leaf, root, shoot, trunk and whole tree DW (Table 2).

Water supply and sampling day did not interact with the amount of excess ^{13}C found in plants and with the fraction respired by roots (Table 3). Unlike stem, the amount of excess ^{13}C allocated into the leaves, roots and shoots was higher in control compared to moderately limited soil water supply (Table 3). During the sixteen days of the investigation, excess ^{13}C decreased in leaves, and was stable in roots, shoots and stem (Table 3). The amount of excess ^{13}C respired by the whole root system increased until day 4, while the amount respired from day 4 to day 16 was not detected (Table 3).

Table 1. Effect of soil water supply and sampling day on pre-down leaf water potential (ψ_w), net photosynthesis rate (Pn), leaf transpiration rate (E) and stomatal conductance (g_s) of potted 'B.9' apple trees.

Water supply (% w:w)	ψ_w (−MPa)	Pn (μmol m^{-2} s^{-1})	E (mmol m^{-2} s^{-1})	g_s (mmol m^{-2} s^{-1})
12	0.575	9.36	1.20	76.8
7	0.737	6.73	0.84	48.4
Significance	*	*	*	*
Sampling day				
1	0.750	7.62	1.10	71.0
2	0.687	6.32	0.73	45.5
4	0.637	10.40	1.23	70.5
16	0.550	6.82	1.03	63.2
Significance	ns	ns	ns	ns
Interaction	ns	ns	ns	ns

ns, *: Effect of treatments not significant or significant at 5 %, respectively. Values are means of 8 (water supply) and 4 (day of sampling) replications.

Table 2. Effect of soil water supply and sampling day on plant organ dry weight (g) of potted 'B.9' apple trees.

Water supply (% w:w)	Leaf (g)	Roots (g)	Shoot (g)	Stem (g)
12	1.98	0.889	0.541	7.82
7	1.40	0.760	0.346	6.77
Significance	ns	ns	ns	ns
Day				
1	1.58	0.655	0.458	7.59
2	1.93	0.830	0.477	7.62
4	1.67	0.585	0.390	6.33
16	1.52	1.230	0.450	7.64
Significance	ns	ns	ns	ns
Interaction	ns	ns	ns	ns

ns: Effect not significant. Values are means of 8 (water supply) and 4 (day of sampling) replications.

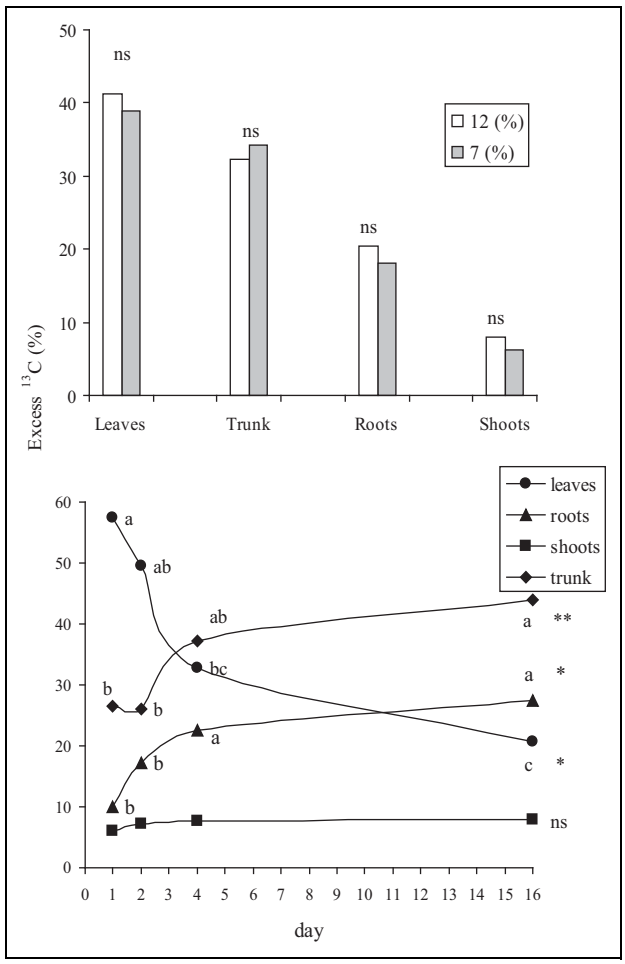


Fig. 1. Effect of soil water supply (above) and sampling day (below) on excess ^{13}C partitioning in different organs of potted 'B.9' apple trees. ns, *, **: Effect of treatments not significant, significant at 5 %, or at 1 %, respectively. Since no interaction between water supply and sampling day were found, only the effect of the main factors is showed: values are means of n = 8 for water supply and n = 4 for sampling day.

