

# THE EFFECT OF BIOSTIMULATORS ON ROOT DEVELOPMENT OF *THUJA OCCIDENTALIS* 'SMARAGD' AND *CHAMAECYPARIS LAWSONIANA* 'ELLWOODII' SHOOT CUTTINGS

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## Abstract

The effect of a foliar treatment with an *Ascophyllum nodosum*-based biostimulant (Yeald Plus) was investigated on stem cuttings of two evergreen varieties. In *Thuja occidentalis* 'Smaragd' 0.15–0.30% biostimulant concentrations resulted in 64–66% rooting within six weeks, while the control group reached 36% only at a later stage. The root mass of rooted cuttings was higher at the 0.15% dose than at the double concentration. *Chamaecyparis lawsoniana* 'Ellwoodii' did not form roots within six weeks; however, differences in shoot biomass and water content were detected among treatments. Based on our results, foliar spraying with a 0.15% Yeald Plus solution can be an effective method in 'Smaragd' arborvitae propagation, whereas 'Ellwoodii' may require a longer rooting period and possibly an additional auxin treatment.

## 1. Introduction

Biostimulants belong to a subgroup of bioregulators that regulate plant development through various physiological processes. Bioregulators – also known as plant growth regulators (PGRs) – are natural or synthetic compounds that can stimulate or inhibit growth and influence the physiological, biochemical and morphological processes of plants [8]. Due to their chemical structure, they are highly effective even in very small quantities [10]. Due to the prioritising of sustainable agriculture and environmentally friendly cultivation, producers increasingly prefer the use of natural compounds that do not damage the environment [3]. Biostimulants typically contain growth-regulating substances, inorganic elements and phenolic compounds that stimulate plant metabolism and physiological activity [4]. Their natural components promote rooting, enhance stress tolerance, and improve water and nutrient utilization. In ornamental horticulture, extracts derived from sea algae (particularly from *Ascophyllum nodosum* and *Ecklonia maxima*) are widely used due to their proven effectiveness in stimulating root development and overall plant vitality. Plants treated with biostimulants generally develop greater root and shoot biomass, exhibit improved nutrient uptake and flower formation, which ultimately leads to higher yields and better product quality [1, 7]. In tree nursery propagation, there is an increasing demand for hormone-free, eco-friendly technologies; therefore, the targeted application of biostimulants on evergreen ornamental species and cultivars is well justified.

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*Chamaecyparis lawsoniana* (A. Murray bis) Oerst. and *Thuja occidentalis* L. belong to the family *Cupressaceae* and the subfamily *Cupressoideae*. The former species is a 2–4 m high, scale-leaved, columnar evergreen tree originated from California [12]. It is easily recognizable by its fine, drooping branches, small (1 cm) globular cones and characteristic X-shaped pattern on the underside of the shoots. Its horticultural importance lies primarily by the numerous ornamental varieties [2, 5, 11]. One of them, the cultivar 'Ellwoodii' is a blue-green to silvery-toned conifer with both needle- and scale-shaped leaves; a sensitive variety that grows best in sheltered, wind-protected locations. *Thuja occidentalis* L. is usually not larger than 10–20 m, native to North America. This conical species produces horizontal branches with dorsiventral shoots in different coloured sides and narrow, bud-liked cones at the same size such as in the case of *Ch. lawsoniana*. It prefers moist, humid soils but relatively hardy regarding nutrient and water supply. The cultivar 'Smaragd' is a columnar, densely branched, 3–4 (even 5–6) m tall plant that retains its glossy, bright green foliage throughout the winter, making it particularly suitable for use as a hedge or landscape boundary plant [5, 6].

As conventional propagation method of both variety, semi-hardwood cutting is effective, however, younger (maximum 3–4-year-old) stock plants of *Chamaecyparis* or wounding the cuttings' basal part and auxin treatments (0.2–0.3 % IBA) usually gives better rooting results [11].

In this trial, our aim was to find the optimal treatment for root stimulation of cuttings of both conifer variety.

