

Aspects of Grafting Influence on Carbon and Nitrogen Movement of Several Pear (*Pyrus sativa*) Cultivars

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Abstract

Among carbon and nitrogen contents, the interaction residing in the “Interdependence Theory” is one of the important components of plants. To elucidate how grafting influences the flow of carbon from shoots to tree roots and nitrogen from the roots to the shoots two sets of tests were carried out that have targeted the dosage of soluble sugars (to emphasize the relative flow of carbon) and nitrogen content dosage around the grafting union area. After many laboratory analyses, we obtained average values that reflect the dynamics of soluble sugars content depending on grafting, namely: 24% in the scion, 41% into the union area and 35% in the rootstock, in a ratio of about 1:1.7:1.4. In what concerns the total nitrogen content, we observed that the values are very similar between variants. Instead, somewhat higher nitrogen quantities (36%) were obtained in the rootstocks compared to the union area (32%) and scions (32%) representing a ratio of 1:1:1.1. Performing our experiments we found that the distribution of soluble sugars and nitrogen, in particular, in the grafting union area and the flow of photoassimilates and mineral elements, in general, for first year grafted trees depends not so much on the compatibility between scion and rootstock, but on grafting itself. Furthermore, we concluded that grafting itself is a barrier in photoassimilates and mineral elements flow in trees.

Keywords: Interdependence theory, plant nutrients, soluble sugars, scion, rootstock

Introduction

Carbon and nitrogen are important components of plants: carbon amounts to 50% and nitrogen up to 7% of the dry matter. Whereas carbon is assimilated as CO₂ from the atmosphere, nitrogen can be taken up as ammonium and nitrate from the soil and as N₂ and ammonia from the gas phase. Carbon reduction is restricted to the green parts of plants, but nitrogen incorporation in organic components can take place in the roots and in the leaves, depending on the nitrogen source, plant species, and nutrient availability. N₂-utilization is only found in plants which are living in a symbiotic relationship with bacteria, such as Rhizobia or cyanobacteria. If leaves are able to fix more CO₂ during the light period than they need for their metabolism, the surplus carbon is exported to support other tissues. Such leaves are called “source” leaves. “Sink” tissues such as growing leaves, flowers, seeds, roots and storage organs are net importers of photoassimilates (Ho, 1988). The primary CO₂ and nitrate fixation reactions and the syntheses of the transported assimilates occur in the mesophyll cells; these contain subcellular compartments which have different functions in the carbon and nitrogen metabolism. Their export to the sink tissues proceeds by mass flow through the sieve tubes.

For mature plants, root growth is shown to be a relatively small sink for carbon, but very sensitive to variations

in assimilate supply. The carbon lost due to respiration directly associated with ion uptake is also very small, although it may represent a significant sink at certain times of the year. Root maintenance, however, represents a large sink, perhaps accounting for more than 50% of the carbon utilized within the root system (Buwalda, 1993).

Nitrogen is commonly associated with tree growth and vigour, two characteristics that, if managed with care, are of the outmost importance for the development of newly planted trees so that they are able to maximize their flower production and yield potential. Too much vigour can be a result of N imbalance and can compromise the onset of bearing or even the yield in the first years (Neto, 2008).

Consequently, rates of N uptake by plant roots depend both on soil factors, determining the rates of supply of available N, and on plant factors, most immediately the activity and numbers of carriers in epidermal and cortical cells of the roots. At plant level, the uptake of N is regulated by the N demand of the plant, which is regarded as a function of plant growth rate (Touraine *et al.*, 1994).

Materials and methods

For the experiment were used four pear cultivars, two of them well-known and cultivated on large areas in Romania (‘Comtesse de Paris’ and ‘Williams’) and two relatively new, autochthonous (‘Trivale’ and ‘Triumf’), grafted onto

one rootstock known as incompatible (quince-*Cydonia oblonga*) and one known as compatible (*Pyrus sativa*) as a control. In Romania pedoclimatic and agro-technical conditions, the cultivars presented different response to the main pear disease (*Erwinia amylovora*) and pest (*Psylla* sp.) attack, and the reaction of the genotypes are influenced also of the rootstock (Sestras *et al.*, 2008; Sestras *et al.*, 2009). The experiments were conducted at the didactic farm "V. Adamachi" of the University of Agricultural Sciences Veterinary Medicine Iasi.

The analyses were accomplished onto the first year grafted pear trees over four generations (from 2006 to 2009). To emphasize the flow of carbon and nitrogen in grafted pear trees on both rootstocks, we made two sets of tests that have targeted dosage of soluble sugars (to emphasize the relative flow of carbon) and nitrogen determination from the grafting union area during two months (July and August). Analysis samples were collected from the scions (just above the grafting union area), from the union area and from the rootstock (just below the grafting union area).

