

## CHOPPER-LIGHT FOR SHOOT CULTURES

Pinker, I.M.

Department of Horticultural Sciences, Humboldt-University of Berlin

Wendenschloss Strasse 254

D-12557 Berlin

Germany

Tel: ++49/30/65 19 640 Fax: ++49/30/65 55 474 E-Mail: ina.pinker@agr.ar.hu-berlin.de

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### **Abstract**

Chopper-Light, a special form of intermittent light, was applied to shoot cultures of deciduous woody plants. Growth parameters such as multiplication rate and biomass production showed that these shoot cultures could be cultured with half or even a third of lighting energy without a decline of these essential parameters. Light conditions were compared between a control treatment with continuous light and the Chopper-Light treatment regarding photon flux density per pulse (40 and 80  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), the total light energy per day (4.6, 2.3 and 1.15  $\text{mol m}^{-2} \text{d}^{-1}$ ) and pulse sequences (20 ms light / 20 ms dark, 100 ms light / 100 ms dark, 20 ms light / 40 ms dark).

### **1. Introduction**

Among research reports on photosynthesis there are repeated indications that intermittent illumination by means of strobe light or rotating discs makes the utilisation of light more effective (Brown and Escombe, 1905, Sager and Giger, 1980, Hieke and Neef, 1989). Until now, large scale application of this knowledge was hampered by the unavailability of illumination systems.

Recently, the Chopper-Light-Technology has been developed by the Oellerich GmbH Berlin, Germany. This technology makes it possible to pulse fluorescent tubes in 20 ms intervals. With this technology the existing fluorescent fixtures in artificial illuminated growth rooms can be easily up-graded. So, it becomes possible to reduce the energy cost considerably, provided plants grow very well under these conditions.

So far no data are known on the response of *in vitro*-cultures to intermittent light. Therefore a long-term study was conducted using Chopper-Light on selected shoot cultures. Results are presented in this article.

### **2. Materials and methods**

#### **2.1. Conditions of the experiments**

Reactions of shoot cultures of *Prunus glandulosa* 'Alboplana' ('Alboplana'), *Prunus cerasifera* 'Nigra' ('Nigra'), *Tilia cordata* 'Wega' (Tilia) and *Amelanchier lamarckii* ('Amelanchier') were investigated under different Chopper-Light conditions (see below) in comparison to the common illumination (continuous control).

Cultures were maintained by transfer of about 10 mm newly formed shoots every 28<sup>th</sup> day on a modified MS-medium supplemented with 0.5 or 1.0 mg  $\Gamma^1$  N<sup>6</sup>-benzylaminopurine and 30 g  $\Gamma^1$  sucrose. Light was supplied with Osram fluorescent tubes (65/25) for 16 hours per day. Temperature was kept constantly at 22°C.

In each treatment, 30 shoots were used and each trial was repeated twice. After each subculture, multiplication rate, number of shoots, shoot length, fresh and dry weight were recorded.

Data were analysed statistically with variance analysis or the Wilcoxon test.

## **2.2. Conditions of experiment 1**

Chopper-Light had the same pulse intensity photosynthetic flux density (PPFD) as the control treatment (80 and 40  $\mu\text{mol m}^{-2} \text{s}^{-1}$  respectively) and half of daily energy supply (control: 4.6 or 2.3  $\text{mol m}^{-2} \text{d}^{-1}$  and Chopper-Light: 2.3 or 1.15  $\text{mol m}^{-2} \text{d}^{-1}$ ). The Chopper sequence was 100 ms light / 100 ms dark.

## **2.3. Conditions of experiment 2**

The daily energy supply in Chopper-Light and control was the same (2.3  $\text{mol m}^{-2} \text{d}^{-1}$ ) but the pulse intensity was twice as high as in the control (Chopper-Light 80  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and control 40  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) Chopper sequence was 100 ms light / 100 ms dark.

