# Effect of Humisol on survival and growth of nursery grafted walnut (*Juglans regia* L.) plants

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**Abstract**

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The effect of Humisol organic fertiliser (150 g humic acid/l + 20 g/l mineral nutrients) on plant survival and growth of five walnut cultivars grown over a period of two years in the nursery were evaluated. Walnut plants treated with Humisol at the total annual rate of 0.75 ml/plant had a significantly higher survival rate in the first year, greater height and diameter in both years, and a higher percentage of first-class plants at the end of the second year, as compared to untreated control plants. Soil application of Humisol led to better performance of nursery grafted plants, compared to foliar application. Nursery plants receiving 1.5 ml Humisol per year through both soil and foliar application exhibited the best performance in the parameters analysed. In the first year, a decrease in plant growth rate in all treatments was observed in August and September; in the second year, growth rate started to significantly decline in the second half of August.

**Keywords**: humic acid; fertilisation; soil application; foliar application

Poor growth of scion shoots and a relatively high graft mortality rate in the first year of growth in the nursery are ongoing problems in the production of grafted walnut trees (Achim, Botu 2001; Porebski et al. 2002; Paunović et al. 2011a). Poor tree growth, shoot dieback and plant death in the first growing season after transplanting are due to transplant stress caused by root loss and slow root recovery of plants after their transfer into a new medium. Apart from providing an optimum supply of water and minerals, diverse biostimulators such as plant hormones, humic substances, marine algae extracts, vitamins, etc. were proved to improve the survival rate and growth of grafted plants in numerous experiments (Ferrini, Nicese 2002; Bostan, Islam 2003; Solar 2003; Salifu et al. 2006). Humic substances (humic acids, fulvinic acids and humines) showed a positive effect on plant nutrition and growth (Tan 1998). Soil or foliar applications of these substances resulted in increased growth in orange (Alva, Obreza 1998), apricot (Fathi et al. 2010), pear (Salem et al. 2010), plum (El-Shall et al. 2010), pistachio (Ismail, Kardoush 2011) and walnut (Bostan, Islam 2003).

Humic substances (HSs) can affect plants indirectly through their positive effect on soil physical, chemical and microbial properties. HSs stabilise soil structure and increase soil water holding capacity; they are important components of the soil redox systems, and they increase the soil cation exchange

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Table 1. Values for major climatic parameters during the experimental period

| Year | Mean annual temperature (°C) | Mean temperature April–October (°C) | Total annual rainfall (mm) | Total rainfall April–October (mm) |
|---|---|---|---|---|
| 2004 | 9.3 | 16.2 | 590.1 | 316.4 |
| 2005 | 10.2 | 16.5 | 707.1 | 442.5 |
| 2006 | 9.7 | 15.5 | 474.3 | 269.5 |

capacity (Piccolo, Mbagwu 1990; Russo, Berlyn 1990; Jiang, Kappler 2008). HSs form complexes with readily soluble ions, and release them in plant-available form when their concentration in the soil solution decreases, thereby preventing their leaching (Schnitzer 1986; Tarafdar, Jungk 1987; Tan 1998). HSs stimulate the microbial activity of saprophytes in the soil, and suppress some soil pathogens, such as *Pythium*, *Alternaria* and *Fusarium* fungi (Nardi et al. 1996; Pascual et al. 2002; Loffredo et al. 2007). HSs can be uptaken by plants and, therefore, may also actively modify the plant metabolism; they can also promote nutrient uptake, as a result of changes in cell membrane function (Chen, Aviad 1990; Varanini, Pinton 1995; Nardi et al. 2002). Plants treated with HSs undergo growth changes similar to those caused by growth regulators (Tattini et al. 1991; Nardi et al. 1996).

In certain studies conducted on soils well supplied with mineral elements, the use of HSs gave no clear positive response in terms of growth, yield and fruit quality; moreover, HSs may be detrimental to growth of some container-grown woody plants (Lee, Bartlett 1976; Laiche 1991). Barnes and Percival (2006) stated that selection of an appropriate biostimulant is critical as effects on growth can vary widely between tree species possibly as a result of (i) the differing active ingredient used in the formulation of a product and (ii) the concentration applied.