Soluble sugar measurement has been made as follows: from grafted trees stem fine grinded samples soluble sugars were extracted by hot ethanol (3 replicates). Five mL of 70% (v/v) ethanol was mixed by shaking it with 300 mg of grinded stem tissues in a test tube. The sample was then incubated at 80 °C for 20 min. by shaking and then centrifuged for 5 min. at 1500×g. Freshly prepared anthrone reagent (0.2% anthrone in concentrated H₂SO₄) was pipetted into a test tube and chilled in ice water. The extract was thoroughly mixed with the anthrone reagent, the tube heated in boiling water for 15 min. and then rapidly cooled. The absorbance was read at 630 nm. Total soluble sugar content was calculated as mg per biomass unit.

Nitrogen concentration in plant tissue was determined by the standard macro-Kjeldahl procedure.

Results and discussions

Carbon and nitrogen are integral components in virtually all essential plant metabolic functions. The carbon is absorbed by plants from the air and the nitrogen from soil solution. Both of these essential chemical elements perform a specific biochemical or biophysical function within plant cells. Hence, the deficiency of one of these elements can impair metabolism and interrupt normal development.

When a graft is made, the injured cells are along the cut margins form a necrotic plate. The surviving cells just inside the necrotic plate respond to the wound signals by exuding suberin and other defensive chemicals. Within a few days, cell division starts and callus eventually forms, depending on the type of plant and the environmental conditions. Up to this point, the plant has responded to wounding just as if it were a cutting. However, since grafting creates a different environment, the subsequent events differ. For a successful graft, the callus from the stock and scion must grow together to form a callus bridge. At this point, cambial strands form in the callus bridge that connects the cambium of the stock and the scion. When the cambial strand is completed, it functions as cambium normally does, forming xylem and phloem. In the last step of graft formation, the formation of vascular connections is considered for most authors, the basic requirement for a successful graft (Moore, 1984 and Wang and Kollmann, 1996). The fact that the new vascular connections could not be well differentiated or weakly established has been postulated as the main reason for incompatibility in woody plants (Mosse, 1962, Errea *et al.*, 1994a) and it was reported to have reduced hydraulic conductivity in the graft union.

Achieving the soluble sugar and total nitrogen contents for elucidating how engrafting influences the flow

Tab. 1. Average values of soluble sugars content into the scion, the union and rootstock over four generations of first year grafted pear trees (years 2006-2009)

Type of grafting		Above union (mg·g ⁻¹ DW)	%	On the union (mg·g ⁻¹ DW)	%	Below union (mg·g ⁻¹ DW)	%
Variant I	'Comtesse de Paris'/ <i>Cydonia oblonga</i>	3.51±0.26	25	5.30±0.37	38	5.02±0.31	36
	'Trivale'/ <i>Cydonia oblonga</i>	3.10±0.23	34	3.58±0.23	39	2.46±0.13	27
	'Triumf'/ <i>Cydonia oblonga</i>	2.78±0.18	26	4.96±0.32	46	3.03±0.17	28
	'Williams'/ <i>Cydonia oblonga</i>	3.53±0.21	21	7.14±0.42	43	5.97±0.34	36
Variant II	Comtesse de Raris/ <i>Pyrus sativa</i>	3.81±0.23	21	7.31±0.57	41	6.72±0.42	38
	'Trivale'/ <i>Pyrus sativa</i>	4.14±0.31	28	4.88±0.33	33	5.64±0.23	38
	'Triumf'/ <i>Pyrus sativa</i>	2.06±0.15	14	7.56±0.49	50	5.56±0.35	37
	'Williams'/ <i>Pyrus sativa</i>	3.34±0.17	32	3.92±0.25	38	3.08±0.16	30
Average value variant I		3.23	26	5.25	42	4.12	33
Average value variant II		3.34	23	5.92	41	5.25	36
Total average value		3.28	24	5.58	41	4.69	35

Each value is shown as the mean ± S.D. of 24 samples; DW-dry weight

Tab. 2. Average values of total nitrogen content into the scion, the union and rootstock over four generations of first year grafted pear trees (years 2006-2009)