## **2.4. Conditions of experiment 3**

Different pulse sequences were compared (20 ms light / 20 ms dark, 100 ms light / 100 ms dark, 20 ms light 40 ms dark). Chopper-Light had the same pulse intensity as the control treatment (40  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) but only the half (1.15  $\text{mol m}^{-2} \text{d}^{-1}$ ) or one third (0.75  $\text{mol m}^{-2} \text{d}^{-1}$ ) of the daily energy supply of the control (2.3  $\text{mol m}^{-2} \text{d}^{-1}$ ).

# **3. Results and discussion**

## **3.1. Experiment 1**

At the same pulse intensity and high PPFD (80  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), the multiplication rate under Chopper-Light was just as good as in the control or considerably better (Fig. 1a). The fresh weight, however, did not always respond in the same manner (Fig. 1b). Despite the higher multiplication rate under Chopper-Light, the fresh weight was significantly lower in *Tilia* than in the control or no differences could be found in *Amelanchier* and 'Nigra'. With the multiplication rate being suppressed (Fig. 1a) and furthermore the chlorophyll bleaching in the control it is assumed that this PPFD level is too high for multiplication of *Amelanchier*, *Tilia* and 'Nigra' under continuous irradiation. Remarkably, under Chopper-Light this PPFD level had no inhibitory influences on the multiplication. These results obtained with a supraoptimal PPFD level indicate, that the light utilisation could be changed under Chopper-Light. Moreover, multiplication and biomass production seems to have other demands with respect to the light conditions. The second observation could not be made at the lower PPFD level that is more common in the commercial labs and is used in the following experiments, should be taken into account in further investigations concerning the action of Chopper-Light.

At a lower PPFD (40  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) the multiplication rate in 'Alboplena' was lower under Chopper-Light (Fig. 2a). But for the other three cultures the same multiplication rate could be achieved under Chopper and control treatment although the daily energy supply was reduced in Chopper-Light. Fresh weight was equal in both conditions (Fig. 2b). This supports the assumption that the PPFD level in continuous light should not exceed 40  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for *Amelanchier*, *Tilia* and 'Nigra' to realise a good multiplication rate. 'Alboplena', however, seems to demand or at least to tolerate higher PPFDs.

### 3.2. Experiment 2

This experiment was aimed to check if the reaction of cultures depends on the total daily energy supply or if the form of light application –continuous or chopped– had the superior effect. Three cultures had the same multiplication rate irrespective the form of light application (Fig. 3a), but ‘Alboplana’ responded under Copper-Light with a considerably reduced multiplication rate. Results for biomass production confirm this (Fig. 3b). Seemingly, the total daily energy supply is the main factor influencing shoot cultures of *Amelanchier*, *Tilia* and ‘Nigra’. The reaction of ‘Alboplana’, however, was different. This could be an indication for the importance of the application form of light at least under certain conditions. An additional experiment with a lower daily energy supply of  $1.15 \text{ mol m}^{-2} \text{ d}^{-1}$  (data not shown) gave further indications, that the application form of light can be very important for the light utilisation by *in vitro* plants.

### 3.3. Experiment 3

The following experiments were conducted at a pulse level of  $40 \mu\text{mol m}^{-2} \text{ s}^{-1}$  but half of daily energy supply with alternation of the pulse sequence. The cultures responded quite different in spite of the same total energy supply under a Chopper sequence of 20 ms light / 20 ms dark (Fig. 4) in comparison with a sequence of 100 ms light / 100 ms dark (Fig. 2a). Because these both experiments did not run simultaneously it is only possible to compare the relation of control and Chopper treatment of the same experiment and not the absolute values of the multiplication rate between both experiments. ‘Alboplana’ reached with shorter sequences (Fig. 4) the same multiplication rate under Chopper-Light as under continuous one. *Tilia* and *Amelanchier* contradictionally had a better reaction with longer sequences than with shorter ones (Fig. 2a and 4). Only ‘Nigra’ did not response to the different sequences of light application. Results shown in Fig. 4 compared with those given in Fig. 2a confirm the assumption that the pulse sequence may have a stronger impact than the total energy supply.

