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THE EFFECT OF pH, KINETIN AND SUCROSE ON THE BRANCHING PATTERN IN LEMNA TRISULCA L.

INTRODUCTION

The inequality in size between daughter fronds, originating from the same mother frond, is one of the known features of members of subfam. Lemnoideae. It is present even in a very early of development (Negelmaier, 1868; Blodget, 1915; Lawalrée, 1943, 1945; Nillman, 1961). This inequality is very conspicuous in *Lemna trisulca* L. the smaller fronds of which may be completely inhibited, resulting in spiral-shaped colonies. Goebel (1921) found that in low intensity light colonies are spiral, whereas in light of higher intensity they are branched on both sides. The presence of sucrose is also necessary for branching of colonies, as in its absence colonies remain spiral (Goebel, 1921; Landolt, 1957; Zurzycki, 1957; Guern, 1965).

Guern (1965) showed the presence of correlative inhibition between the dominant, left-side and the inhibited right-side fronds. He also found that inhibited fronds can be released by sucrose, cytokinins and gibberellins added to the culture medium.

We have also studied the development of assymetric colonies in a solution without sucrose and noticed that cytokinins had a remarkable effect on branching. We have also found that the branching pattern was influenced by pH of the medium. These results, concerning the effect of sucrose, kinetin and pH on the branching are described in the present paper.

MATERIAL AND METHODS

Lemna trisulca L. was isolated from a local pond near Beograd and axenic cultures established as previously described (Bata and Nešković, 1974). The cultures have been routinely grown in modified Bonner-Devirian solution (Gupta and Maheshwari, 1968), 1/2 Hutner solution (Hillman, 1969) and Hoagland solution (Esashi

and O d a, 1964), containing 1% sucrose. Growth substances, IAA, GA_3 or kinetin were added before autoclaving, pH was adjusted to 5,4—8,0, using 1,0 KOH. At the higher pH the same precipitate was formed in the medium.