## 2. Material and method

During 2022–2023, the experiment was carried out in Vojvodina, Serbia, in an unheated greenhouse with regular ventilation. Inside the greenhouse, under a foil tunnel, cuttings of *Thuja occidentalis* 'Smaragd' and *Chamaecyparis lawsoniana* 'Ellwoodii' were used to examine the effect of biostimulant treatments on rooting. We collected 10–12 cm sized semi-hardwood cuttings from 15-year-old *Chamaecyparis* and 15 yearold *Thuja* stock plants in September.

We established three groups:

- 30 cuttings per cultivar treated with 0.15% Yeald Plus
- 30 cuttings per cultivar treated with 0.3% Yeald Plus
- 30 untreated cuttings per cultivar (control)

Yeald Plus® is a foliar fertilizer in which zinc is the main active component, present in zinc ammonium acetate form. It is reported to stimulate auxin synthesis, increase root fresh weight, and improve chlorophyll content and nutrient uptake [13]. The 0.15% concentration corresponds to the manufacturer's recommended dose [10] and was therefore used as the reference treatment; the 0.30% solution represented a double dose to evaluate potential dose-dependent effects, while the 0% treatment (tap water only) served as the control.

The rooting medium consisted of a 1:1 mixture of sand and perlite, which was pre-moistened, compacted and levelled into the trays before planting. The cuttings were inserted 2–3 cm deep into the substrate using a dibber stick. We ventilated the foil tunnel daily to prevent fungal infections. Yeald Plus was applied as a foliar spray once per week during the first four weeks of the experiment. None of the treatments received auxin application. Throughout the experimental period, all cuttings were misted daily with tap water.

Before to make cuttings, we measured five cuttings from each variety in order to collect data of fresh weight, then dried and reweighed to determine water content. Rooting evaluation was performed in the sixth week after planting. From each treatment group, five samples per species were selected, and the following parameters were recorded:

- percentage of rooted cuttings (%),
- fresh and dry weight of shoot and root parts,
- water content of shoots and roots, calculated from the difference between fresh and dry weight.

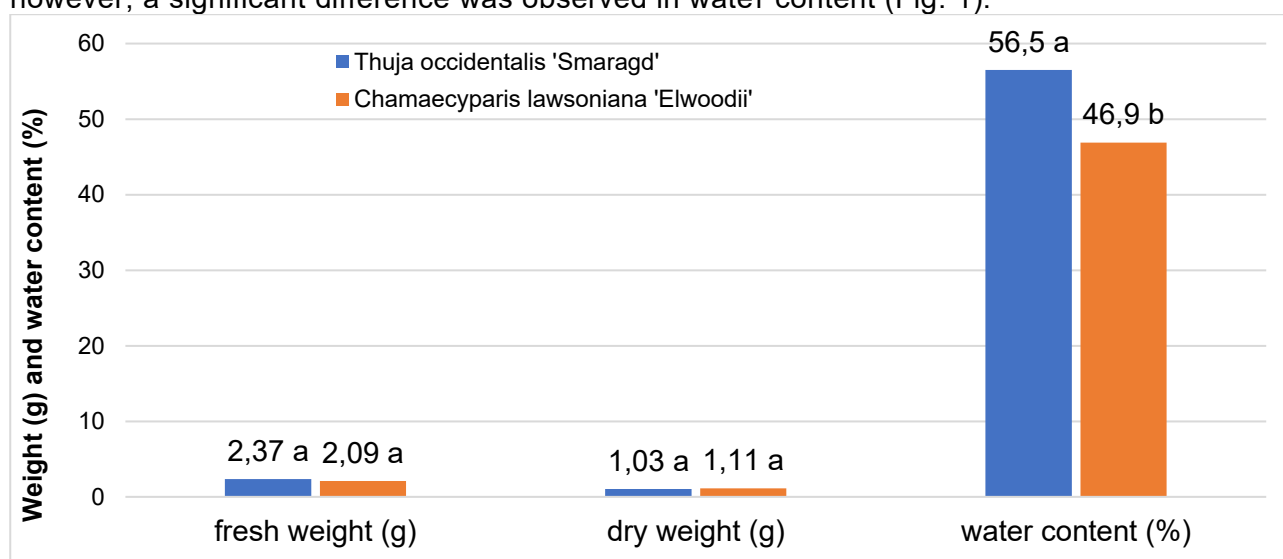
The rooting percentage for both species was calculated based on the full set of 30 cuttings per treatment, whereas fresh and dry mass as well as water content were measured on 5 representative samples per treatment group. In the case of *Chamaecyparis lawsoniana* 'Ellwoodii', the absence of rooting allowed only the determination of shoot mass and shoot water content.