In the next experiment the light / dark-regime was changed (20 ms light / 40 ms dark). So the total lighting period was reduced to a third. Despite the lower energy supply ‘Alboplana’ and *Tilia* had the same multiplication under Chopper-Light and in the control (Fig. 5). This is an unexpected result considering the foregoing investigations leading to the conclusion that ‘Alboplana’ demands a higher light supply while *Tilia* prefers lower PPFD for good multiplication rates (see experiment 1).

Apparently, there is only one publication referring to the influence of the timing in light/dark regime (Hieke and Neef, 1998). They found in chloroplast suspensions the highest light utilisation efficiency with pulse sequences of 10 ms light interrupted by 150 ms dark periods. Most of the investigations, however, were conducted with the equal time interval for light and dark period (Sager and Giger, 1980, Tennessen *et al.*, 1995). Our results underline the importance of light/dark-regime. Further experiments are necessary to investigate the optimal light/dark sequence *in vitro*.

## 4. Conclusions

Shoot cultures can be grown under Chopper-Light for a period of more than 18 months without any impairment of the cultures. Chopper-Light is therefore a useful tool for reducing lighting time to half or even a third. The calculation of exact figures for the reduction of energy input by Chopper-Light based on these initial experiments is impossible and requires investigations under large scale production. But we know that a Chopper sequence of 100 light / 100 ms dark did not take the expected 50 % energy input for lighting but only 44.15 % while 20 ms light /20 ms dark needs even 30.61 % of the control (Oellerich, personal communication).

The interpretation of our results concerning the impact of Chopper-Light on the development of *in vitro* cultures becomes difficult because the utilisation of light seems to be quite different under Chopper conditions and morphogenetic parameters like shoot

development respond in an other way than biomass production for example. Additionally each culture investigated demanded special conditions to achieve the maximal economy effect. The PPFD could be the same at  $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ , but the Chopper sequence for 'Alboplana' should be 20 ms light / 40 ms dark, for Amelanchier and Tilia, however, 100 ms light / 100 ms dark. 'Nigra' did not react so strong on the Chopper sequence. But from the results presented here it seems to be very important to investigate intensively the relation of light and dark periods at least for tissue cultures.