Type of grafting		Above union (mg·g ⁻¹ DW)	%	On the union (mg·g ⁻¹ DW)	%	Below union (mg·g ⁻¹ DW)	%
Variant I	'Comtesse de Paris' / <i>Cydonia oblonga</i>	54±3.18	32	53±2.97	31	62±4.82	37
	'Trivale' / <i>Cydonia oblonga</i>	34±2.54	32	33±2.11	31	39±2.76	37
	'Triumf' / <i>Cydonia oblonga</i>	46±2.87	32	43±3.79	30	55±4.22	38
	'Williams' / <i>Cydonia oblonga</i>	34±1.98	30	39±2.69	34	42±3.16	37
Variant II	Comtesse de Raris / <i>Pyrus sativa</i>	42±3.12	31	46±4.21	34	47±3.59	35
	'Trivale' / <i>Pyrus sativa</i>	50±3.81	34	47±3.53	32	51±4.02	34
	'Triumf' / <i>Pyrus sativa</i>	45±3.17	32	46±2.98	33	49±3.34	35
	'Williams' / <i>Pyrus sativa</i>	38±2.63	31	40±3.24	33	43±3.75	36
Average value variant I		42	31	42	31	50	37
Average value variant II		44	32	45	33	48	35
Total average value		43	32	43	32	49	36

Each value is shown as the mean ± S.D. of 24 samples; DW – dry weight

of carbon from the shoots to the roots and nitrogen flow from the roots to the shoots, we pointed out a series of results that reflect the specific behavior of these substances in the area of junction between scion and rootstock.

By analyzing the data presented in Tab. 1 and Fig. 1 we notice a significant difference in soluble sugar content above, below and at the junction area between scion and rootstock. For pear varieties engrafted on quince (*Cydonia oblonga*), combinations that show low affinity between scion and stock, the average values of soluble sugars are 26% above the union area (in scion), 42% in the union area and 33% below the union area (in rootstock). Dynamics of content of soluble sugars for the pear varieties engrafted on *Pyrus sativa*, combinations known to be compatible, was as follows: 23% in scion, 41% in the union area and 36% in rootstock. If we compare the results of these two variants we can see small differences between them that can be neglected. Instead, we see that both variants maintain the same ratio of soluble sugar content in the regions of fruit-trees stem: above, below and the union area.

Therefore, we can say that both the distribution of sugars around the union area and the circuit of this photoassimilate in the first year grafted trees depends not so much on the compatibility between scion and rootstock (in case the degree of incompatibility allows cohabitation between scion and rootstock), as on the grafting itself. In other words, the sieve tubes recovery after grafting seems not to be perfect. The tubes present some barriers in the photoassimilates migration to the roots.

Furthermore, by joining the values of these two variants we obtained other average values reflecting that the dynamics of soluble sugars content depends on grafting, namely: 24% in scion, 41% at union and 35% in stock, in a ratio of about 1:1.7:1.4.

Concerning the total nitrogen content (Tab. 2, Fig. 2) in the fruit-tree stem areas mentioned before (scion, the union area and rootstock), as in the case of soluble sugars,

the values between variants are very similar. Instead, we obtained somewhat higher quantities of nitrogen in rootstock (36%) than in the union area (32%) and scion (32%) representing a ratio 1:1:1.1. This is very interesting because it confirms the hypothesis of some impediments that exist in the grafting junction areas for both soluble sugars, transported by the elaborated sap through sieve tubes and nitrogen transported by the raw sap through the xylem vessels and tracheids.

Therefore, by applying induction to the obtained data it follows that generally the grafting itself may be considered an impediment in photoassimilates and mineral elements flow in grafted trees. The weakening of the elements flow equilibrium in the grafted trees reduces the stability of the tree as a whole, thus becoming more vulnerable to exogenous and endogenous stress factors.

One of these imbalances, as a grafting result, is the one we highlighted after our analysis, namely the imbalance between C and N. This fact negatively affects trees growth and development, knowing that there is an interaction be-

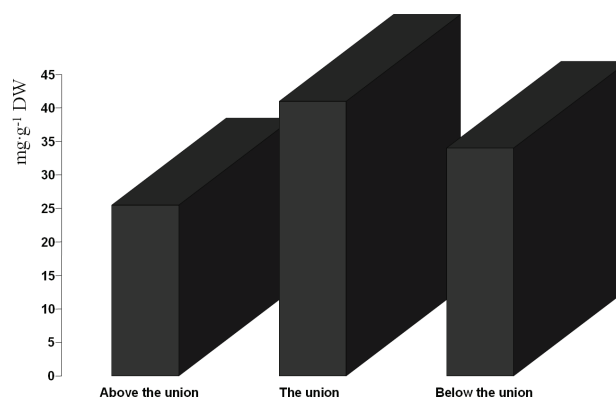


Fig. 1. Average soluble sugars contents into the scion, the union and rootstock over four generations of first year grafted pear trees (years 2006-2009)

