Ann Sci For (1994) 51, 373-379

Original article

Lipid utilization and carbohydrate partitioning during germination of English walnut *(Juglans regia)*

D Chenevard, JS Frossard *, A Lacointe

INRA–Université Blaise-Pascal, Unité Associée de Physiologie Intégrée de l'Arbre Fruitier,
Centre Clermont-Ferrand-Theix, Domaine de Crouelle, 63039 Clermont-Ferrand Cedex 02, France

(Received 22 March 1993; accepted 20 September 1993)

Summary — Conversion of reserve lipids in the seed, and carbohydrate and dry matter partitioning during germination were studied in walnut (*Juglans regia* L cv Franquette) seedlings. Nuts showed a gradual decrease in lipid content with a concomitant rise in carbohydrates (fig 2); starch appeared to be a transient sink for the end products of the degradation of lipid reserves. During germination, tap root elongation was preferential over stem growth (fig 3). The tap root accounted for most of the seedling dry matter increase and carbohydrate accumulation mainly as starch (table I). The other organs accumulated essentially soluble carbohydrates. At the end of the experiment, only 26.7% of the carbohydrates (starch + soluble carbohydrates) from lipid conversion were recovered in the seedling and the nut. A similar discrepancy was found in the energy budget. The energy loss from the nut (76.391 kJ) and the total energy recovered in the seedling (30.985 kJ) differed markedly at the end of the experiment (fig 4); this difference of 59% can be attributed to the metabolic lipid conversion, respiration (growth and maintenance) and translocation (table II).

germination / lipid utilization / carbohydrate / energy / English walnut / *Juglans regia*

Résumé — Utilisation des réserves lipidiques et répartition des glucides pendant la germination du noyer commun *(Juglans regia)*. *La germination du noyer (*Juglans regia *L cv Franquette) a été étudiée au niveau de la dégradation des lipides dans le cerneau ainsi que de la répartition des glucides et de la matière sèche dans la jeune plante. La teneur en lipides dans le cerneau diminue progressivement tandis que la concentration en glucides augmente (fig 2). Dans le cerneau, l'amidon semble être une forme transitoire de la dégradation des réserves lipidiques. Durant la phase de germination, le pivot présente une élongation préférentielle par rapport à la tige (fig 3). Par ailleurs, le pivot est le principal organe de la jeune plante tant au niveau de la matière sèche que de l'accumulation de glucides, principalement sous forme d'amidon (tableau I). Il est vraisemblable que le pool d'amidon dans le pivot soit responsable de l'absence du rythme nycthéméral de respiration racinaire. Il doit jouer un rôle tampon vis-à-vis du flux de glucides provenant de la partie aérienne. À la fin de l'expérience, seulement 26,7% des glucides provenant de la dégradation des lipides se retrouvent dans la plante et dans le cerneau. Cette différence se retrouve lors de la réalisation du bilan énergétique (fig 4). À la fin*

---

* Correspondence and reprints

de l'expérience, les pertes énergétiques dans le cerneau sont égales à 76 391 J tandis que l'énergie présente dans la plante n'est que de 30 985 J. Cette différence peut être attribuée aux processus de conversion des lipides dans le cerneau, à la respiration et à la translocation (tableau II).

***germination / conversion lipidique / glucides / énergie / noyer /* Juglans regia**

INTRODUCTION

The lipid reserves of oilseeds transferred from the cotyledons to the different parts of the growing seedling originate from gluconeogenesis (Moreau and Huang, 1977) with an accumulation of starch in the seed (Bory *et al,* 1990). The seedling roots are very important organs for the storage of food reserves, particularly during the early years of development to the woody plant. In walnut, during the first year, the carbon fixed through photosynthesis is mainly accumulated in the tap root (Lacointe, 1989). In addition, 6 weeks after germination, the relative independence of root respiration with respect to current photosynthesis was shown to be related to the size of the tap root (Frossard *et al,* 1989).

The present study was undertaken to characterize the changes in lipids, starch, soluble carbohydrates and energy in the cotyledons and in the different parts of the walnut seedling during germination. This study also provides a likely biochemical explanation for the absence of root respiration rhythm, which was observed in earlier studies.

MATERIALS AND METHODS

Plant material and germination conditions

Nuts from English walnut (*Juglans regia* L, cv Franquette) were soaked in running tap water for 72 h at room temperature (20°C). Germination was carried out in moist vermiculite at saturation in a growth cabinet at 25°C and 90% relative air humidity, with a 12 h light period (250 µm•m^{-2}•s^{-1} PAR) for 26 d.

