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## Enhancing Rooting of Vegetatively Propagated Cannabis sativa 'BaOx' Cuttings

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## Enhancing Rooting of Vegetatively Propagated Cannabis sativa 'BaOx' Cuttings

### Cover Page Footnote

We would like to thank Broadway Hemp Co., Triangle Hemp, Galema's Greenhouse, and Fine Americas Inc. for contributing to this research.

## Introduction

Hemp (*Cannabis sativa* L.) has recently become legalized in the United States (U.S.) (Congress, 2014 & 2018). With this recent legislation, the growing of industrial hemp is now legal throughout the US. To grow hemp, often propagation occurs via asexual means through rooting vegetative tip cuttings. To produce plants, meristems are excised from stock plants and propagated to produce transplants for greenhouse or field-production. Successfully rooting vegetative cuttings can vary widely among growers due to greenhouse propagation environmental conditions, cultural inputs and practices, and vegetative cutting quality. To overcome rooting challenges, the impact of cutting stem caliper (diameter) and the use of root-zone heating (RZH) on rhizogenesis were investigated. Results indicated that large (2.9 – 3.2 mm) caliper cuttings and the use of RZH of 27.8°C resulted in a higher quality rooted cutting.

Hemp is often propagated vegetatively for cannabinoid (CBD) production given that seeds are highly dioecious (Harita, 1927). Dioecious seeds can result in sub-optimal conditions for flower production due to fertilization. To produce the largest flowers with high concentrations of CBD only female plants are grown. When propagating hemp vegetatively, uniformity in rooting and rooting success varies widely (Caplan, 2018; Coffman and Gentner, 1979). Cutting losses of up to 35% have been observed by the authors. Previous work reported that cutting location, leaf number per cutting, and the use of IBA (indolebutyric acid) can impact rooting success and vigor (Caplan, 2018). In asexually propagated crops, it is a common practice to standardize rooting uniformity and to utilize root-zone heating to hasten rhizogenesis (Janes et al., 1980; Sadhu, 1989). Additionally, cutting caliper (stem diameter) has been shown to impact rhizogenesis (Chaturvedi, 2001; Gehlot et al., 2015; Li et al., 2008). Thus, this study aimed to

determine the impacts of cutting stem caliper (CSC) and root-zone heating (RZH) on rhizogenesis of vegetatively propagated shoot-tip cuttings of hemp.

## **Experimental Outline**

### *Methods Used in Both Experiments*

For both experiments, Shoot-tip cuttings were excised from ‘BaOx’ hemp mother stock plants (Figure 1) and grouped by cutting caliper. Shoot-tips excised from secondary, tertiary, and quaternary branches had an average of 35.5 cuttings per mother stock plant. Each cutting with 4 nodes were excised 4 cm below the (second or third node), resulting in uniform unrooted shoot-tip cuttings with 1 fully developed leaf and a length of  $\approx 10$  cm. Upon obtaining cuttings and sorting by caliper size (see protocol in Experiment 1), the cuttings (8 cuttings per size) were inserted ( $\approx 1.54$  cm) into Ellepots (Esbjerg, DK) (H x W: 6.35 cm x 2.54 cm in 72 trays) and placed in a glass-glazed greenhouse with a propagation tent (white plastic suspended on a frame L x H x W: 2.44 m x 0.91 m x 1.52 m) to maintain humidity provided by a humidifier (Herrmidifier 707U: Trion; Sanford, NC) with an on/off frequency of one min. every 15 min.

### *Experiment 1: Shoot Caliper Study*

Upon obtaining shoot-tip cuttings from the ‘BaOx’ mother stock plants, the cutting’s stems were measured at the middle of the cutting ( $\approx 5$  cm from the proximal end of the cutting) with a vernier caliper (Mitutoyo, 530-104; Aurora, IL), and sorted according to size (in mm) small (1.7 – 2.0), medium (2.3 – 2.6), and large (2.9 – 3.2), respectively. Cuttings were then placed into their Ellepots and placed in the propagation unit as described above. All cuttings in

the shoot caliper study were subjected to no RZH and were rooted at ambient temperatures (25.6 °C).

