Polyphenolic and enzymatic characterization of ageing and rejuvenation of hybrid walnut trees (Juglans nigra x Juglans regia). Relationship to growth

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Polyphenolic and enzymatic characterization of ageing and rejuvenation of hybrid walnut trees (*Juglans nigra* x *Juglans regia*): relationship to growth

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Introduction

This paper is mainly devoted to the polyphenolic and enzymatic characterization of hybrid walnut rejuvenation in order to find better plant materials, treatments and conditions which are propitious to propagation. Consequently, a comparative study of adult and rejuvenated annual shoots was undertaken.

Rejuvenation, widely linked to vegetative propagation, was described by Bonga (1982) and it has been shown on hybrid walnut that the propagation success by *in vitro* culture of cuttings was strongly dependent upon severe annual coppicing (Cornu, 1977). First results with phenolic compounds and enzymes were obtained during the annual growth of walnut: 1) juvenility and rejuvenation seemed to be linked to high values of the ratio of typical polyphenols during the first stages of growth after bud burst (Jay-Allemand *et al.*, 1987; 1988); 2) enzyme activity changes were found between adult and rejuvenated shoots, while no difference was noted in the total protein content (Drouet *et al.*, 1989).

Key results and a brief discussion on different factors involved in walnut ageing and rejuvenation will be presented.

Materials and Methods

Two clones of hybrid walnut (*Juglans nigra* x *J. regia*), which were grown in a nursery in Orleans, were used. Each clone was represented by 2 different physiological situations: 1) a rejuvenated 13 yr old tree obtained by annual coppicing in March for 10 yr, each stump producing at least 60 sprouts; 2) 10 yr old adult form obtained from a cutting of a previous stump sprout.

A detailed study of growth was undertaken to ensure that samples were shoots at the same growth stage and to study the relationship between growth rate and biochemical factors. For each sample, 5 shoots were cut, defoliated and immediately immersed in liquid nitrogen for subsequent lyophilization. Samples were taken on 5 days during shoot elongation (10, 18 and 24 June; 2 July and 7 August) (Drouet *et al.*, 1989). Extraction, purification and high performance liquid chromatography (HPLC) methods were reported by Jay-Allemand *et al.* (1988) and enzyme analyses were described by Drouet *et al.* (1989).

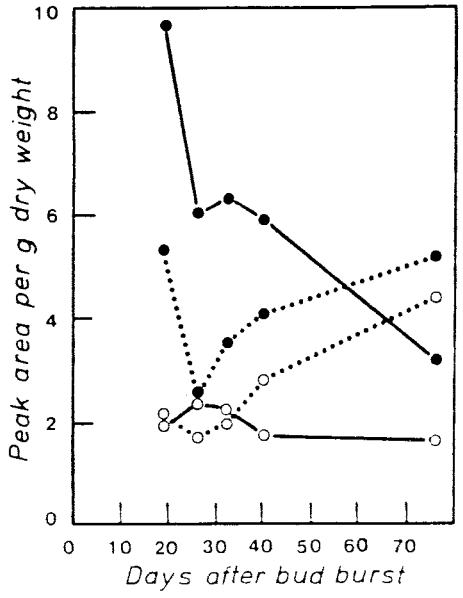

Fig. 1. Evolution of 2 phenolic compounds A (●) and B (o) of adult (···) and rejuvenated (—) shoots of the hybrid walnut *J. nigra* x *J. regia* 34. Quantitative data

Results

Phenolic compounds and phenylalanine ammonia lyase (PAL)

Results on polyphenols are based on 2 main compounds (A and B), elucidated by a previous study using canonical discriminant analysis (Jay-Allemand *et al.*, 1988). The evolution of these compounds during elongation of adult and rejuvenation shoots is described in Fig. 1. A high level of compound A (undetermined structure) at the beginning of growth characterized rejuvenated shoots, while adult shoots were characterized by late accumulation of compound B (flavonol). Moreover, the ratio *A/B* was always higher in rejuvenated shoots than in adult shoots. These results confirm and specify previous data. On the other hand, during the first growth wave, rejuvenation was marked by 2 PAL peaks which were absent in adult shoots (Fig. 2). However, no simple relationship has been found between this enzyme activity and the accumulation of studied polyphenols.