Data were recorded in Excel and evaluated by using SPSS Statistics 27 software. One-way analysis of variance (ANOVA) was applied to identify differences among treatments, and group means were compared using Duncan's and paired t-tests ( $p \leq 0.05$ ). Significant differences in the graphs and tables were indicated by different letters.

### 3. Results

#### Initial weight and water content of the cuttings

At the beginning of the experiment, the average fresh weight of *Thuja occidentalis* 'Smaragd' cuttings was 2.37 g, the dry weight was 1.03 g, and the water content was 56.5%. In the case of *Chamaecyparis lawsoniana* 'Ellwoodii', the fresh weight slightly exceeded 2 g, while the dry weight was somewhat higher at 1.11 g, resulting in a lower water content of 46.9%. There was no significant difference between the two variety in terms of fresh and dry weight; however, a significant difference was observed in water content (Fig. 1).



**Figure 1:** Fresh and dry weight, water content of *Thuja occidentalis* 'Smaragd' and *Chamaecyparis lawsoniana* 'Ellwoodii' cuttings at the beginning of the trial

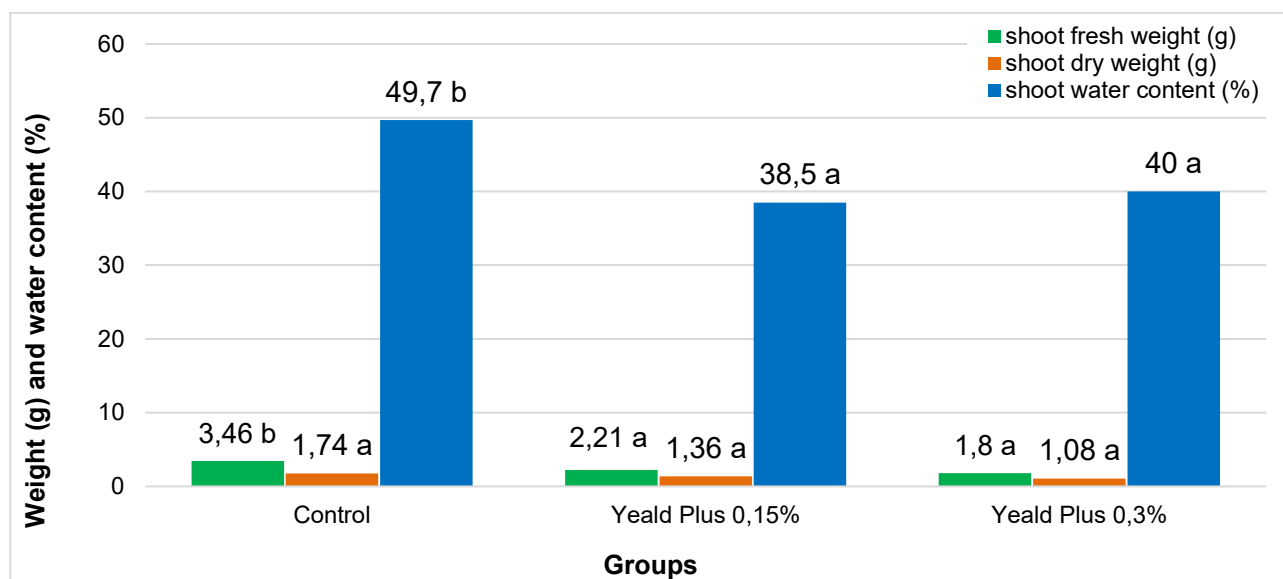
#### Weight and water content of the shoot part of *Thuja occidentalis* 'Smaragd' cuttings after treatments