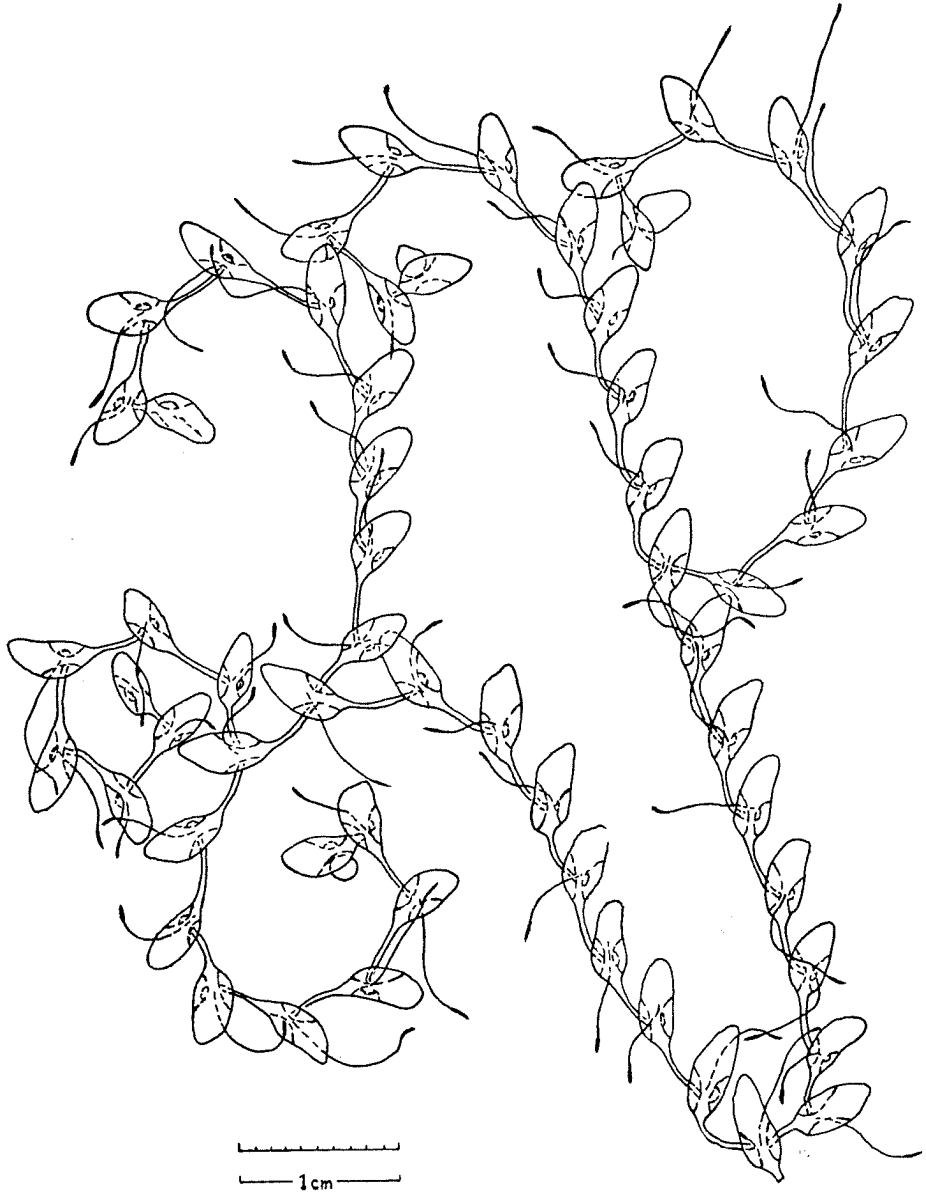

Fig. 1. — Colony aged 153 days, grown in liquid modified Hoagland's medium, without sucrose, at pH 5,4—5,6.

Kolonija stara 153 dana, rasla u tečnom modifikovanom Hoagland-ovom medijumu bez saharoze, na pH 5,4—5,6.

Colonies were cultivated in 100 ml erlenmayer flasks, containing 50 ml of nutritive solution, and kept in diffuse,white fluorescent light of 550 lux at the plant level. The lenght of day was 16 hours. Day temperature was 26°C, night temperature 22°C.

The inoculation of cultures was performed by transfering a three-membered colony mother frond and two daughter fronds, emerging from the pockets.