Data on the effect of humic substances on walnut growth are quite scarce. The objective of the present study was to evaluate the survival rate and growth of nursery plants of five walnut cultivars as affected by the method of application (foliar and soil application) of Humisol organic fertiliser containing humic acids.

## MATERIAL AND METHODS

The present experiment was performed at Fruit Research Institute, Čačak, Western Serbia, over 2004–2006, using cvs Šeinovo, Ovčar, Elit, G-286 and G-139, grafted by whip-and-tongue method onto a one-year-old local-type walnut (*Juglans regia* L.) seedling rootstock. Upon callusing in a temperature- and humidity-controlled chamber, high-quality grafts uniform in rootstock development and callus quality at the graft union were selected for further testing. The grafts were planted in the nursery at a spacing of 1 m between the rows and 0.25 m within the row, in a randomised block design (5 cultivars × 4 treatments × 4 replications, with each replication comprising 34 plants). The experiment was conducted over two two-year growing cycles. Each cycle lasted from graft planting in the nursery until the end of the second growing season i.e. from 20 May, 2004 through October 2005, and from 25 May, 2005 through October 2006, respectively.

In terms of its physicochemical properties, the soil where the experiment was carried out was a slightly acid alluvial loamy soil (pH 6.3), which contained 2.8% organic matter, 1,300 ppm N, 178 ppm $P_2O_5$ and 283 ppm $K_2O$ in the 0–30 cm layer. Values for major climatic parameters during the experimental period are given in Table 1.

Table 2. Humisol treatment (mg/plant) of nursery walnut plants at the end of the first and second growing seasons

| Treatment | 1st growing season | | | 2nd growing season | | | Total |
|---|---|---|---|---|---|---|---|
| | 4 weeks after planting | 6 weeks after planting | 8 weeks after planting | 15–20 May | 18–20 June | 18–20 July | |
| (1) Control | – | – | – | – | – | – | 0 |
| (2) Soil application | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 1.5 |
| (3) Foliar application | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 1.5 |
| (4) Soil + foliar application | 0.25 + 0.25 | 0.25 + 0.25 | 0.25 + 0.25 | 0.25 + 0.25 | 0.25 + 0.25 | 0.25 + 0.25 | 3 |

The grafted plants were grown in the nursery over a period of two years, using standard cultivation practices. The experiment involved the use of the following treatments: Treatment 1 – walnut cultivation without Humisol fertilisation (control); Treatment 2 – soil application of Humisol; Treatment 3 – foliar application of Humisol; Treatment 4 – soil and foliar application of Humisol.

Humisol is a commercial organic fertiliser manufactured by Nomit, Sremska Mitrovica, Serbia. Its chemical composition is as follows: humic acids 15%, N – 0.5%, $K_2O$ – 2%, and other elements (Ca, S, Mg, Zn, Fe, Cu, B, Mn) – 2%. Humisol was applied to the soil using the drip irrigation system; its foliar application to the grafts involved spray of 2.5% Humisol solution using a hand sprayer. Instead of Humisol solution, the control plants received water only. The plants were treated in accordance with the dates given in Table 2.

The following parameters were tested:
– percent survival of grafted plants at the end of the first growing season in the nursery;
– plant height at the end of the first and second seasons (as measured from the graft union up to the top of the grafted plant);
– trunk diameter at 3 cm above the graft union of the rootstock and the scion;
– number of first-class nursery plants (plants with a height of more than 120 cm above the grafting point) at the end of the second season;
– plant growth dynamics – plant height measured from the graft union to the top at 20-day intervals during the growing season.

The results obtained were statistically analysed using the analysis of variance, and means were compared by the Tukey's test at the significance level of 0.05.