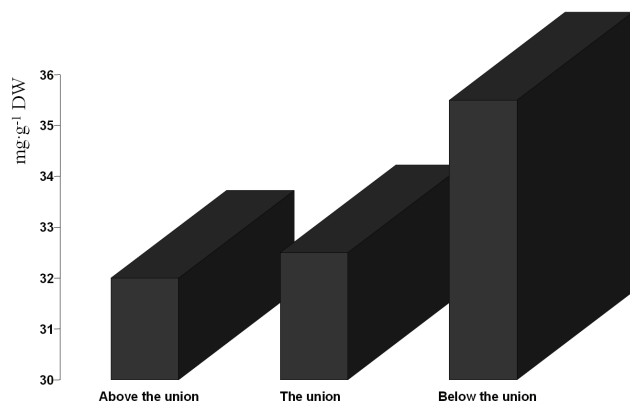


Fig. 2. Total nitrogen average content into the scion, the union and rootstock over four generations of first year grafted pear trees (years 2006-2009)

tween carbon and nitrogen content in plants, which comes from the “Interdependence Theory”. Through the work of a number of research groups, it is known that shoot and root growth are coordinated (Brouwer and De Wit, 1969; Ingestad and Lund, 1979). For a given growth environment and developmental stage, there will be a characteristic shoot and root dry matter distribution ratio. If the shoot and root dry matter ratio is disrupted, for example by excision of a major portion of either plant part like stem excision after chip-budding grafting, then disproportionate regrowth occurs until the characteristic shoot and root distribution pattern is reestablished. Further, if the growth environment is altered, such as by altering nutrient availability, the shoot to root growth ratio (S/R) adjusts to a new characteristic set point. It has been proposed that the physiological basis for shoot and root coordination is the ‘functional equilibrium’ between processes controlling acquisition of carbon and nitrogen. Carbon is acquired primarily by the shoot, and carbohydrate provision to the root controls the rate of root growth and the capacity for N uptake; N is acquired primarily by the root, and supply of N to the shoot determines carbon fixation by controlling the rate of shoot growth and the associated expansion of photosynthetic capacity. Interdependence theory also assumes that a hierarchy of carbon and nitrogen utilization exists. When photosynthetic rates are low, a greater proportion of the acquired carbon is utilized for sustaining shoot growth, less is supplied to the root, and a higher S/R growth ratio results. When limited amounts of nitrogen are available to the plant, a larger proportion of absorbed nitrogen is retained by the root and less is transported to the shoot. With slower shoot growth, a larger proportion of available carbohydrate ‘spills-over’ to the root, and the S/R growth ratio declines. The interdependence between carbon and nitrogen processes is a logical way to explain the empirical observations on shoot and root growth coordination. It has become a central component in models characterizing whole plant growth (Thornley, 1976; Wann

and Raper, 1979) and, to borrow from Lambers (1983), a virtual paradigm in whole plant physiology.

An important assumption of Interdependence Theory is that growth of the root system is primarily controlled by carbohydrate being supplied from the shoot. This is logical, since carbohydrate pools in the root system are relatively small. Soluble carbohydrate and starch pools together seldom comprise more than 5% of the root dry weight. Furthermore, a large majority of the carbohydrate reserves are relatively unavailable for metabolism, evidently being sequestered in vacuolar storage pools or, in the case of starch, being slowly degraded (Farrar and Williams, 1991; Jackson *et al.*, 1980). Thus, root metabolism and growth are dependent on a sustained flux of carbohydrate from the shoot. Beyond being a source of energy and carbon skeletons, carbohydrate arriving in the root may directly control growth processes.

A substantial amount of evidence indicates that nitrate uptake by existing root is dependent on photosynthesis and carbohydrate supplied from the shoot. Nitrate uptake is lower when plants are exposed to low light or low aerial carbon dioxide concentrations, and during the dark phase of the diurnal cycle (Clement *et al.*, 1978; Hansen, 1980; Rideout and Raper, 1994). Also, nitrate uptake rapidly decreases following shoot and stem excision (after chip-budding grafting), stem ringing and, in young plants, removal of the endosperm (see references in Jackson *et al.*, 1980), apparently reflecting the limited availability of carbohydrate reserves in the root.

Conclusions

If the degree of incompatibility allows cohabitation between scion and rootstock the distribution of soluble sugars around the grafting junction area and the movement of photoassimilates and mineral elements through this area, in the first year grafted trees, depends not so much on the compatibility between scion and rootstock as on the grafting itself.

Grafting is a kind of impediment in photoassimilates and mineral elements flow in trees and therefore, it weakens the elements movement equilibrium in trees, reducing the stability of the tree as a whole, thus becoming more vulnerable to exogenous and endogenous stress factors.

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