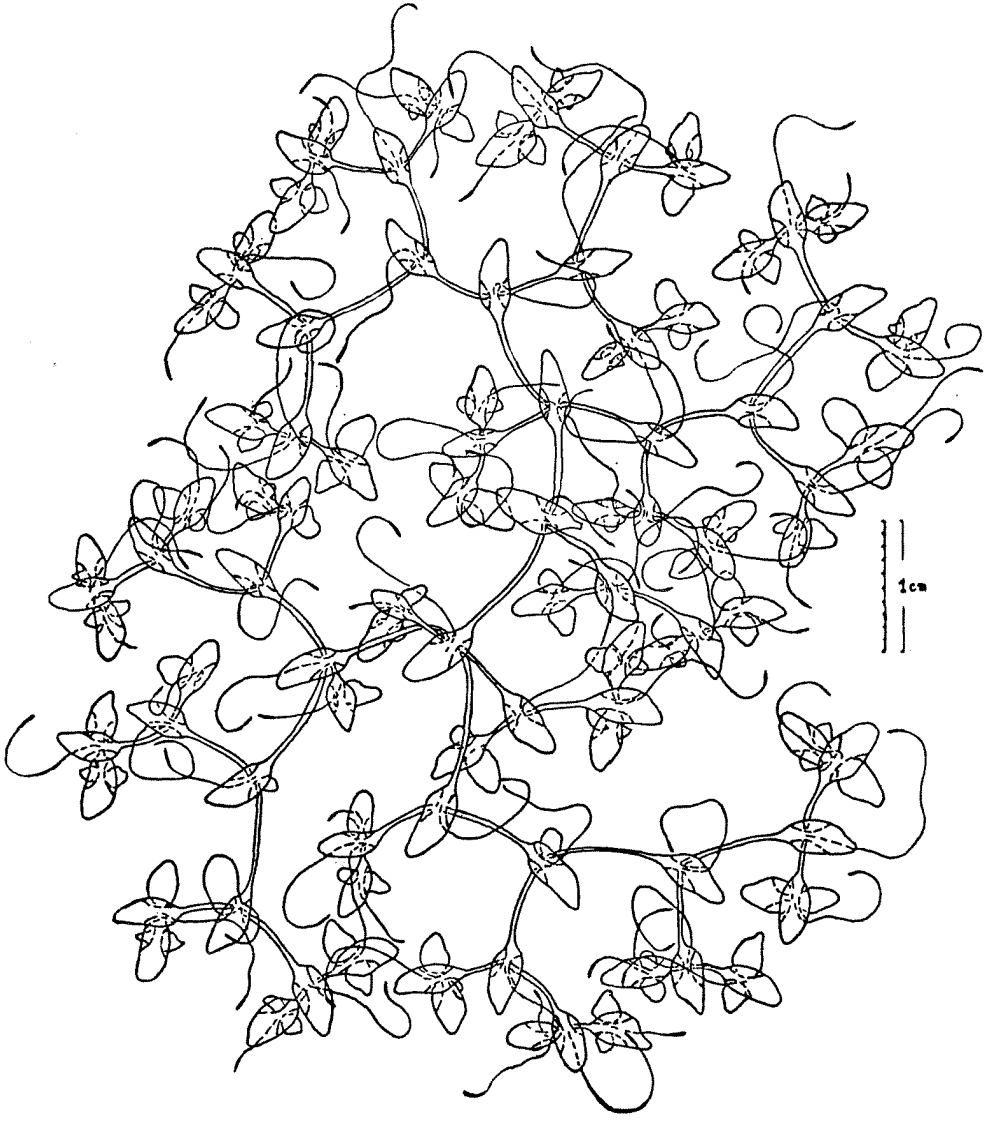

Fig. 2. — Colony aged 36 days, grown on liquid modified Hoagland's medium, supplement by 1% sucrose, at pH 5,4—5,6.

Kolonija stara 36 dana, rasla u tečnom modifikovanom Hoagland-ovom medijumu sa 1% saharozom i pH 5,4—5,6.

RESULTS AND DISCUSSION

L. trisulca, cultivated in a medium without sucrose, at pH 5,4—5,6 develops only assymetric colonies, since the domination of the left-side fronds is complete. Right-side fronds do not emerge from their pockets (Fig. 1). Occasionally in older cultures a right-side frond may develop, but the colony remains essentially assymetric. Colonies were pale, slow-growing and could be kept for months without formation of anthocyanins and other signs of ageing. The same aspect was also obtained in other mineral solutions (1/2 strength Hutner or Hoagland).

The addition of sucrose caused the opposite effect, namely the branching of colonies on both sides, although daughter fronds were not equal (Fig. 2). The growing of colonies was much faster, the loss of chlorophyll and the appearance of anthocyanin occurred after 2—3 weeks.

Kinetin was applied in conc. 0,001, 0,01, 0,1 and 1,0 mg/1, but only the highest conc. (1,0 mg/1) was effective. In cultures without sucrose, kinetin strongly stimulated the growth of right-side fronds and produced normally branched colonies (Fig. 3). The inequality in size was still present. In aged cultures younger fronds tended to be assymetric.

