Juvenility and Rooting Potential on the Stem Cuttings of *Pyrus betulaefolia*

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To establish a method for cutting propagation of *Pyrus betulaefolia*, an attempt to understand the biochemical mechanism of juvenility which increases the rooting potential was made by analyzing the endogenous polyamines in cuttings taken at different stages during the rooting process. As expected, cuttings taken from the juvenile phase showed higher rooting potential than those from the mature phase. When treated with 30 ppm IBA, endogenous putrescine (Put) increased in parallel with increased rooting. In contrast to juvenile cuttings, changes in Put, spermidine (Spd), and spermine (Spm) in mature cuttings were less even with IBA treatment.

INTRODUCTION

Pyrus betulaefolia is a superior rootstock for Japanese pear because of its well developed root system which helps prevent certain physiological disorders of the fruit, e.g. yuzuhada (rough skin). Because of these advantages, it is desirable that the seedling rootstocks currently used be replaced by clonal rootstocks in order to avoid the wide variation in characteristics found in seed-propagated rootstocks.

Many factors are important in the rooting of cuttings, however, juvenility is one of the most important. In general, the rooting potential of cuttings from the basal part of a plant (juvenile region) is higher than from the apical part of a mature plant (adult region). The differences in the biochemical and physiological status between different growth phases is not known. The objectives of this study were: (1) to determine the difference in rooting capability on the cutting of juvenile and mature *P. betulaefolia,* (2) to investigate the biochemical significance in both types of cuttings of endogenous polyamines which have a role in senescence of plants (Galston and Sawhney, 1987) and possibly rooting, and eventually (3) to establish the methodology for cutting propagation of *P. betulaefolia.*

MATERIALS AND METHODS

Cuttings were prepared from current shoots arising from basal and terminal parts of a 20-year-old *P. betulaefolia* plant—the basal portion with thorny growth and round leaves (characteristics associated with the juvenile phase) and the apical portion from the mature phase. Green stem cuttings, approximately 12 cm long with 3 to 4 leaves were taken. After submerging the base of the cutting in 30 ppm IBA solution for 24 h, they were transferred to the propagation bench in a glasshouse equipped with a mist propagation system.

Polyamine extraction and analyses were carried out on the stems and leaves of both cutting types—with or without IBA treatment—as follows. Tissue samples were homogenized and extracted with 5% $HClO_4$ at 4C. After centrifugation at

20,000 g for 20 min, the supernatant was benzolated by a reaction of NaOH and benzoyl chloride, and then subjected to HPLC analysis. The HPLC conditions were: mobile phase, acetonitrile and water (1:1, v/v); column, C 8 reverse phase; detection, UV at 254 nm (McDonald and Kushad, 1986).

RESULTS AND DISCUSSION

Rooting was significantly faster and higher in basal cutting than from apical cuttings, although percent rooting in both was relatively low which was probably an inherited quality from the mother plant (Fig. 1). Since the apical part of the mother plant was already mature ontogenically, it was expected that the rooting potential would show a difficult-to-root character (Heuser, 1976; Smith, 1985).

Table 1. Changes in endogenous putrescine level in stems of *Pyrus betulaefolia* cuttings taken from basal portion of mother plant (referred to as juvenile phase) during rooting process.

Days after planting	IBA	Control
	nmol g^{-1} FW	
0	28.63	
10	4.663	9.697
20	9.411	7.301
30	15.028	6.001
40	20.027	7.832
50	20.950	11.822

The polyamines were separable under reverse phase HPLC (Fig. 2) and putrescine (Put), spermidine (Spd), and spermine (Spm) were identified as the major polyamine by comparing retention times with benzoyl standards. Changes in Put contents in IBA-treated and non-treated cuttings from juvenile phase cuttings during the rooting process are shown in Table. 1. Put decreased after sticking and then increased with time; the content of which was greater in IBA-treated than in non-treated cutting 20 days after sticking. This increase coincided with root initiation in the cuttings. These results are similar to those shown by Jarvis et al. (1983), who observed, using stem cuttings of mung bean, enhanced levels of polyamines in the hypocotyl prior to primordium formation when IBA was applied. They implied this response might be associated with the changes in RNA metabolism leading to the initiation of adventitious root primordia. The changes in polyamine contents expressed as nmol per g fresh weight showed different tendencies between juvenile phase and mature phase cuttings during the rooting process (Fig.3). Put in leaves and stems gradually increased at the later period in juvenile cuttings. Despite IBA-treatment Put in mature cutting did not increase. In contrast, Spd tended toward a decrease on both cutting types with an exception of a conspicuous increase in leaves of cuttings from juvenile phase 30 days after planting. These changes was similar to that of Spm, however, Spm in leaves and stems of mature cutting and in stems of juvenile cutting was mostly stable until