Compositional analysis

A total of 240 nuts were used in germination experiments; 20 seedlings (4 samples of 5 seedlings) were harvested at regular intervals (2–3 d) up to 26 d. After harvest, the seedlings were rapidly measured and dissected into cotyledons, tap roots of diameter < 3 mm, tap roots of diameter > 3 mm, lateral roots, stem, and leaves when present (fig 1). These different parts were immediately frozen in liquid nitrogen and freeze-dried. The dry matter content of the different organs was determined.

Before biochemical analysis, the organs were ground and passed through a 125-µm-mesh screen. The lipid content of the nut was evaluated by a Bruker Spectrospin NMR analyser (Minispec 10), using crude walnut oil as reference.

For each sample, soluble carbohydrates were extracted in boiling ethanol (80% v/v) and assayed by the anthrone method (Halhoul and Kleinberg, 1972). Starch was assayed in the ethanol-

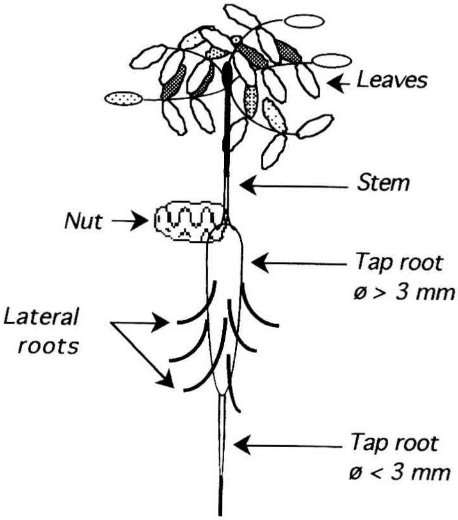

Fig 1. The different parts of a 26-day-old seedling of English walnut.

extracted residue, as previously reported (Frossard and Friaud, 1989). Soluble carbohydrates and starch were both expressed as glucose equivalents.

An analysis of variance was carried out on the starch content of the cotyledons.

Energy content

The energy content of each organ was determined using a bomb calorimeter (model CB-100, Gallenkamp, London, UK). The carbon dioxide produced from each combustion was trapped in soda lime, which was then weighed to determine the carbon content.

RESULTS AND DISCUSSION

Lipid utilization and carbohydrate partitioning

The main biochemical component in the walnut seed fraction are lipids (71% of the nut dry matter). These are stored mostly as triglycerides (Labavitch and Polito, 1985) and represent a very concentrated source of energy, since considerable reducing power is used to form them.

From the beginning of the germination, the lipid content of the cotyledons (nut) gradually decreased whereas their soluble carbohydrate and starch contents increased significantly (fig 2). Soluble carbohydrate and starch accumulation accelerated from the 10th day after soaking. The general pattern reported here was similar to that observed in castor bean (Desvaux and Kogane-Charles, 1952; Beevers, 1975; Reibach and Benedict, 1982), jojoba (Moreau and Huang, 1977), soybean (Adams *et al,* 1980; Brown and Huber, 1988), cotton (Doman *et al,* 1982), hazel (Li and Ross, 1990a,b) and bush butter tree (Bory *et al,* 1990). It is well known that in oilseeds, lipids are degraded to fatty acids, and are then converted into glucose by the glyoxylate cycle and gluconeogenesis (Beevers, 1961, 1975; Mazliak and Tchang, 1983).

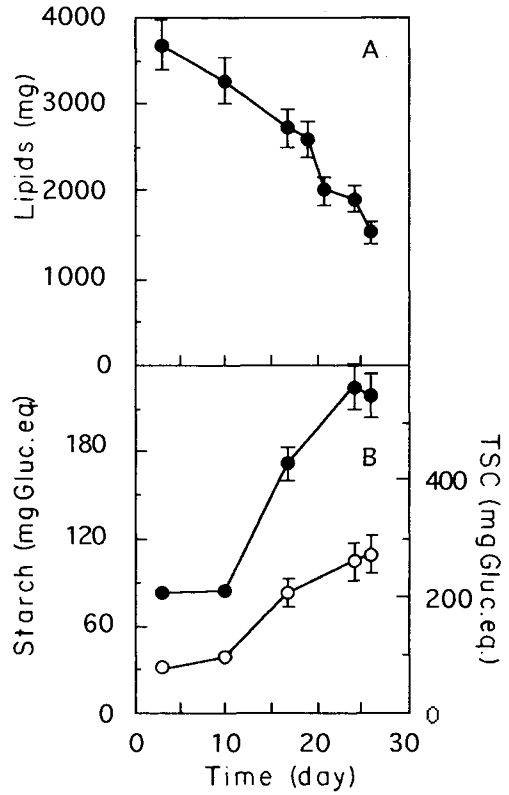

Fig 2. Changes in (A) lipid (●) or (B) starch (○) and total soluble carbohydrate content TSC (●) in the nut during germination and seedling emergence. Starch and TSC are expressed as glucose equivalents (GLUC eq). Vertical bars represent standard deviation (*n* = 4), when greater than the symbol size.