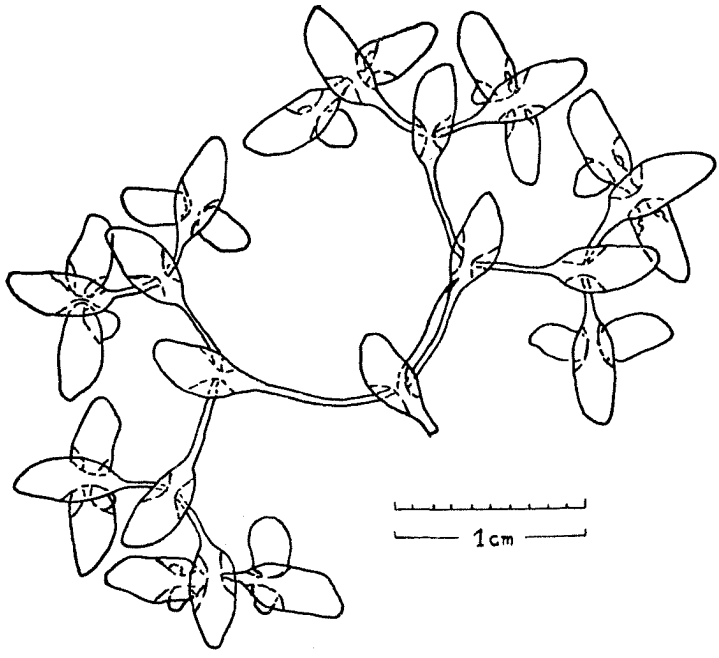

Fig. 3. — Colony aged 50 days, grown on modified Bonner-Devirian liquid medium, without sucrose, supplement by 1,0 mg/l kinetin, at pH 5,4—5,6.

Kolonija stara 50 dana, rasla u tečnom modifikovanom Bonner-Devirian medijumu bez saharoze sa 1,0 mg/l kinetina i pH 5,4—5,6.