In the control group, the average fresh weight of the shoot part was 3.46 g and dry weight was 1.74 g. After treatment with 0.15% Yeald Plus, these values were 2.21 g and 1.36 g, while in group treated by double dosage, they were 1.80 g and 1.08 g, respectively. There was a significant difference among the treatments: the control group exhibited a higher water content (49.7%), which can be attributed to the lack of root development, whereas the lower water content of the treated cuttings (38.5% and 40%) may indicate changes in water and nutrient flow associated with the beginning of root formation. No significant differences were found in dry mass (Fig. 2).

#### Rooting characteristics of *Thuja occidentalis* 'Smaragd' cuttings after treatments

The control group did not develop roots by the sixth week, whereas 0.15% Yeald Plus resulted 64% rooting rate and not significantly higher (66%) by 0.3% concentration. In the control, weak rooting appeared only two weeks later, reaching not more than 36%; therefore, root weight and water content data of the control were not evaluated due to the delayed root formation.

The fresh root weight was 0.166 g in the 0.15% Yeald Plus treatment and 0.118 g at 0.3%, with corresponding dry weights of 0.134 g and 0.094 g. The manufacturer-recommended 0.15% concentration produced higher values than the double dose, indicating that an excessive level of active ingredients may inhibit root formation. Root water content was 19.3% at 0.15% and 20.3% at 0.3% dosage, showing no notable difference between treatments (Table 1).



**Figure 2:** Fresh and dry shoot weight, water content of the shoot part of *Thuja occidentalis* 'Smaragd' cuttings under different treatments

**Table 1.** Rooting parameters of *Thuja occidentalis* 'Smaragd' cuttings

Treatment	rooting rate (%)	root fresh weight (g)	root dry weight (g)	root water content (%)
control	-	-	-	-
Yeald Plus (0.15%)	64	0.166 b	0.134 b	19.3 a
Yeald Plus (0.3%)	66	0.118 a	0.094 a	20.3 a

### Weight and water content of the shoot part of *Chamaecyparis lawsoniana* 'Ellwoodii' cuttings after treatments

The cuttings of this variety did not develop roots by the end of the sixth week, even in the treated groups. Therefore, no data were recorded about rooting rate, root mass and root water content for this species. The fresh and dry weights, water content of the shoot parts are presented in Table 2.

**Table 2.** Fresh and dry weight, water content of the shoot part of *Chamaecyparis lawsoniana* 'Ellwoodii' cuttings after treatments

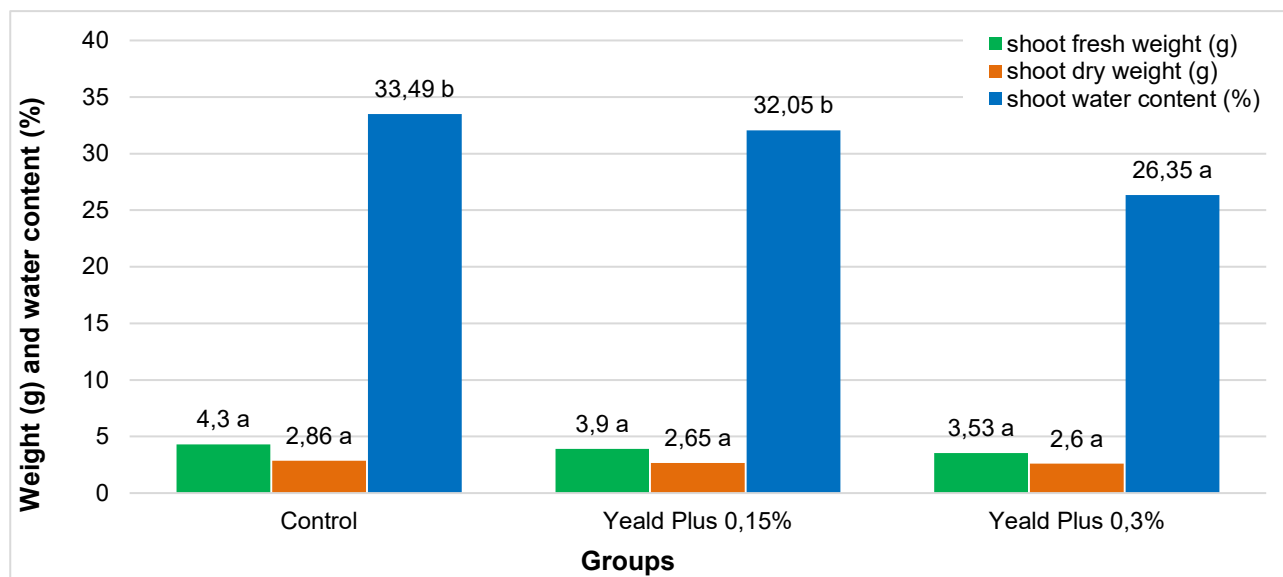
treatment	shoot fresh weight (g)	shoot dry weight (g)	shoot water content (%)
control	4.30 a	2.86 a	33.49 b
Yeald Plus (0.15%)	3.90 a	2.65 a	32.05 b
Yeald Plus (0.3%)	3.53 a	2.60 a	26.35 a