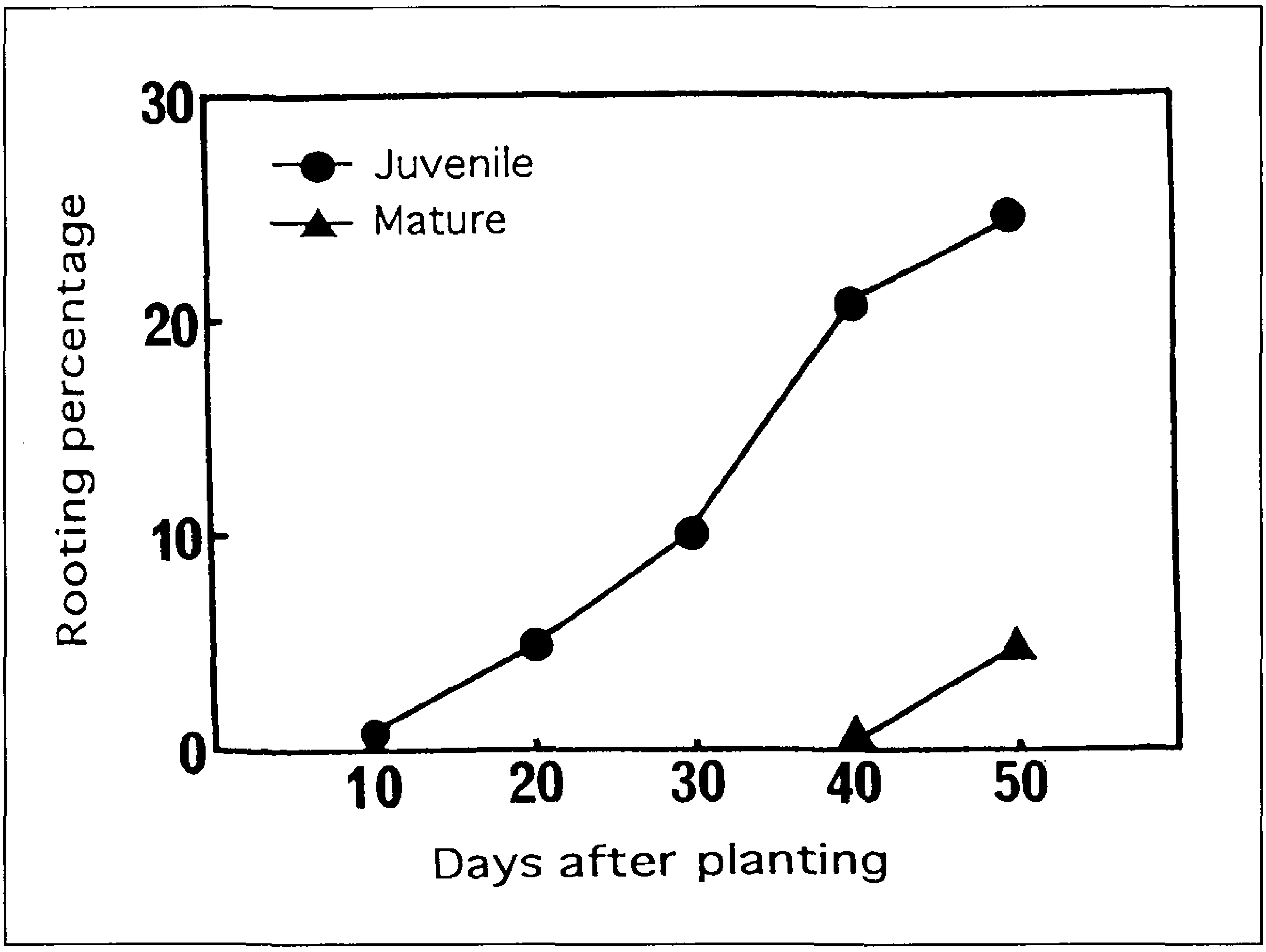

Figure 1. Rooting response of juvenile and mature *Pyrus betulaefolia* cuttings.