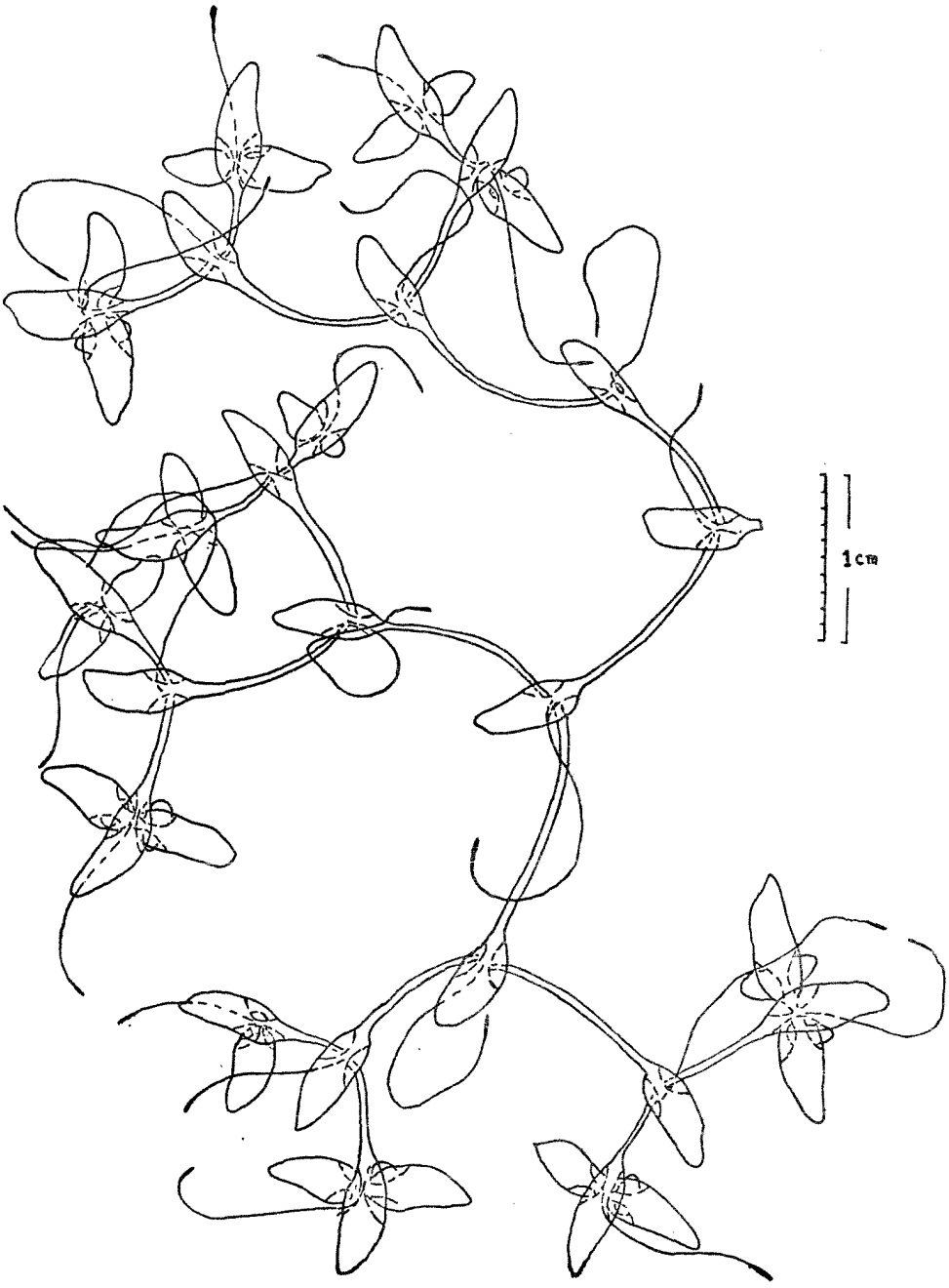

Fig. 4. — Colony anged 25 days, grown on modified Bonner-Devirian liquid medium without sucrose, at pH 6,0.

Kolonija stara 25 dana, rasla u tečnom modifikovanom Bonner-Devirian medijumu bez saharoze, na pH 6,0.