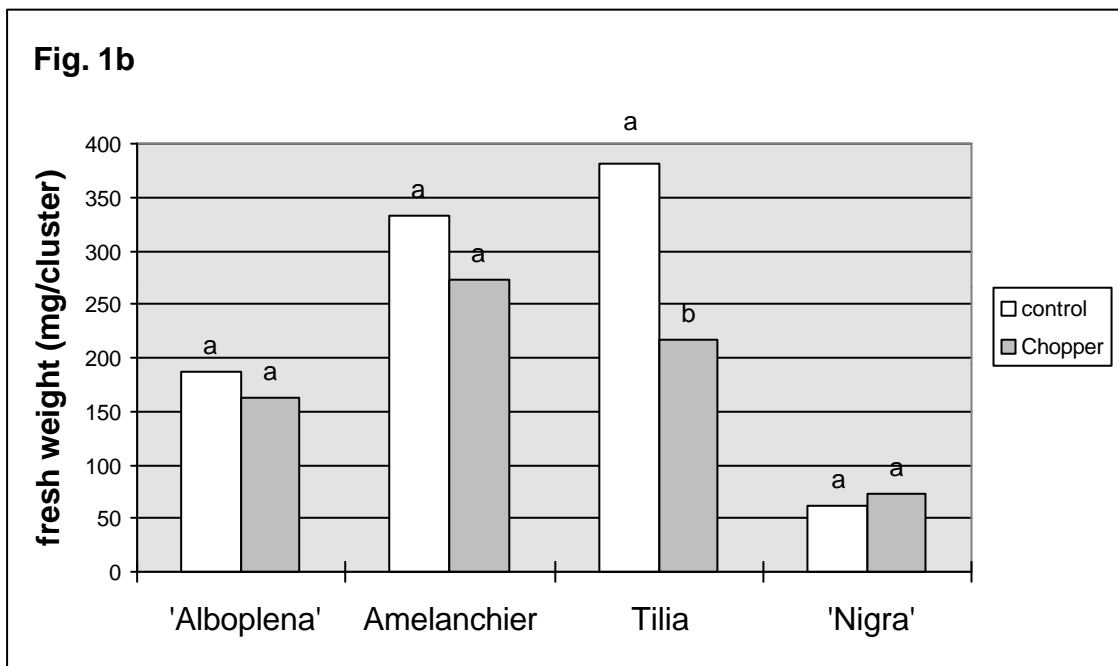
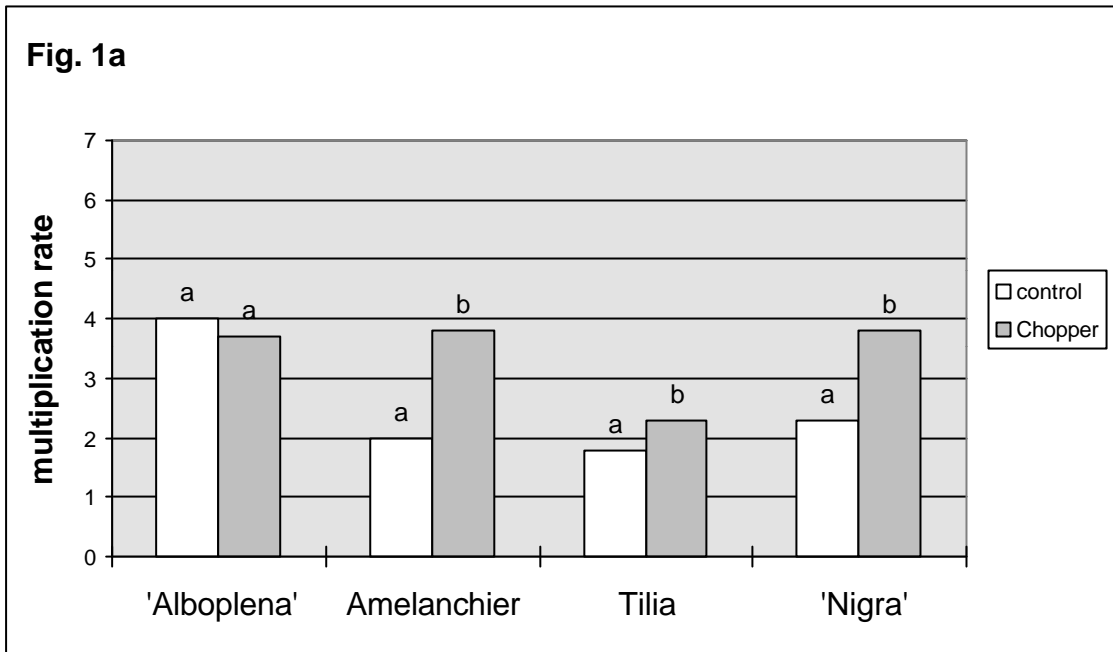
Apparently, Chopper-Light cannot be used only to reduce the energy cost but also as a tool to learn how the intermittent light influence the plant development.

### **Acknowledgements**

We are grateful for the support of the Oellerich GmbH for Chopper-Light Technique.