When the relative partitioning of excess ^{13}C was analyzed, no effect of soil water supply was observed (Fig. 1, upper). In fact, in both treatments, the percentage of excess ^{13}C decreased in leaves over time and increased in roots and trunk while was steady in shoots (Fig. 1, lower). The reduction of water supply decreased the rate of excess $^{13}CO_2$-C respired by root (Table 4), while no effect of sampling days was observed. Specifically the use of SEM as minimum significant difference between two means (Saville and Rowarth 2008) showed a root respiration rate of control, 24 and 48 hours after the $^{13}CO_2$ pulse, higher than that of trees exposed to moderately limited soil water supply 24 hours after the pulse (Table 4). Both water supply and sampling day affected the percentage of excess ^{13}C respired by the roots, with no interaction between factors (Table 4). The percentage of total excess $^{13}CO_2$ fixed by leaves and respired by control tree roots increased significantly with the day: at day 1 it accounted for about 10 %, in both irrigation treatments, then at day 4, while steady in moderately limited soil water supply plants, it increased to up to 50 %, and was significantly higher than in low soil water supply (Table 4). From day 4 to day 16 it was not possible to estimate the percentage of excess ^{13}C respired. A linear relation was found between the evolution of excess $^{13}CO_2$-C and the excess ^{13}C allocated to the root at day 1, 2 and 4 (Fig. 2).

Discussion

The purpose of this experiment was to induce conditions of mild soil water depletion, so that we could monitor the physiological adjustments of young apple trees as when the water restitution does not meet entirely the evapotranspiration rate. Consequently the reduction of predawn leaf ψw and the decrease of leaf C assimilation were considered as parameters to define soil water content and were maintained constant through the experiment. The tree physiological adjustments to soil water depletion had a direct consequence on the reduction of excess ^{13}C allocation to the whole tree and particularly to leaf, root and shoot, which were organs in active growth. The soil water reduction was not sufficient to modify significantly tree growth and organ DW, or to change the new fixed ^{13}C partitioning, that was allocated to the different tree districts with the same proportion in well watered and in moderately limited soil water supply. However, the reduction of soil moisture was effective in reducing the percentage of excess ^{13}C respired by roots. Consequently, in the present condition of moderate limiting soil water con-

Table 3. Effect of soil water supply and sampling day on amount of excess ^{13}C allocated in potted 'B.9' apple tree organs and respired by the whole root system.

Water supply	Excess ^{13}C (mg)					
(% w:w)	Leaf	Root	Shoot	Stem	Root respiration	Total
12	4.66	2.27	0.964	3.72	4.89	16.4
7	2.84	1.13	0.432	2.00	1.04	7.5
Significance	*	*	*	ns	*	*
Day						
1	6.18 a	1.13	0.638	2.54	1.15 b	11.0
2	4.80 ab	1.82	0.797	3.14	2.42 ab	13.0
4	2.55 b	1.61	0.577	2.54	4.15 a	11.4
16	1.84 b	2.25	0.780	3.20	–	–
Significance	*	ns	ns	ns	*	ns
Interaction	ns	ns	ns	ns	ns	ns

ns, *: Effect of treatments not significant or significant at 5 %, respectively. Values are means of 8 (water supply) and 4 (day of sampling) replications.

Table 4. Effect of soil water supply and sampling day on excess $^{13}CO_2$-C and percentage of the total $^{13}CO_2$-C fixed, respired by root of potted 'B.9' apple trees.

Hours	Excess $^{13}CO_2$-C root respiration			
	(µg g root DW^{-1} h^{-1})		(% of total $^{13}CO_2$ fixed)	
	12 % w:w	7 % w:w	12 % w:w	7 % w:w
7	1.29	1.85		
24	3.23	0.13	10.7	10.0
48	3.85	0.65	22.6	6.8
96	2.65	0.61	49.3	16.5
384	0.00	0.00	–	–
Significance				
Water supply	*		*	
Sampling day	ns		*	
Interaction	ns (SEM = ± 1.5)		ns (SEM = ± 4.4)	

ns, *: Effect of treatments not significant or significant at 5 %, respectively. Values differing by 2 standard error of means (SEM) are statistically different. Values are means of 8 (water supply) and 4 (day of sampling) replications.