The breakdown of a mixture of triglycerides can be represented by the equation (Penning de Vries and Van Laar, 1975):

$$1\ \text{g triglycerides} \longrightarrow 1.430\ \text{g glucose} + 0.754\ \text{g}\ CO_2 + \text{energy} \quad [1]$$

From the beginning of germination, the total lipid of the cotyledons (nut) decreased by 2.139 g. The theoretical amount of glucose resulting from lipid conversion is 3.059 g. By the end of the experiment, starch accumulation in the nut accounted for 2.6% of the theoretical amount of glucose derived from lipid conversion, and soluble carbohydrates for 11.2%. The remainder was translocated into the seedling to support growth, or consumed in the nut and for seedling maintenance processes.

The transitory accumulation of starch in the nut may be interpreted in 2 ways:

1. Starch can be considered as an internal sink for soluble carbohydrates, which would thus allow a more active lipid conversion in the seed and prevent accumulation of soluble sugars to an inhibitory osmotic level (Li and Ross, 1990b).

2. Alternatively, this accumulation could be related to a saturation of the utilization capacities within the seedling with the absence of feedback response from this saturation on gluconeogenesis.

During germination, tap root elongation was preferential over stem growth (fig 3). The tap root accounted for most of the dry matter seedling growth (58% of total dry weight) with intermediate amounts in leaves and stem and small amounts in lateral roots after 26 days (table I). Carbohydrate accumulation was very high in the tap root where it occurred mainly as starch. In contrast, carbohydrate accumulation was low in the lateral roots and lower part of the tap root (diameter < 3 mm). It occurred mainly as soluble carbohydrates in lateral roots. Stems contained equivalent levels of starch and soluble carbohydrates whereas in leaves and lateral roots most of the carbohydrates were in the soluble form. At the end of the experiment, only 12.9% of theoretical carbohydrates originating from lipid conversion had accumulated in the seedling.

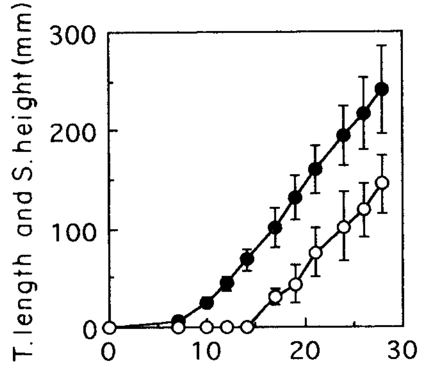

Fig 3. Evolution of tap root length (●) and stem height (O) during the germination of English walnut (*n* = 20).

With the further 13.8% recovered in the nut itself, this gives a total of 26.7% of the carbohydrates released from lipid conversion recovered in the system (nut + seedling). Presumably, the rest (73.3%) was lost in the processes of growth respiration, maintenance respiration and the translocation to the seedling.

The preferential accumulation of soluble carbohydrates in lateral roots and young leaves is consistent with sink behaviour, which is classical for growing organs.

The large amounts of starch accumulated in the tap root from the very beginning of its formation suggests than this organ is a potential source of carbohydrates for the seedling. The role of the tap root as a storage organ continues for later growth stages in walnut (Lacointe, 1989). These reserves could play an important role in stress conditions such as root damage. However, the functional importance of the lateral roots should not be neglected. In the young carrot plant, which has a root morphology similar to that of the walnut seedling, pruning lateral roots reduced leaf growth and altered the assimilate partition to the different organs, without any modification in the efficiency of carbon fixation by the leaves; pruning the tap root had a slight effect (Benjamin and Wren, 1980).

Table I. Allocation of dry matter, starch and total soluble carbohydrates to various parts of the seedlings at the end of experiment (26 days).