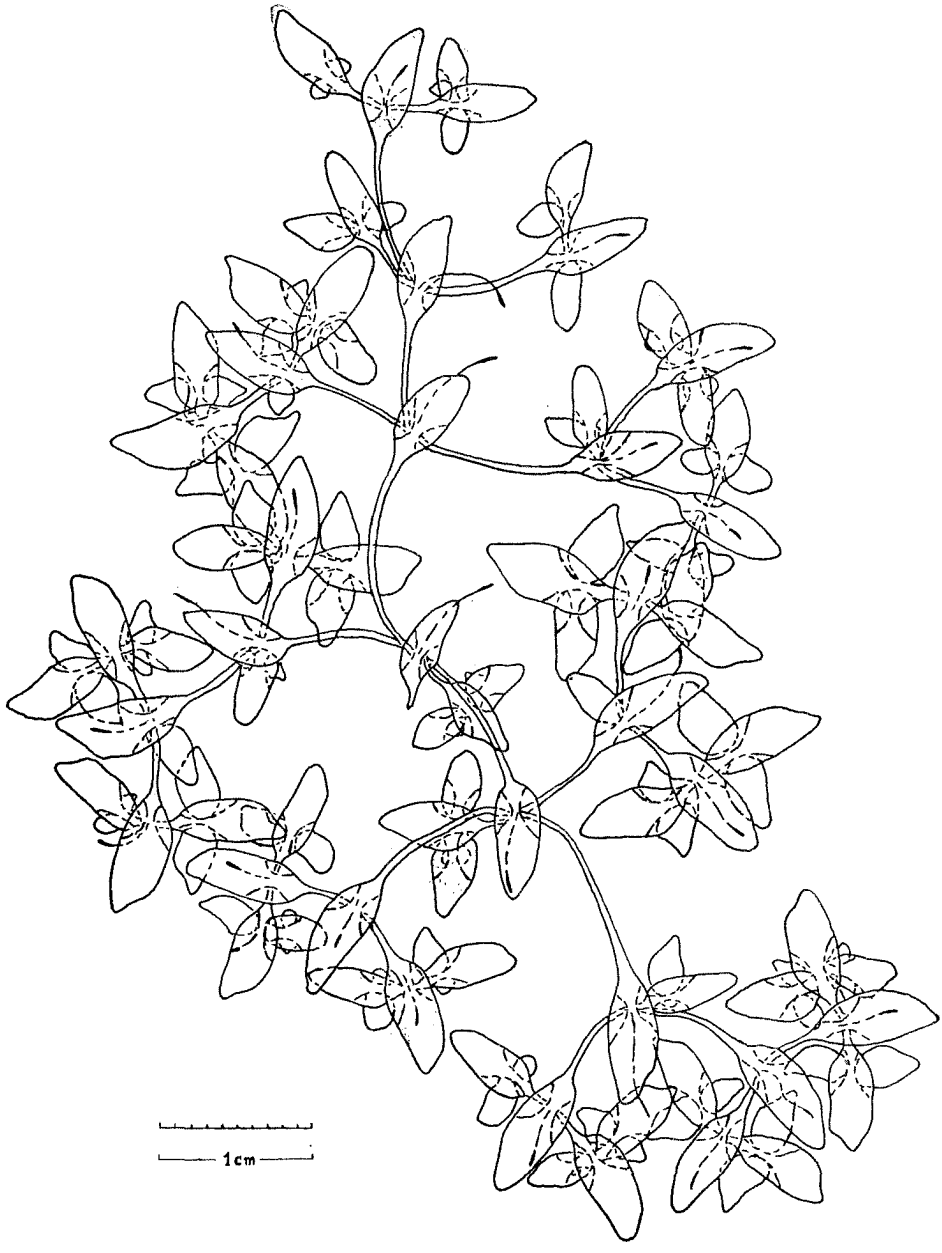

Fig. 5. — Colony aged 26 days, grown on mod.fied Bonner-Devirian liquid medium without sucrose, supplement by 1,0 mg/l kinetin, at pH 6,6.

Kolonija stara 26 dana, rasla u tečnom modifikovanom Bonner-Devirian medijumu bez saharoze sa 1,0 mg/l kinetina i pH 6,6.

Colonies were less green from those with sucrose. The growth of roots was markedly inhibited. IAA and GA_3 had some influence on growth, which will be described elsewhere, but do not affect the branching pattern.

The increase in pH had, however, remarkable effect on branching, which was apparent already at pH 6,0, even in the absence of sucrose (Fig. 4). Colonies were green and well developed. The size of fronds, particularly younger ones, was increased and reached 8,0—9,0 mm, compared to 6,0—7,0 mm at pH 5,4—5,6. Rots were also long.

At pH 6,6 and without sucrose, the addition of kinetin caused further stimulation of branching. Axillary fronds from both pockets also developed, the left-side pocket sometimes gave three daughter fronds in older cultures. Fronds were large in size, with long petioles, while roots were short (Fig. 5).

Guern (1965) has shown that both trophic and hormonal factors are involved in the correlative inhibition between daughter fronds in *Lemna trisulca.* Our results are in good accordance with these data. The development of right-side fronds canbe released by sucrose, as well as by kinetin. It does not seem likely that the presence of sucrose enables the colony to synthesize cytokinins, since cytokinins have some other effects, as the inhibition of roots, which are not produced by sucrose alone. The fact that high pH can produce the same effect as sucrose or kinetin cannot be explained at the moment. It is possible that at higher pH the plant becomes capable of synthesizing some factor necessary for branching. Whether this factor is of trophic or hormonal nature is the subject of further research.

SUMMARY

The branching pattern in *Lemna trisulca* L. is dependent on environmental conditions. In the of low intensity the effect of sugars, growth hormones and pH has been investigated. In the medium without sucrose, the colonies are spiral in shape, sine only the left-side fronds develop. Kinetin (1,0 mg/1) induces branching on both sides under the same conditions, while IAA and GA_3 are ineffective. The dominance of the left-side frond is also markedly reduced by higher pH of the medium (6,0—8,0). The addition of kinetin or the increase of pH in the presence of sucrose brings about further stimulation of branching, and 2—3 fronds are frequently observed in the same pocket.