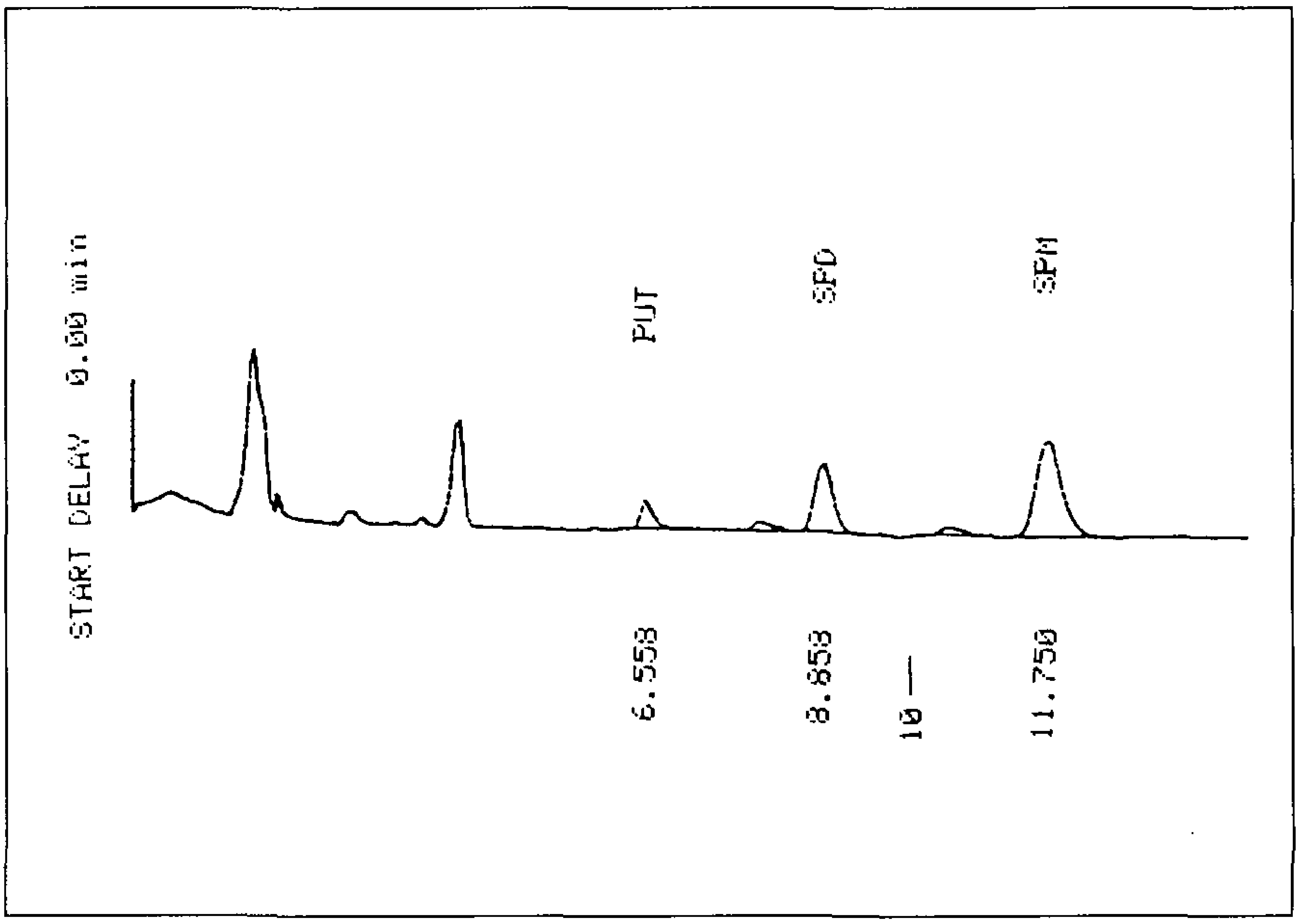

Figure 2. HPLC chromatogram showing the extracted sample containing putrescine (Put), spermidine (Spd), and spermine (Spm) identified as major polyamines.