## RESULTS AND DISCUSSION

As regards the percent survival of nursery plants at the end of the first season, no significant interaction was observed between Humisol application and cultivar or year (Table 3). In 2004, the average survival percentage at the end of the first growing season was 76.5%, which was rather significantly lower (9.3) than in 2005. The lowest survival was found in control plants (81.2%). Compared to the control treatment, foliar and soil Humisol application led to a 1.5% and

Table 3. Survival percentage and growth of nursery walnut plants at the end of the first growing season

| Factor | | Survival (%) | | | Plant height (cm) | | | Plant diameter (mm) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 2004 | 2005 | average | 2004 | 2005 | average | 2004 | 2005 | average |
| Cultivar (A) | Šeinovo | 82.1[a] | 91.8[a] | 86.9[a] | 16.8[a] | 20.8[a] | 18.8[a] | 7.9[a] | 7.8[ab] | 7.8[ab] |
| | Ovčar | 74.9[b] | 85.9[b] | 80.4[b] | 16.5[a] | 20.0[abc] | 18.3[b] | 7.7[a] | 7.6[ab] | 7.7[abe] |
| | Elit | 74.0[b] | 82.9[c] | 78.4[c] | 15.3[b] | 19.0[cd] | 17.2[c] | 7.1[b] | 7.0[c] | 7.0[c] |
| | G-139 | 73.6[b] | 81.4[c] | 77.5[c] | 15.4[b] | 19.6[bcd] | 17.5[c] | 6.8[c] | 6.7[d] | 6.8[d] |
| | G-286 | 78.0[c] | 87.4[b] | 82.7[d] | 17.5[c] | 20.7[ab] | 19.1[a] | 7.7[a] | 7.6[b] | 7.6[be] |
| Treatment (B) | control | 71.9[a] | 81.8[a] | 76.9[a] | 14.4[a] | 18.2[a] | 16.3[a] | 7.0[a] | 6.9[a] | 7.0[a] |
| | foliar | 73.6[b] | 83.2[a] | 78.4[b] | 15.2[b] | 19.2[b] | 17.2[b] | 7.2[b] | 7.1[b] | 7.2[b] |
| | soil | 79.6[c] | 88.6[b] | 84.1[c] | 17.5[c] | 21.2[c] | 19.4[c] | 7.7[c] | 7.6[c] | 7.7[c] |
| | foliar + soil | 80.9[c] | 89.8[b] | 85.3[d] | 18.1[d] | 21.6[c] | 19.8[d] | 7.8[c] | 7.7[c] | 7.8[c] |
| Growth cycle (C) | 2004/2005 | 76.5[a] | – | 81.2 | 16.3[a] | – | 18.2 | 7.4[a] | – | 7.4 |
| | 2005/2006 | – | 85.8[b] | | – | 20.0[b] | | – | 7.3[b] | |
| A × B | | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| A × C | | – | – | * | – | – | * | – | – | ns |
| B × C | | – | – | ns | – | – | ns | – | – | ns |
| A × B × C | | – | – | ns | – | – | ns | – | – | ns |

values followed by the same letter do not differ significantly at $P \leq 0.05$; ns – non-significant at $P \leq 0.05$; *significant at $P \leq 0.05$

Table 4. Growth and quality of nursery walnut plants at the end of the second growing season