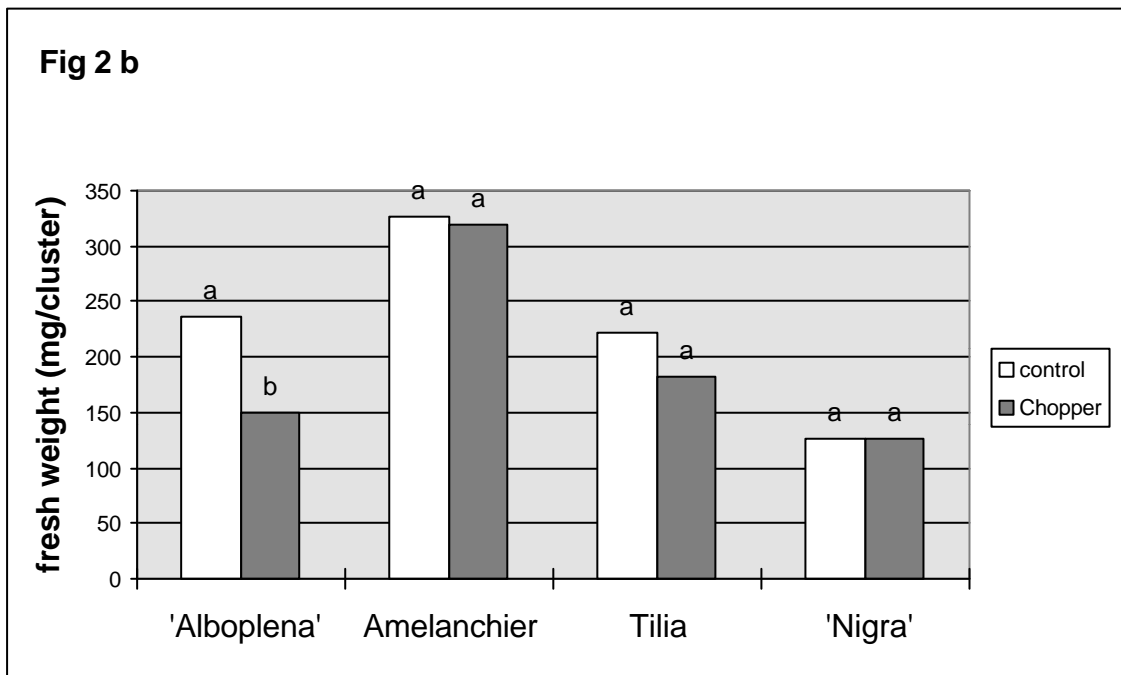
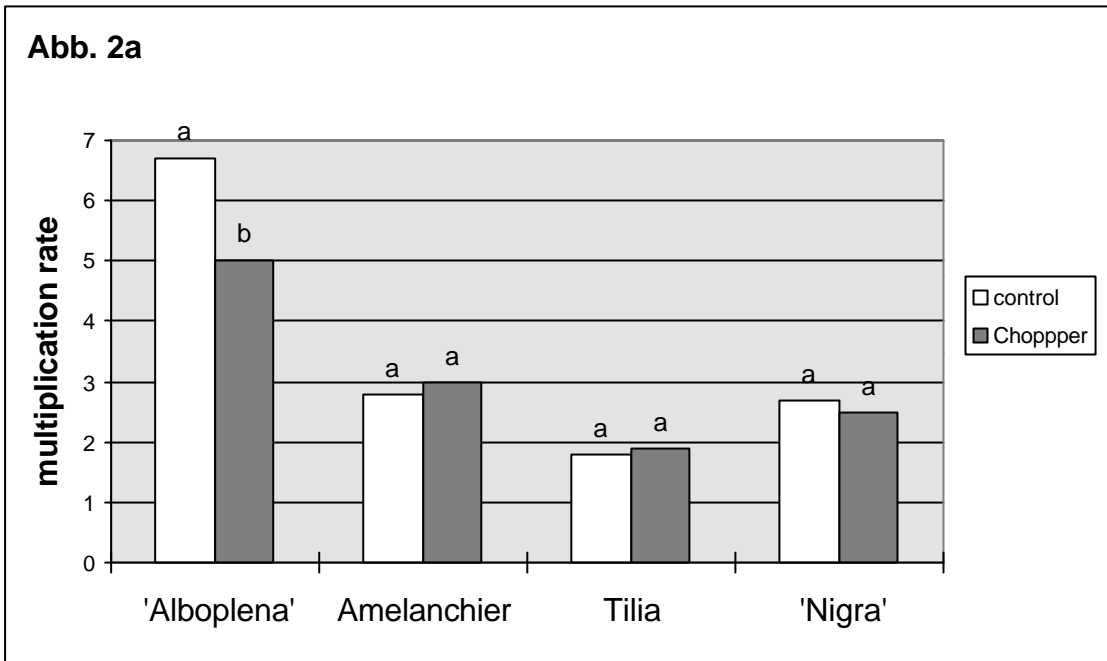
### **References**