tent, C was not allocated preferentially to the roots and was not used to provide energy through respiration. In fact, the decrease of excess ^{13}C allocated to the roots, as a response to soil water depletion, was effective in promoting a reduction of excess ^{13}C root respiration, which decreased from 50 % of the newly fixed C to less than 20 % in moderately limited soil water content. These results are consistent with those found in a number of tree species, where soil moisture was lower than in the present study. This is the case of citrus (Bryla et al. 1997), where roots in the upper layer of soil was maintained without water for 110 days, grape (Huang et al. 2005), where root respiration decreased logarithmically with depletion of soil moisture (100 mL d^{-1} in the drought treatment versus 2000 mL d^{-1} in the well watered control). Beech sapling (Gansert 1994) and maple forest (Burton et al. 1998) that during period of natural water stress showed a decline of in situ fine-root ($\leq$ 1.0 mm

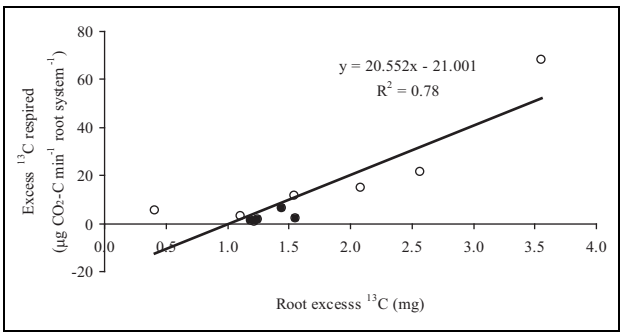


Fig. 2. Correlation between excess ^{13}C root respiration and excess ^{13}C root concentration. Each point refers to a tree; data of 3 sampling days (1, 2 and 4 days) are pooled together. Open circles = well watered trees, close square = low water supply trees. ***: Correlation significant at 0.1 %, r = Pearson correlation coefficient.

diameter) respiration with decreasing soil moisture. In addition in hardwood forest in central Massachusetts, during periodic natural moisture deficit (Davidson et al. 1998), an exponential relationship between reduction in soil matrix potential and the decline in CO_2 flux was reported, suggesting a mechanistic effect of drought stress on root respiration. This decrease of root respiration was related to the increase in the amount of carbohydrates (mainly glucose) necessary for osmotic adjustment, that were likely used to synthesize compatible solutes, such as sorbitol (Lambers et al. 1991). On the contrary in water stressed apple plots (soil moisture 20 % v:v, compared to 40 % v:v in control), in northern Italy, an increase in rhizosphere respiration, calculated as the difference between total soil respiration and C efflux from soil organic C, was observed (Panzacchi et al. 2012).

In our experimental conditions, we observed that under mild soil water depletion, tree adjustments did not include a change in C partitioning, rather a limitation of root metabolic activity, as a consequence of the reduction of tree CO_2 assimilation rate. Some papers report a different partitioning of C during periods of drought stress. Buwalda and Lenz (1992), for instance, found a 7–22 % increase in the proportion of total tree biomass allocated in the root system of apple trees grown in lysimeters and subjected to water deficit. Panzacchi and co-workers (2012) found that in water stressed trees, above ground net primary production was decreased, while below ground net primary production was increased compared with control trees, with a significant variation in the ratio between above and below net primary productivity that decreased from 1.88 (in control) to 0.98 (in water stressed trees). We did not observe this response, in fact our data showed the same reduction of excess ^{13}C in the different organs of trees grown in low moisture and well watered soil, suggesting that, in response to mild water limiting conditions, 'B.9' apple rootstock does not invest a larger portion of assimilates in root growth and root respiration. This can be explained considering that our soil water supply was still sufficient to maintain unaltered the metabolic activity in all tissues with no change in sink strength.

Sixteen days after the $^{13}CO_2$ pulse, the highest percentage of excess ^{13}C was found in the trunk (44 % of the total excess ^{13}C in the tree) followed by roots (27.5 %) and leaves (20.7 %), and herbaceous shoots (less than 8 %). It's important to point out, however, that the organ mass fraction to the whole tree mass was approximately 72, 17, 7, and 4 % for trunk, leaves, roots and shoots, respectively. Considering that leaves are the source of carbohydrates, which are then rapidly exported to the sink tissues, we observed that, in both soil water supply rates, roots and shoots were stronger sinks compared to the trunk.

In conclusion, the moderate reduction of soil water supply of our experimental conditions did not promote a preferential C partitioning to the roots, rather, it induced a reduction of net CO_2-C fixation and consequently a decrease of the new assimilated C allocation to the different organs of the tree, without a change of their strength as a C sink. A change in tree growth would be expected, with no preferential effect on one organ rather than another.

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Received 03/13/2014 / Accepted 06/02/2014

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