| | | Plant height (cm) | | | Plant diameter (mm) | | | 1st class plants (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 2005 | 2006 | average | 2005 | 2006 | average | 2005 | 2006 | average |
| Cultivar (A) | Šejnovo | $225.9^{a}$ | $178.3^{a}$ | $202.1^{a}$ | $22.2^{a}$ | $18.3^{a}$ | $20.3^{a}$ | $65.7^{a}$ | $80.4^{a}$ | $73.1^{a}$ |
| | Ovčar | $217.5^{a}$ | $174.8^{ab}$ | $196.1^{b}$ | $20.0^{b}$ | $18.1^{a}$ | $19.1^{bcd}$ | $52.2^{b}$ | $67.4^{b}$ | $59.8^{b}$ |
| | Elit | $203.9^{b}$ | $169.5^{bc}$ | $186.7^{c}$ | $21.0^{b}$ | $18.1^{a}$ | $19.5^{bc}$ | $49.1^{c}$ | $63.3^{c}$ | $56.2^{c}$ |
| | G-139 | $204.1^{b}$ | $170.6^{bc}$ | $187.4^{c}$ | $20.8^{b}$ | $17.9^{a}$ | $19.4^{bc}$ | $46.9^{c}$ | $62.3^{c}$ | $54.6^{d}$ |
| | G-286 | $223.1^{a}$ | $174.4^{abc}$ | $198.7^{ab}$ | $20.1^{b}$ | $17.1^{b}$ | $18.6^{bd}$ | $58.4^{d}$ | $71.3^{d}$ | $64.8^{e}$ |
| Treatment (B) | control | $185.6^{a}$ | $162.5^{a}$ | $174.0^{a}$ | $18.4^{a}$ | $16.8^{a}$ | $17.6^{a}$ | $49.6^{a}$ | $62.7^{a}$ | $56.1^{a}$ |
| | foliar | $207.0^{b}$ | $169.6^{b}$ | $188.3^{b}$ | $19.4^{b}$ | $17.1^{a}$ | $18.3^{b}$ | $52.2^{b}$ | $66.1^{b}$ | $59.1^{b}$ |
| | soil | $226.7^{c}$ | $177.9^{c}$ | $202.3^{c}$ | $22.5^{c}$ | $18.8^{b}$ | $20.7^{c}$ | $57.1^{c}$ | $72.6^{c}$ | $64.9^{c}$ |
| | foliar + soil | $240.2^{d}$ | $184.1^{d}$ | $212.2^{d}$ | $22.9^{c}$ | $19.0^{b}$ | $21.0^{c}$ | $58.9^{d}$ | $74.3^{d}$ | $66.6^{d}$ |
| Growth cycle (C) | 2004/05 | $214.9^{a}$ | – | 192.4 | $20.8^{a}$ | – | 19.4 | $54.4^{a}$ | – | 61.7 |
| | 2005/06 | – | $173.5^{b}$ | | – | $17.9^{b}$ | | – | $68.9^{b}$ | |
| A ×B | | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| A × C | | – | – | * | – | – | * | – | – | * |
| B × C | | – | – | ns | – | – | * | – | – | ns |
| A × B × C | | – | – | ns | – | – | ns | – | – | ns |

values followed by the same letter do not differ significantly at $P \leq 0.05$; ns – non-significant at $P \leq 0.05$

8.4% increase in survival percentage, respectively. Survival percentage was the highest (76.9%) in the treatment involving both soil and foliar applications of Humisol. With an average of 86.9%, cv. Šeinovo showed the highest percent survival of nursery plants, in contrast to the lowest survival rate in cvs Elit (78.4%) and G-139 (77.5%).

In all cultivars, in both growing cycles, the lowest height and diameter growth in the first year was observed in control plants. Soil Humisol application caused significantly higher growth of nursery plants compared to control plants, by an average of 19% and 10% in terms of height and diameter, respectively. Foliar Humisol spraying gave a significant increase of 5.5% in plant height, and a non-significant increase of 2.9% in plant diameter compared to control groups. Plants receiving Humisol through both the soil and foliage attained the highest growth, superior by 21.5% in height and 11.4% in diameter compared to control plants. The average plant height at the end of the first growing season was 16.3 cm in 2004, i.e. by 3.7 cm lower than in 2005. Cvs Elit and G-139 achieved significantly lower average height, compared to cvs G-286, Šeinovo and Ovčar. A similar effect was observed in plant diameter.

Table 5. Effects of different Humisol application rates on percent increment for height and diameter growth in walnut nursery plants

| Treatments | Total rates for 2 years (ml/plant) | Height growth | | Diameter growth | | 1st class nursery plants | |
|---|---|---|---|---|---|---|---|
| | | increment in relation to control (%) | effectiveness* | increment in relation to control (%) | effectiveness* | increment in relation to control (%) | effectveness* |
| Control | 0 | – | – | – | – | – | – |
| Foliar | 1.5 | 8.2 | 5.5 | 4.0 | 2.7 | 5.3 | 3.5 |
| Soil | 1.5 | 16.3 | 10.9 | 17.6 | 11.7 | 15.7 | 10.5 |
| Foliar + soil | 3 | 22.0 | 7.3 | 19.3 | 6.4 | 18.7 | 6.2 |

*ratio of increment (%) divided by the total rate of Humisol per plant (ml)