### *Experiment 2: Root Zone Heating Study*

The RZH study consisted of a sub-set of the medium sized cuttings (8 cuttings for each treatment RZH verses No-RZH) which were subdivided into two treatments. RZH (Redi-Heat; Earth City, MO) was maintained at an average substrate temperature of 27.8 °C and without RZH with an average temperature of 25.6 °C. Data were collected 14 d after cuttings were placed under propagation conditions and transplants were assessed by root evaluation (indicative of rooting vigor with higher numbers representing a greater establishment of roots) and the percentage of transplants with visible roots on the outside or bottom of the Ellepot (Table 1).

At four weeks after sticking, transplants from both treatments were sorted into groups based on rooting quality to establish a subjective visual rating (Figure 2) scale from 0 to 5, with 0 indicating the least amount of rooting and 5 being the greatest amount for rooting vigor (Table 2). Data were analyzed using SAS (version 9.4; SAS Inst. Inc., Cary, NC) general model procedure (PROC GLM) and a *t*-tests were used to determine significant differences between means. Statistical significance indicated at  $P \leq 0.05$ .

## **Results**

### *Experiment 1:*

Large caliper (2.9 – 3.2 mm) cuttings resulted in the highest rooting percentage with an average root rating of 0.90 (Table 1 & Figure 3) compared to the smallest caliper (1.7 – 2.0 mm) cuttings with an average root rating of 0.73 (Table 1). However, no statistical differences in rooting vigor were determined between cuttings with large and medium caliper (2.3 – 2.6 mm). Small and medium calipers cuttings were statistically similar in rooting (Table 1).

### *Experiment 2:*

RZH increased rooting vigor after 28 days of cuttings with a medium shoot caliper by 70.0% (Table 2 & Figure 4) compared to the unheated control (Table 2). While rooting vigor was higher by four weeks under RZH, the use of RZH also hastened the initial root initiation and development beginning at 2 weeks after placement under propagation conditions compared to the unheated control (Table 2). After 28 d, all cuttings were rooted.

## **Conclusions**

Results from these trials indicate that cuttings with a larger shoot caliper establish more vigorously rooted hemp cuttings. It is speculated that the larger caliper cuttings may contain more carbohydrate reserves in the shoot and the roots create a greater source-sink relationship, resulting in higher rooting success (Gehlot et al., 2015; Li et al., 2008; Chaturvedi, 2001). The developmental rate of adventitious root initiation is temperature-dependent, and subsequent development of organized adventitious root initials gives rise to adventitious root formation, thus influencing rooting of cuttings (Owen, 2017). Similar to previous research (Owen, 2017), the use

of RZH resulted in more vigorously rooted cuttings. When these results are taken together, hemp shoot-tip cuttings with a large shoot caliper are preferred and the addition of RZH improved early root development as compared to the non-heated conditions. Utilizing these two factors will aid cannabis propagators in improving their rooting success and avoid plant losses.

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## Appendix



*Figure 1.* Architecture of a mother stock hemp (*Cannabis sativa* 'BaOx') plant. The red line indicates the primary stem, the orange is a secondary branch, the yellow indicates the tertiary branches, and the magenta shows quaternary branches.

