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SOME ASPECTS OF FLOWERING, GIBBOSITY AND TURION FORMATION IN LEMNACEAE

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SUMMARY

Literature on Lemnaceae is reviewed with regard to experimental control of flowering, gibbosity and turion formation by chelating agents, metal ions and growth regulators. Possible mechanisms by which chelating agents and metal ions influence flowering are discussed.

1. INTRODUCTION

The Lemnaceae (Duckweeds) are excellent experimental materials for morphological, physiological, and biochemical research on higher plants due to rapid growth, small size and structural simplicity. Each plant, usually called a frond, produces a daughter frond within three to four days. The plants can be grown aseptically in small flasks and maintained under controlled environmental conditions on a defined medium. The reproduction is usually vegetative, and therefore a single clone can be used for all experiments.

In nature, however, the Lemnaceae are harmful in certain areas because of their excessive growth. They frequently block the grids of small domestic dams and are troublesome amongst irrigated tropical crops. In particular rice fields are often completely covered by various species of duckweeds.

Comprehensive monographs on the morphology and systematics of the Lemnaceae have been published by HEGELMAIER (1868), SCHULZ (1962), DAUBS (1965) and DEN HARTOG & VAN DER PLAS (1970), while a very extensive review of the descriptive and experimental literature was written by HILLMAN (1961d).

The family is divided by den Hartog and van der Plas into two subfamilies, Lemnoideae with genera *Spirodela* and *Lemna*, the latter with two subgenera, *Lemna* and *Staurogeton*, and Wolffioideae with the genera *Wolffia*, *Wolffiella*, *Wolffiopsis* and *Pseudowolffia*. The fronds of the Lemnoideae are flat, more or less oval in outline and leaf-like. *Spirodela* has two or more thread-like roots on each frond, while *Lemna* has only one. The Wolffioideae are thalloid and have no roots. The Lemnoideae produce daughter fronds from two pockets, one on each side of the mother frond. Each daughter frond undergoes vegetative reproduction when still attached to its mother frond. The fronds of the Wolffioideae have only one reproductive pocket. In *Wolffiella*, mother and several generations of daughter fronds may be attached for a considerably long time,

thus forming colonies. In most species of the Lemnaceae flowering is extremely rare. In one species, *Wolfiella gladiata*, flowering has never been reported. The flower arises from one of the pockets in the Lemnoideae. Each flower consists of one pistil and two stamens. During early development these organs are surrounded by a membranous spathe. The flowers of the Wolffioideae differ from those in the other subfamily in having a single stamen and lacking a spathe. The flower in the Wolffioideae arises from the upper side of a frond.

During the last ten years extensive research has been carried out on the physiology and biochemical aspects of the Lemnaceae. The object of the present paper is to review some literature on morphological aspects and physiology of flowering in Lemnaceae with special reference to chelating agents. This review has four parts. The first part describes experimental control of flowering in Lemnaceae by chelating agents and metal ions, the second section summarizes the effects of growth regulators on flowering in Lemnaceae, the third discusses the gibbosity in *Lemna gibba*, and the last section gives a brief summary on turion formation in Lemnaceae.

2. EXPERIMENTAL CONTROL OF FLOWERING IN LEMNACEAE BY CHELATING AGENTS

Control of flowering in Lemnaceae was first reported by KANDELER (1955) who showed that several strains of *Lemna gibba* behaved as long-day plants under certain conditions. Flowering in long days under white fluorescent light occurred only in aged media, i.e., media in which the plants had grown for more than two weeks. Flowering could not be induced under any photoperiod if the medium was changed every week.

HILLMAN (1961a, b), working with *Lemna gibba* strain G3, confirmed the results of Kandeler. Moreover, he discovered that addition of EDTA (ethylenediaminetetraacetic acid) to fresh media enabled *Lemna gibba* G3 to flower as a long-day plant, thus imitating the effect of aged medium. Hillman suggested that the effect of aged medium on flowering is perhaps caused by natural chelating agents produced by plants.

A conditional short-day response was discovered by HILLMAN (1959a, b) in *Lemna perpusilla* 6746. This plant was day-neutral when grown in a Hoagland type medium, but the flowering under long days was inhibited when EDTA was added to the medium.

HILLMAN (1962) showed that greater purification of the macronutrient salts in a modified Hoagland's medium (M medium) had the same effect as adding EDTA, i.e., inducing of flowering under long days in *Lemna gibba* G3 and changing *Lemna perpusilla* 6746 from a day-neutral to a short-day plant. Low concentrations of cupric ions (2 μM/liter) added to purified medium reversed this effect while Cd^{++}, Co^{++}, Cr^{+++}, Hg^{++}, Mn^{++}, Ni^{++}, Pb^{++}, and Zn^{++} ions were not effective. It was not possible for HILLMAN (1962) to test the effect of increased iron concentration in M medium because this led to precipitation.

An unexpected phenomenon was encountered by HILLMAN (1961a, b, c) with regard to the flowering in *Lemna gibba* G3. This plant rarely flowered in Hutner's medium (HUTNER 1953) which contains EDTA. *Lemna perpusilla* 6746, which behaved as a short-day plant in 0.8 strength Hutner's medium containing a high concentration of EDTA, exhibited less flowering when the iron content of the medium was reduced form 6 mg/l to 0.6 mg/l although the iron concentration was still sufficiently high not to impair vegetative growth (HILLMAN 1961a). It was further suggested that copper may block the reversible phytochrome system possibly accomplishing this effect by interfering with iron metabolism (HILLMAN 1962). Iron has been shown to influence flowering in *Xanthium pennsylvanicum* (SMITH et al. 1957).