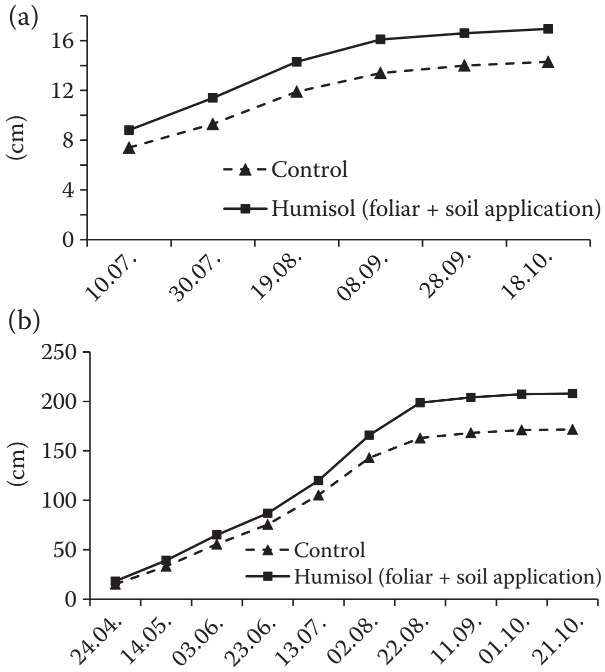


Fig. 1. Height growth of nursery plants during the first (a) and second (b) growing season

The average plant height at the end of the second growing season in 2005 was 214.9 cm, with nursery plants being 41.4 cm higher than in 2006 (Table 4). In both research cycles, nursery plants treated with soil-applied Humisol solution attained significantly higher growth, compared to control plants, the increase being 16.3% and 17.6% in terms of height and diameter, respectively. Plants foliarly sprayed with Humisol grew significantly higher (8.2%) compared to control plants, the difference in plant diameter of 4% being non-significant. Plants supplied with Humisol through both soil and foliar application attained the highest height and diameter growth. Compared to control plants, they were 22% higher, and 19.3% thicker. In the second season, cvs Šeinovo, Ovčar and G-286 achieved a significantly superior increase in height in both 2005 and 2006 compared to cvs Elit and G-139, whereas growth in plant thickness was quite uniform, particularly in 2006. In 2005, cv. Šeinovo exhibited significantly superior diameter growth than did the other cultivars, with no significant differences observed.

At the end of the 2004/2005 growing cycle, the proportion of the first-class nursery plants was 54.4%, and was significantly lower by 14.5% than at the end of the 2005/2006 cycle. In all cultivars, in both cycles, the differential methods of plant fertilisation with Humisol gave significant differences in the percentage of first-class plants at the end of the second year of growth in the nursery. The lowest percentage of first-class plants was found in the control. As compared to the control, soil application and foliar spray of Humisol resulted in a 15.7% and 5.3% increase in number of first-class plants, respectively. The combined soil and spray application of Humisol gave the highest percentage of first-class plants, which was 18.7% higher than in unfertilised plants. The significantly highest percentage of first-class plants was obtained by cv. Šeinovo, and the lowest by cvs Elit and G-139.

Dividing the percent increase in the test parameters relative to the control with the total Humisol rate (ml) per plant results in relationships that enable comparison of the effects of the treatments used (Table 5). The highest efficiency in terms of growth and percentage of first-class nursery plants was obtained by soil Humisol application, whereas the foliar application was the least effective. Regardless of the two-fold higher rate of Humisol per plant in the combined soil and foliar application, this treatment was 50–80% less effective, depending on the parameter tested, compared to the treatment involving soil application only. This suggests that soil application of Humisol solution to grafted plants was found to be the most cost-effective fertilisation method employed.

During the first year of growth in the nursery, until 19 August, nursery plants showed intense and quite uniform height growth, which started to weaken in the last ten days of August and September, becoming very poor in October. The highest rate of growth of nursery plants was observed from 30 July to 19 August. Humisol fertilisation had no substantial impact on the shape of the growth curve of nursery plants. The difference in plant growth rate between Humisol-treated plants and control plants was evident from 10 July to 19 August, with growth curves for both groups of plants through the rest of the growing season becoming almost parallel. In the second season, plant height increased intensively until 22 August, decreasing thereafter. Up until 22 August, the highest differences in growth rate between Humisol-treated and control plants were observed. In the second season, the highest growth rate was observed over the period 13 July–2 August (Fig. 1).