- Brown, H.T., Escombe, F.T.. Research on some of the physiological processes of green leaves, with special reference to the interchange of energy between the leaf and the surrounding. Proc. R. Ser. B., 76: 29-111, The Royal Society, London, 1905
- Hieke, B., Neef, E. 1989. The rate-limiting step of DCPIP photoreduction by isolated chloroplasts of different plant species, characterized by measurements under intermittent irradiation with variable flash and dark interval. Photosynthetica, 23 (4): 524-536
- Sager, J.S., Giger, W. 1980. Re-evaluation of published data on the relative photosynthetic efficiency of intermittent and continuous light. Agr. Meteorology, 22: 289-302
- Tennessen, D.J., Bula, R.J., Sharkley, T.D. 1995. Efficiency of photosynthesis in continuous and pulsed light emitting diode irradiation. Photos. Res., 44: 261-269



Different letters indicate the significant differences, P=0.05.

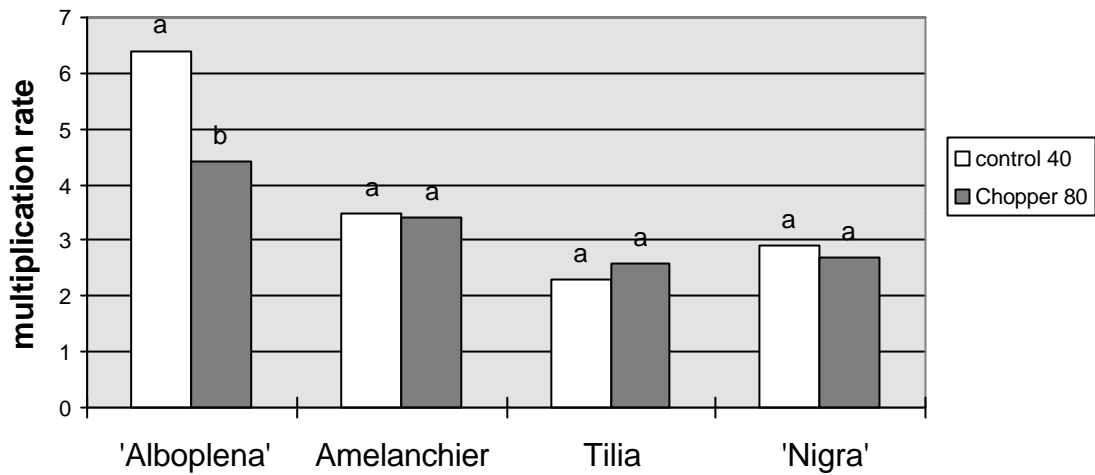
1. Influence of Chopper-Light with the same pulse intensity like control on shoot multiplication (1a) and biomass production (1b) of various shoot cultures at high PPFD. PPFD:  $80 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; Chopper sequence: 100 ms light / 100 ms dark; daily energy supply: Control:  $4.6 \text{ mol m}^{-2} \text{d}^{-1}$ , Chopper:  $2.3 \text{ mol m}^{-2} \text{d}^{-1}$