Organs	*Dry matter (mg)*	*Starch* *	*Soluble carbohydrates* *
Stem	323	25	37
Largest part of tap root	944	179	68
Smallest part of tap root	58	1	10
Secondary roots	149	1	32
Total seedling	1 782	210	186

* In mg glucose equivalent/organ.

Furthermore, in relation to this starch accumulation, the tap root may act as a buffer for carbohydrate transfer to the metabolic respiratorally active roots. This is consistent with the absence of a nycthemeral rhythm of root respiration observed in walnut seedlings that are 6 weeks older (Frossard *et al,* 1989). The absence of such a rhythm could reflect the relative independence of root respiration from carbohydrate transport from the aerial part to the root system which originated from the daily pattern of carbon assimilation by the leaves and its transport from the leaves to the roots.

Energy budget

The energy budget of the system (nut + seedling) between 2 dates can be determined because the system is closed. The seedling photosynthetic gains were negligible throughout the experiment because leaf growth was just starting. The substrate was inert (vermiculite + water).

The relationship is (in J):

$$\Delta E_{\text{seedling}} = \Delta E_{\text{nut}} - \Sigma E_{\text{losses}} \qquad [2]$$

where $\Delta E_{\text{seedling}}$ is the change in the energy content of the seedling (sum of the energy of the differents parts of the plant); ΔE_{nut} is the change in the energy content of the nut; and ΣE_{losses} is the sum of respiratory losses of the system: growth respiration + maintenance respiration + translocation + metabolic lipid conversion.

The energy lost from the nut greatly differed from the energy content of the seedling at the end of the experiment (fig 4). The cumulative energy in the seedling represents only 19% of the energy losses in the nut on the 10th day, but up to 41% on the 26th day (table II), whereas seedling

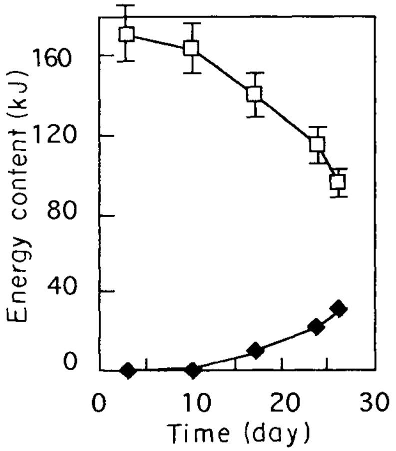

Fig 4. Changes of energy in the nut (□) and in the seedling (◆) during germination and seedling emergence. Vertical bars represent standard deviation, when greater than the symbol size ($n = 4$).

Table II. Change in total energy content in the nut and the seedling (kJ) during germination and seedling emergence.

Period (d)	*Change in nut*	*Change in seedling*	*Total losses*
3rd–10th	– 7.243	+ 1.368	5.875
3rd–17th	– 31.159	+ 10.041	21.118
3rd–24th	– 57.188	+ 21.755	35.433
3rd–26th	– 76.391	+ 30.985	45.406

energy content remained stable (17 kJ/g DM). This is not surprising because important respiratory processes are known to take place at the beginning of germination.

The energy level was in good agreement with the biochemical composition described above: the highest values are found for the nut and the tap root of diameter > 3 mm, which also contain the highest amounts of energetic compounds (lipids and carbohydrates).

There was also a close correlation between total carbon content (C, g DM) and total energy (E, kJ) in the seedling and the nut:

$$E_{seedling} = 40.60 \times C_{seedling} - 0.004 \quad [3]$$
with $R^2 = 0.997$

$$E_{nut} = 53.43 \times C_{nut} - 13.49 \quad [4]$$
with $R^2 = 0.999$

The quality of the relationship is in good agreement with that reported by Vertregt and Penning de Vries (1987) on reserve organs: it is possible to evaluate seedling energy and nut energy from carbon content.

CONCLUSIONS

From the beginning of the germination, the lipid content of the cotyledons (nut) gradually decreases whereas their soluble carbohydrate and starch contents increase. The carbohydrates present in the nut are used for the growth of the seedling. Much energy loss occurred in the nut during germination, and there remained large amounts of non-mobilized energy (lipids and carbohydrates) in the nut at the end of the experiment. Since further seedling growth rate is not modified by nut removal at this period (Frossard, unpublished results), the question of the exact role of such reserves remains open.

The study presented here was performed at 25°C in a growth cabinet. In natural or nursery conditions, the temperature would be lower. Would the germination pattern be the same under these conditions?

In oak (Levert and Lamond, 1979) and apple (Come, 1975), lowering temperature during germination delays seedling growth without any change in the final size or morphology of the seedling. In apple (Come, 1975), the total of oxygen consumption is not affected by the temperature over the range 4 to 20°C.