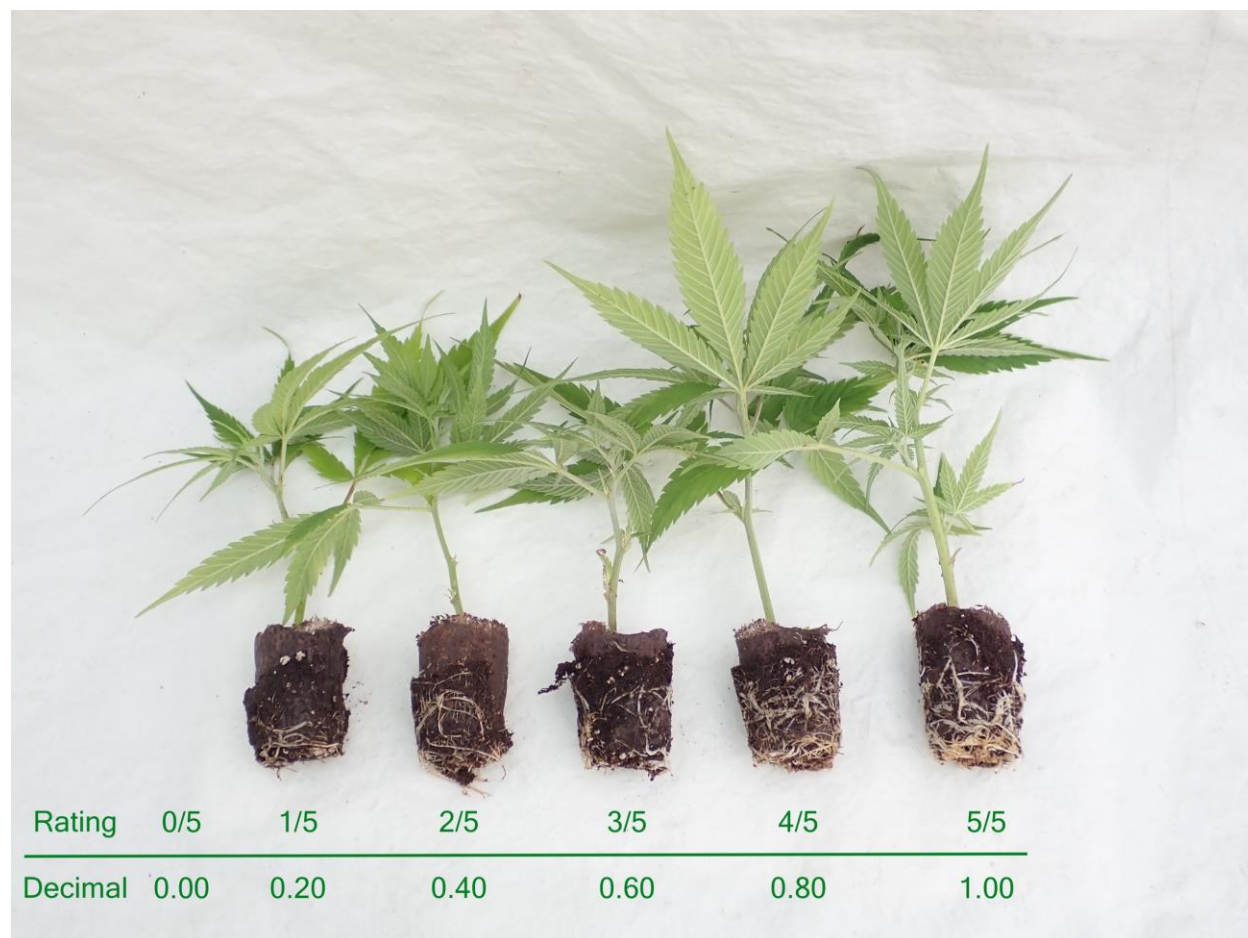
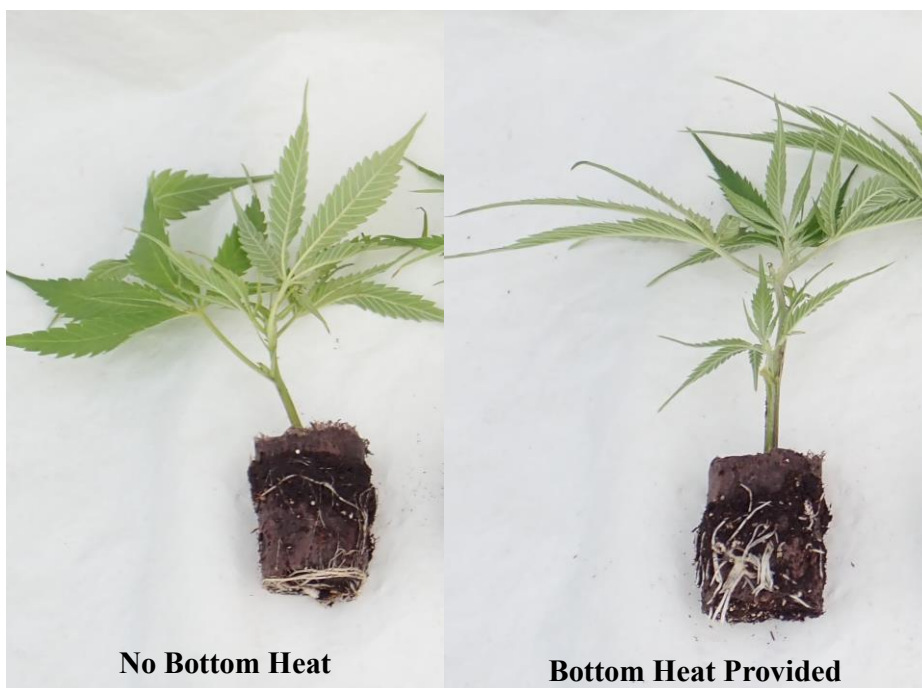