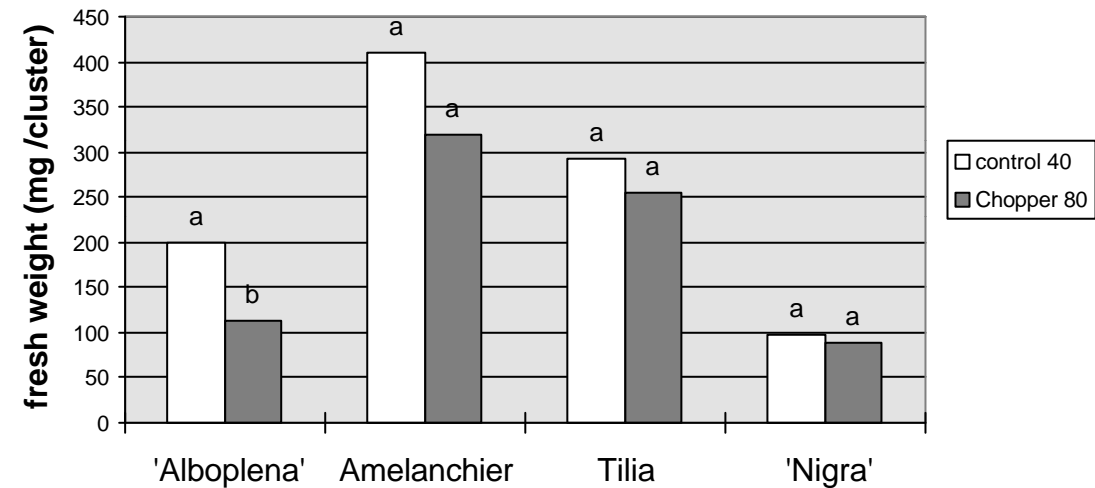
Different letters indicate significant difference,  $P=0.05$ .

- Influence of Chopper-Light with the same pulse intensity like control on shoot multiplication (2a) and biomass production (2b) of various shoot cultures. PPF<sub>D</sub>:  $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; Chopper sequence: 100 ms light / 100 ms dark; daily energy supply: control:  $2.3 \text{ mol m}^{-2} \text{d}^{-1}$ , Chopper:  $1.15 \text{ mol m}^{-2} \text{d}^{-1}$