In all methods of Humisol application, the average survival rate, growth and percentage of first-class plants were the highest in cv. Šeinovo, and the lowest in cvs G-139 and Elit. These results are in agreement with the findings of Paunović et al. (2010) who suggested that genetic traits of a cultivar have a significant effect on survival rate and growth of nursery plants, and that cultivars exhibiting better graft-take success and plant growth in the first season show identical performance and yield high quality plants in the second season. All parameters, except thickness of one-year-old nursery plants, showed year × cultivar interactions, due to the specific response of cultivars to differing values for climate factors in certain years. Over the three-year experimental period (2004–2006), the most favourable hydrologic conditions were observed in 2005. In June through August 2004 and 2006, there was 80 and 100 $l/m^2$ less rainfall than in the same period in 2005. Therefore, the results on survival, height and diameter growth, and percentage of first-class plants were significantly most favourable in 2005. The stronger effects of fertilisation (particularly soil application of Humisol) on diameter of two-year-old nursery plants in 2005 compared to 2006 indicate the presence of year × fertilisation interaction, as confirmed by statistical analysis. The results obtained suggest that fertilisation of nursery plants with Humisol had a stronger positive effect on plant diameter in the growing season that had favourable hydrologic conditions.

Employing the same grafting technology and cultivation practices without the use of humic substances, Paunović et al. (2011a) obtained a survival percentage of 68.7 to 86.5% and plant height of 14.7 to 17.8 cm at the end of the first growing season, plant height of 170.0 to 172.4 cm at the end of the second growing season, and percentage of first-class plants of 50.9 to 63.4%, depending on the cultivar, under Čačak (Western Serbia) conditions. The results obtained are comparable to those for the control plants in the present study.

Humisol application in this experiment led to a significant increase in survival rate, growth and percentage of first-class plants compared to the control plants. Soil Humisol application gave better results than foliar spray. The best results were obtained with the treatment involving both foliar and soil application of Humisol to nursery plants. Fathi et al. (2010) treated apricot trees during the growing season with weekly soil and spray applications of Actosol® complex organic fertiliser solution (2.9% humic acid), which improved growth and fruit yield in treated trees. Soil applications were more effective than spray ones; whereas the highest effect was obtained with both soil and foliar applications. The spray application of the complex organic fertiliser containing humic substances in the studies by Paunović et al. (2011b) led to an average plant growth of 16.9 and 207.8 cm at the end of the first and second growing season, respectively, as compared to the treatment without foliar application, which is in agreement with the results of the present experiment.

## CONCLUSION

The results obtained in this study suggest that the increase in Humisol rate from 1.5 to 3 ml/plant did not result in a proportional increase in growth and percentage of first-class nursery plants. Similar effects were obtained by Bostan and Islam (2003) in one-year-old walnut seedlings. They used soil applications of humic substances three times during the growing season, and significantly improved height and diameter growth in treated seedlings compared to untreated seedlings. The most effective stimulation of walnut seedling growth was obtained at the total rate of 1.5 ml/plant, followed by 3, 0.75 and 6 ml/plant. The authors found the most rapid growing period for young walnut seedlings to be from 15 June to 15 July, followed by 15 July–15 August, and the slowest period from 15 August to 15 September. In this experiment, the highest growth rate in the first season occurred several weeks later, due to the fact that walnut grafts take longer for the rootstock and the scion to fuse and for the vascular tissues to completely join together at the graft union.

Davies et al. (2002) stated that transplanting woody plants into an outdoor environment leads to a large reduction in the root system which fails to supply the aboveground parts with sufficient amounts of water, resulting in water deficit in plant tissues. Water requirements increase during the growing season due to increasing leaf surface area. Rapid root regeneration in newly transplanted seedlings is an important step in establishing normal root/shoot ratio and reducing transplant losses (Davies et al. 2002). Tattini et al. (1990, 1991) reported that humic acid increased the root/shoot ratio as well as the production of thin lateral roots of olive plants; according to Sammonds and Struve (2004), humic acid increased root growth and water uptake of red oak. Humic acid treated avocado trees were larger and the root system was better developed than the untreated trees (Phanuphong, Partid 2003).

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