Enzymes: L-glutamate:NAD$^+$ oxidoreductase (GDH) and D-glucose-6-phosphate:NADP$^+$ oxidoreductase (G6PDH)

The evolution of the 2 enzyme activities in rejuvenated and adult shoots is presented in Fig. 3. Ageing induced an accelerated and asynchronous functioning of these 2 enzymes. While GDH decreased steadily after bud burst. G6PDH activity increased during the first 20 d. On the contrary, the rejuvenation treatment induced both a late (40 d) and synchronous increase of these 2 enzyme activities.

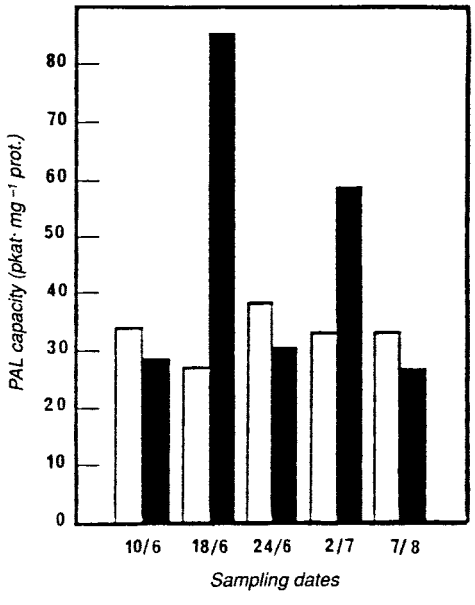

Fig. 2. PAL capacity of adult (❑) and rejuvenated (■) shoots during their elongation. This study was made with the hybrid walnut *J. nigra* x *J. regia* 34.

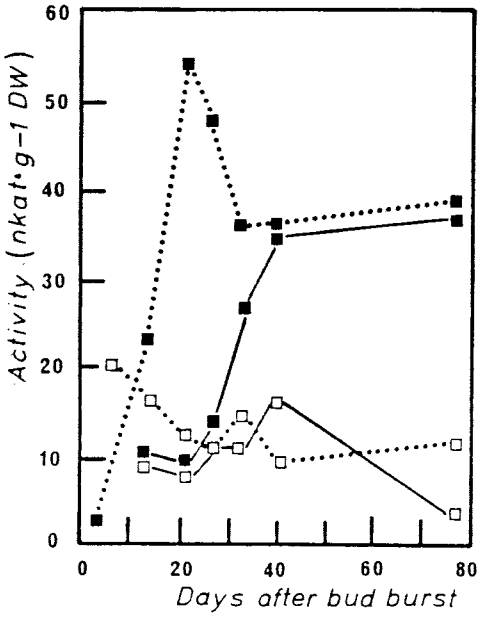

Fig. 3. Evolution of enzyme activity in adult (···) and rejuvenated (—) shoots during the growth period of the hybrid walnut *J. nigra* x *J. regia* 31. GDH (□) and G6PDH (■). (From Drouet *et al.*, 1989.)

Discussion

Ageing and rejuvenation of walnut trees has been characterized by different biochemical factors. In addition, studies undertaken on *Prunus avium* showed an increase of different flavonoids in phloem with ageing (Treutter *et al.*, 1987). Moreover, Zimmerman *et al.* (1985) reported changes of enzyme activities (ribonuclease, phosphatase, phenolase) during the different stages of development of woody plants. Recently, a specific protein of cell walls has been found to be associated with juvenility of *Sequoia sempervirens* (Bon, 1988).

All these data suggest that biochemical characteristics of ageing and rejuvenation could be related to root effects, tissue quality and gene activation.

In walnut, the accumulation of phenolic compounds (A and B) was not directly controlled by PAL activity. This enzyme was linked to growth acceleration (results not published) and involved in lignification processes, while compound A decreased steadily and compound B remained stable when the elongation rate increased during the first growth period of stump sprouts.

On the other hand, the initiation of growth and its acceleration seem to be linked to enzyme changes (GDH and G6PDH). Protein content decreased because of the synthesis of non-protein substances during growth (mainly lignin). Correlatively, activities of G6PDH (pentose phosphate pathway) and GDH (cellular detoxification of NH_4^+) increased and ensured, respectively, the production of NADPH and NADH which are needed for active growth (Drouet *et al.*, 1989).

The identification of the structure of the phenolic compounds associated with rejuvenation and their enzymatic regulation (PAL and chalcone synthetase) remains a high priority. It will also be necessary to specify relationships between typical phenolic compounds, their metabolism, growth and rooting.

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