Therefore, the germination of English walnut at temperatures other than 25°C should present the same final growth and energy budget, the growth pattern being delayed at low temperature.

ACKNOWLEDGMENTS

We would like to thank B Saint-Joanis and M Crocombette for technical assistance.

REFERENCES

Adams CA, Rinne RW, Fjerstad MC (1980) Starch deposition and carbohydrase activities in developing and germinating soya bean seeds. *Ann Bot* 45, 577-582

Beevers H (1961) Metabolic production of sucrose from fat. *Nature (Lond)* 191, 433-436

Beevers H (1975) Organelles from castor bean seedlings, biochemical roles in gluconeogenesis and phospholipid biosynthesis. *In: Recent Advances in the Chemistry and Biochemistry of Plant Lipids* (T Gaillard and El Mercer, eds) Academic Press, New York, 287-299

Benjamain LR, Wren MJ (1980) Root development and source–sink relations in carrot, *Daucus carota* L. II. Effects of root pruning on carbon assimilation and the partitioning of assimilates. *J Exp Bot* 31, 1139-1446

Bory G, Youmbi E, Clair-Maczulatjys D (1990) Evolution des réserves cotylédonnaires au cours de la germination de *Dacroydes edulis* (Don) Lam. *Bull Soc Bot Fr* 137, 5-12

Brown CS, Huber SC (1988) Reserve mobilization and starch formation in soybean *(Glycine max)* cotyledons in relation to seedling growth. *Physiol Plant* 72, 518-524

Come D (1975) Rôle de l'eau, de l'oxygène et de la température dans la germination. *In: La Germination des Semences* (R Chaussat and Y Le Deunff, eds) Gauthier-Villars, Paris, 27-44

Desvaux R, Kogane-Charles M (1952) Étude sur la germination de quelques graines oléagineuses. *Ann Inst Natl Agron Série A*, 3, 385-387

Doman DC, Walker JC, Trelease RN, Moore BD (1982) Metabolism of carbohydrate and lipid reserves in germinating cotton seeds. *Planta* 155, 502-510

Frossard JS, Friaud JF (1989) Root temperature and short-term accumulation of carbohydrates in maize hydrids at early growth stage. *agronomie* 10 (9), 941-947

Frossard JS, Cruziat P, Lacointe A *et al* (1989) Biologie racinaire d'un jeune noyer, germination, croissance, relations hydriques, rôle dans la gestion des réserves. *8e Coll Recherches Fruitières, INRA-CTIFL*, Bordeaux, December 1988, 15-25

Halhoul MN, Kleinberg I (1972) Differential determination of glucose and fructose, and glucose- and fructose-yielding substances with anthrone. *Anal Biochem* 50, 337-343

Labavitch JM, Polito VS (1985) Fruit growth and development. *In: Walnut Orchard Management* Cooperative extension. University of California, Division of Agriculture and Natural Resources 90-94

Lacointe A (1989) Assimilate allocation and carbon reserves in *Juglans regia* L seedlings. *Ann Sci For* 46 (suppl), 832s-836s

Levert J, Iamard M (1979) Température et germination du chêne pédonculé. *CR Acad Agric Fr* 65, 1006-1017

Li L, Ross JD (1990a) Lipid mobilization during dormancy breakage in oilseed of *Corylus avellana. Ann Bot* 66, 501-505

Li L, Ross JD (1990b) Starch synthesis during dormancy breakage in oilseed of *Corylus avellana. Ann Bot* 66, 507-512

Mazliak P, Tchang F (1983) Installation et utilisation des réserves lipidiques dans les graines oléagineuses. *Bull Soc Bot Fr, Actual Bot* 3/4, 49-56

Moreau RA, Huang HC (1977) Gluconeogenesis from storage wax in the cotyledons of jojoba seedlings. *Plant Physiol* 30, 329-333

Penning De Vries FWT, Van Laar HH (1975) Substrate utilization in germinating seeds. *In: Environmental Effects on Crop Physiology* (JJ Landsberg, CV Cutting, eds). Academic Press, London, 217-228

Reibach PH, Benedict CR (1982) Biosynthesis of starch in proplastids of germinating *Ricinus communis* endosperm tissues. *Plant Physiol* 70, 252-256

Vertregt N, Penning De Vries FWT (1987) A rapid method for determinating the efficiency of biosynthesis of plant biomass. *J Theor Biol* 128, 109-119