Figure 2. Root rating scale for both trials propagating hemp (*Cannabis sativa* L.) shoot-tip cuttings. The scale indicates rooting vigor on a subjective visual and decimal system.



*Figure 3.* Photo taken 28 d of rooting of small, medium, and large shoot caliper sized hemp (*Cannabis sativa* L.) cuttings. Results indicate an increase in rooting vigor in medium to large caliper shoot cuttings compared to small caliper cuttings.



*Figure 4.* Comparison of rooting vigor of hemp (*Cannabis sativa* L.) cuttings at 28 days after stick and placement under propagation conditions with root-zone heating (left) or unheated control (right).

Table 1

Rhizogenesis of Hemp (*Cannabis sativa* L. 'BaOx') Shoot-tip Cuttings, as Affected by Stem Caliper

	Caliper size (mm)		
	Small (1.7 – 2.0)	Medium (2.3 – 2.6)	Large (2.9 – 3.2)
Plugs Rooted (%) (14 d <sup>a</sup> )	60.0%	40.0%	40.0%
Root Rating (28 d <sup>b,c</sup> )	0.73*	0.77*	0.90*

*Note:* <sup>a</sup>Percent of transplants with visible roots on the outside or bottom of the Ellepot and their associated subjective evaluations.

<sup>b</sup>Root rating averages (n = 8) from rating scale expressed as a decimal (e.g. 2/5 rating = 0.40).

<sup>c</sup>Results with asterisks (\*) are statistically significant ( $P \leq 0.05$ ).

Table 2

Rooting of Hemp (*Cannabis sativa* L. ‘BaOx’) Shoot-tip Cuttings, as Affected by Root-zone Heating (RZH at 27.8 °C and No-RZH at 25.6 °C)

Treatment	RZH	No-RZH
Plugs Rooted (%) (14 d <sup>a</sup> )	66.7%	12.5%
Root rating (28 d <sup>b,c</sup> )	0.68 <sup>*</sup>	0.40 <sup>*</sup>

*Note:* <sup>a</sup>Percent of medium (2.3 – 2.6 mm) caliper transplants showing roots on the outside or bottom of the Ellepot and their associated subjective root evaluations.

<sup>b</sup>Root rating averages (n = 8) from rating scale expressed as a decimal (e.g. 2/5 rating = 0.40).

<sup>c</sup>Results with asterisks (\*) are statistically significant ( $P \leq 0.05$ ).