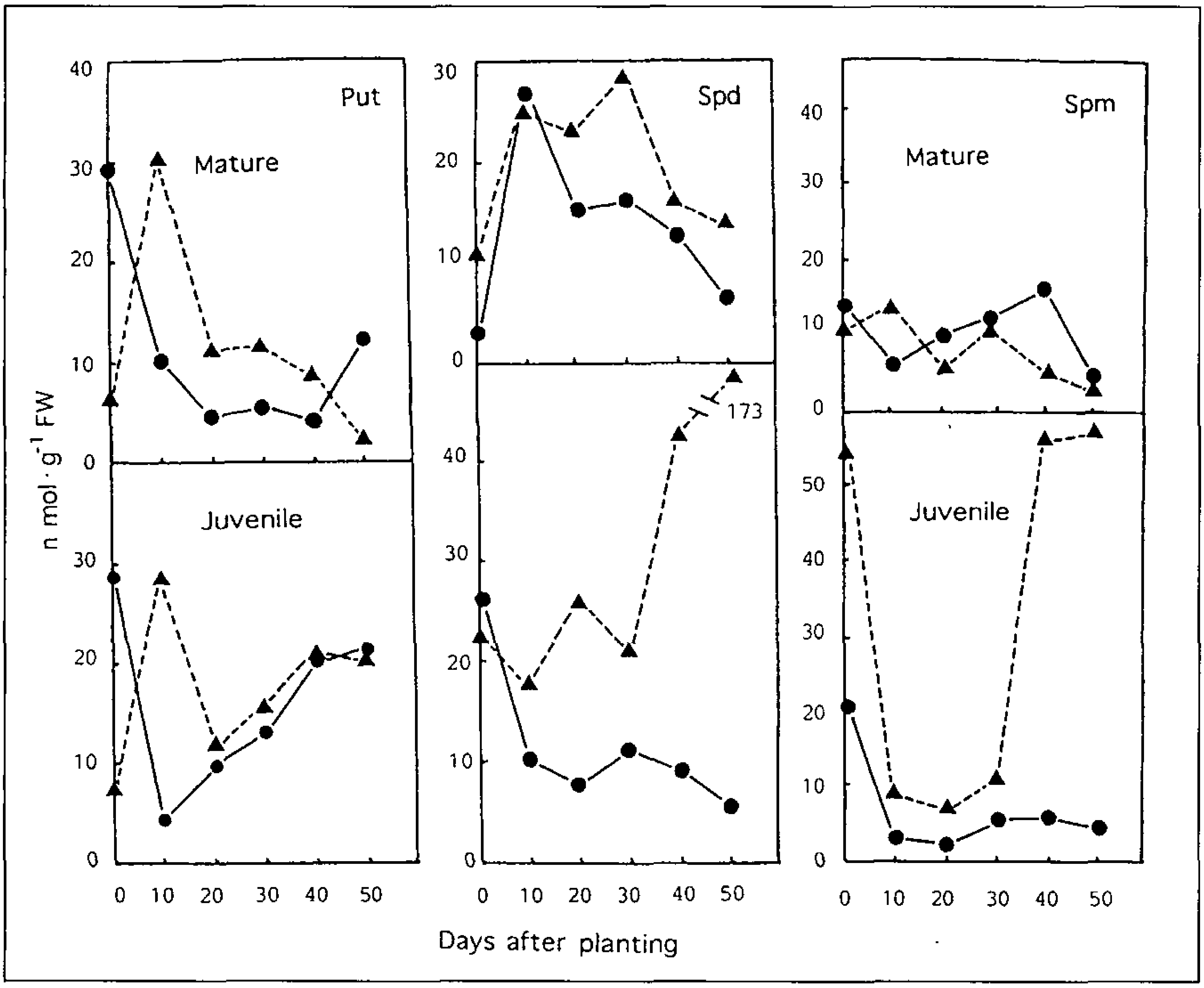

Figure 3. Changes in polyamine levels in stem (-●-) and leaves (--▲--) of *Pyrus betulaefolia* cuttings from juvenile (bottom) and mature phase (top) during rooting process.

termination of the rooting period.

As to differences in rooting potential between cuttings taken from different growth phases (juvenile and mature phases), it has been postulated to result from significant difference in rooting cofactor (Heuser, 1976) or endogenous ethylene level (Geneve et al., 1990). However, it also could be polyamine level is associated with juvenility in relation to rooting potential.

Polyamine levels have been reported to increase considerably in parallel with root formation of apple seedlings (Wang and Faust, 1986) and adventitious root formation in sweet cherry shoot cultures (Biondi et al., 1990). The result of this study was mostly in agreement with their data. Particularly, Biondi et al. (1990) emphasized the involvement of polyamines in root initiation which could be accompanied by action of ethylene based on the competitive relation in both biosythetic pathways (Galston and Sawhney, 1987). Nevertheless the role of ethylene in adventitious root formation is still unknown. The pattern of ethylene production stimulated by exogenous auxin application was significantly different in juvenile and mature debladed petioles of English ivy suggesting reduced ethylene level might be a prerequisite for root initial out-growth (Geneve et al., 1990). As shown in this study, Spd and Spm contents in leaves of juvenile cuttings, which show a good-rooting potential, increased markedly at the later rooting period. This seemed to be in accord with hypothetical ethylene function in the rooting process on the basis of polyamine-ethylene competition in their synthesis pathway. In addition, it could also be considered that higher Put levels in juvenile cuttings involve easy- or different-to-root potential as mediated by RNA metabolism. For better understanding of juvenility and rooting potential further studies concerning auxin, ethylene, and polyamine relations are necessary.

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