**Fig. 3a**

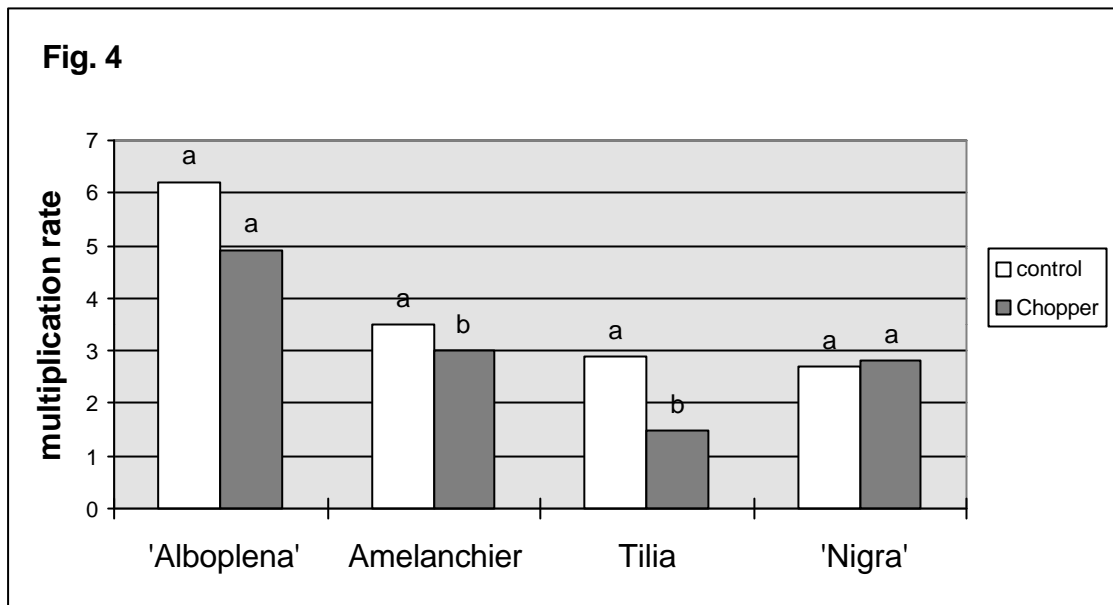


**Fig. 3b**



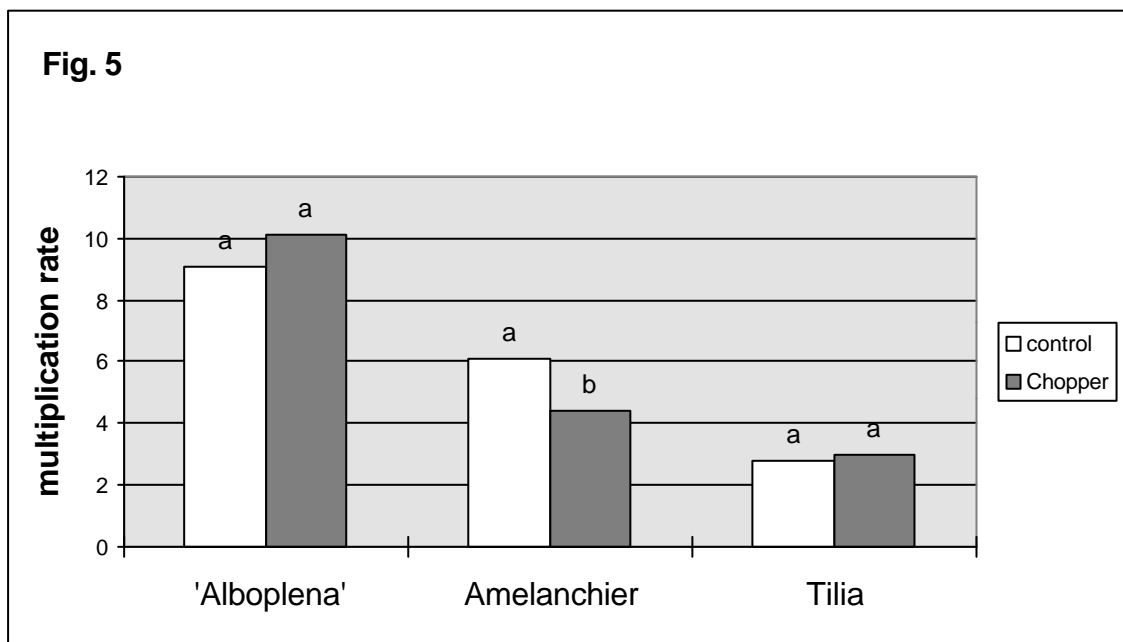
Different letters indicate significant difference, P=0.05.

3. Influence of Chopper-Light with the same daily energy supply like control on shoot multiplication (3a) and biomass production (3b) of various shoot cultures. PPFD: control  $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ , Chopper  $80 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; Chopper sequence: 100 ms light / 100 ms dark; daily energy supply:  $2.3 \text{ mol m}^{-2} \text{d}^{-1}$



Different letters indicate significant difference, P=0.05.

4. Influence of Chopper-Light with a shorter Chopper sequence on shoot multiplication of various shoot cultures. PPFD:  $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; Chopper sequence: 20 ms light / 20 ms dark; daily energy supply: control  $2.3 \text{ mol m}^{-2} \text{d}^{-1}$ , Chopper  $1.15 \text{ mol m}^{-2} \text{d}^{-1}$



Different letters indicate significant difference, P=0.05.

5. Influence of Chopper-Light with changed light/dark interval on shoot multiplication of various shoot cultures. PPFD:  $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; Chopper sequence: 20 ms light / 40 ms dark; daily energy supply: control  $2.3 \text{ mol m}^{-2} \text{d}^{-1}$ , Chopper  $0.75 \text{ mol m}^{-2} \text{d}^{-1}$