The average fresh shoot part weight of cuttings in the control group was 4.30 g, while their dry weight was 2.86 g. As a result of 0.15% Yeald Plus treatment, these values decreased to 3.90 g and 2.65 g, and a further reduction was observed at the 0.3% concentration (3.53 g and 2.60 g).

Water content of the shoot part was also declined in response to the treatments. The highest water content (33.49%) was measured in the control group, which decreased to 32.05% with the 0.15%, and to 26.35% at the 0.3% concentration (Fig. 3).

Root formation of cuttings did not occur during the experimental period, likely due to the cultivar's inherently slower rooting dynamics. Successful root induction may require a longer rooting

period or the application of an additional auxin treatment. The use of younger stock plants could also give better results, as the mother plants used in this experiment were 15 years old, probably overaged.



**Figure 3:** Fresh and dry weight, water content of the shoot part of *Chamaecyparis lawsoniana* 'Ellwoodii' cuttings under different treatments

#### 4. Conclusions and recommendations

Based on the results, foliar application of the Yeald Plus biostimulant had a positive effect on the rooting of *Thuja occidentalis* 'Smaragd'. Without treatment, no roots developed by the sixth week, while at a concentration of 0.15%, 64% of the cuttings formed roots. Higher (0.3%) dosage did not result in a significant improvement, and the observed reduction in root mass indicates that excessive dosage may exert an inhibitory effect. This phenomenon is likely associated with dose-dependent physiological responses and increased osmotic stress.

In both treated groups, the fresh and dry weight of the shoot part of the cuttings decreased, suggesting a redistribution of assimilates and nutrients towards root formation. The lower water content of treated plants may also reflect the initiation of root development and changes in water transport activity.

Cuttings of *Chamaecyparis lawsoniana* 'Ellwoodii' were not rooted during the six-week experimental period, even after Yeald Plus treatments. This may be attributed to a variety-specific rooting behaviour, higher temperature and humidity requirements, or the advanced age of the mother plants. It is also possible that the biostimulant alone was insufficient to trigger root initiation.

##### **Our recommendations are the followings.**

For *Thuja occidentalis* 'Smaragd', foliar application of 0.15% Yeald Plus once a week is recommended, as it provided the best rooting rate and root biomass.

The 0.3% concentration is not advised, as it offered no additional benefit and may negatively affect plant water balance.

For *Chamaecyparis lawsoniana* 'Ellwoodii', a longer rooting period, combined use of a low-dose auxin treatment (for example with the use of IBA or NAA), maintaining high humidity and optimal temperature, and the use of younger stock plants are suggested.

The effects of biostimulants are species- and/or cultivar-dependent; therefore, further repetitions, longer experimental periods and comparative studies with different types of biostimulants are required before broader implementation in ornamental nursery propagation practices.

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