REFERENCES

Bata, J. and Nešković, M. (1974): The effect of gibberellic acid and kinetin on chlorophyll retention in *Lemna trisulca* L. — Z. Pflazenphysiol.*73:* 66—88.

Blodget, F. H. (1915): Morphology of the *Lemna* frond. — Bot. Gaz. *60:* 383—390.

Esashi, Y. and Oda, Y. (1964): Effects of light intensity and sucrose on the flowering of *Lemna perpusilla.* — Plant and Cell Physiol. *5:* 513—516.

Goebel, K. (1921): Zur Organographie die Lemnaceen. — Flora *114:* 278—305.
Guern, J. (1965): Correlation de croissance entre fronds chez Lemnacees. — Ann. Sc. Nat. Bot., Paris, *12*: 1—156.
Gupta, S. and Maheshwari, S. C. (1969): Induction of flowering by cytokinins in a short day plant, *Lemna paucicostata.* — Plant Cell Physiol., Jap., *10:* 231—233.
Hegelmaier, F. (1968): Die Lemnaceae. Eine monographische Untersuchungen. — 169 p. Wilhelm Engelman, Leipzig.
Hiilman, W. S. (1961): The Lemnaceae. . Bot. Rev. *27:* 221—287.
Hillman, W. S. (1969): *Lemna perpusilla* Torr, strain 6746. — L. T. Evans: The induction of flowering, 186—204.
Landolt, E. (1957): Physiologische und ekologische Untersuchungen an Lemnaceen. — Ber. Schweiz. Bot. Ges. *67:* 271—410.
Lawalrée, A. (1943): La multiplication vegetative des Lemnacees, en particulier chez *Wolffia arrhiza.* La Cellule *49: 335—387.*
Lawalrée, A. (1945): La position systematique des Lemnacees et leur classification. — Bull. Soc. Roy. Bot. Belg. *77:* 27—38.
Lawalrée, A. (1952): L' embriologie des Lemnacees. Observation sur *Lemna minor* L. — Cellule *54:* 303—326.
Zurzycki, J. (1957): Formative effects of various spectral regions of light on *Lemna trisulca* L. — Med. Land. Wageningen *57:* 1—14.

Rezime

JOVANKA BATA

UTICAJ pH, KINETINA I SAHAROSE NA NAČIN GRANANJA LEMNA TRISULCA L.

Nejednakost u veličini kćeri frondova poreklom od iste majke, karakteristična za članove podfam. Lemnoideae, veoma je izražena kod *Lemna trisulca* L., naročito u zavisnosti od uslova pod kojima se razvija. Dominacija jednog fronda može da bude tako velika da se dobijaju asimetrične spiralnog izgleda kolonije za razliku od normalnih, koje se granaju sa obe strane. Poznato je da ovu pojavu dominacije jednog fronda redukuju 3 faktora: svetlost visokog intenziteta, šećeri i kinetin.

U ovom radu je ispitivan uticaj izvesnih faktora na način grananja kolonija. Pod uslovima slabog (nedovoljnog) osvetlenja, u otsustvu saharoze i pri pH 5,4—5,6 *L. trisulca* L. razvija izrazito asimetrične kolonije. Pod istim uslovima kultivisanja, aplikacijom materija rastenja (IAA, GA_3 i kinetina) pokazalo se da je samo kinetin u konc. 1,0 mg/1 imao izraziti efekat na grananje i redukciju dominacije. Zabeležen je isti efekat sa povećanjem pH medijuma od 6,0—8,0. Aplikacija kinetina ili povećanje pH medijuma u prisustvu saharoze izaziva stimulaciju grananja i čestu pojavu 2—3 fronda iz jednog džepa.