Lemna perpusilla behaved as a short-day plant in the presence of EDTA in M medium (modified Hoagland's) or Hutner's medium. *Lemna gibba* G3, however, behaved as a long-day plant in M medium with EDTA, but rarely flowered in Hutner's medium. Thus, EDTA's effect on *Lemna gibba* G3 depended upon whether the medium was M or Hutner's. Hutner's medium contains EDTA and high concentrations of micronutrients as compared to M medium.

ODA (1962) was unable to confirm Hillman's report that the photoperiodic response of *Lemna perpusilla* 6746 is affected by the presence of EDTA in M medium. In Oda's cultures this strain behaved as a typical short-day plant under white fluorescent light even in the absence of EDTA. In red or green light a short-day response was observed, while in blue or far-red light the plant was day-length indifferent; in no case was the response modified by adding EDTA to the medium. Further doubts about the effects of EDTA were raised by UMEMURA et al. (1963) who found that *Lemna gibba* G3 behaved as a long-day plant in the presence or absence of EDTA in M medium.

HILLMAN (1965) suggested that ODA (1962) and UMEMURA et al. (1963) had used rigorously purified macronutrient salts, in other words, that these chemicals contained such low amounts of copper that the "copper effect" was not discernible.

On the other hand, SCHUSTER (1968), working in Kandeler's laboratory, confirmed the results of HILLMAN (1962) with *Lemna perpusilla* 6746. Addition of copper to a purified medium changed this plant from a short-day to a day-neutral plant. However, this effect was only apparent at high light intensity, since under low light intensity (1500 lux and less) *Lemna perpusilla* 6746 continued to flower only under short days. Thus, in line with HILLMAN's (1962) earlier suggestion, copper does not seem to promote or inhibit flower induction. Copper rather appears to affect the photoperiodic control of flowering. *Lemna perpusilla* 6746, a short-day plant in a medium containing a small quantity of copper, becomes day-neutral when the copper concentration is increased to 2 μM/l. On the other hand, *Lemna gibba* G3, which flowers under long days in a medium containing a low concentration of copper (0.3 μM/l), does not flower when the amount of copper is increased, i.e., the ability to flower under long days is lost.

A change in light intensity was found to have a marked effect upon the

photoperiodic behaviour of *Lemna perpusilla* 6746 (ESASHI & ODA 1964). In M medium without EDTA it behaved as a short-day plant at 1400 lux, but acted as a day-neutral plant at 7000 lux. SCHUSTER (1968) suggested that the effect was not only due to the increase of the light intensity only, but to a combination of light and copper. Schuster could not induce flowering in *Lemna perpusilla* 6746 by increasing the light intensity in the absence of copper ions under long days in medium containing purified macronutrients. *Lemna perpusilla* 6746 flowered in long days under high light intensity only when copper was added to the medium.

MAHESHWARI & CHAUHAN (1963) induced flowering in *Wolffia microscopica* when EDTA was added to the nutrient medium. When EDTA was added to Bonner-Devirian medium (another modification of Hoagland's medium), flowering occurred regardless of the photoperiod. However, the percentage of plants which flowered increased with increasing length of the dark period. In the absence of EDTA the plants continued to grow and multiply but there was no flowering.

MAHESHWARI & VENKATARAMAN (1966), by using Hoagland's major and Heller's minor salts supplemented with EDTA, induced flowering in *Wolffia microscopica* under short-day conditions (see also MAHESHWARI et al. 1967; VENKATARAMAN et al. 1970). However, they could not induce flowering without EDTA. The difference between the two media used by Maheshwari and co-workers was in the concentration of major salts and in the presence of an additional ion, Cl^-, in the Bonner-Devirian medium. However, addition of Cl^- to Maheshwari's medium did not affect the photoperiodic response of *Wolffia microscopica* in this medium (VENKATARAMAN et al. 1970). Presumably, therefore, the difference in concentration of macronutrients is the controlling factor in determining the photoperiodic behaviour of *Wolffia microscopica.*

MAHESHWARI & SETH (1966) demonstrated that when EDTA in Maheshwari's medium was replaced by Fe-EDDHA (the iron salt of ethylenediamine-di-o-hydroxy-phenylacetic acid), *Wolffia microscopica* flowered under long days, i.e., the species acted as a day-neutral plant when Fe-EDDHA was present. However, the percentage of plants which flowered under long days was lower than under short days. Fe-EDDHA proved to be more effective on flowering than EDTA under short-day conditions. High concentrations of ferric citrate also induced flowering in *Wolffia microscopica* under short-days (SETH et al. 1970). Ferric citrate was, however, less effective than EDTA or Fe-EDDHA.

Lemna paucicostata, another duckweed which was studied extensively by Maheshwari and his students, behaved almost exactly like *Wolffia microscopica.* When EDTA was added to Bonner-Devirian medium, Maheshwari's medium or M medium flowering took place under short-day conditions (MAHESHWARI & GUPTA 1967; GUPTA & MAHESHWARI 1970a). The photoperiodic behaviour of *Lemna paucicostata* is thus similar in Bonner-Devirian medium and Maheshwari's medium. When EDDHA (ethylenediamine-di-o-hydroxyphenylacetic acid) instead of EDTA was added to the nutrient media the percentage of flowering increased, while a combination of EDDHA and a high concentration

(5×10^{-4} M) of ferric citrate permitted flower induction even under long-day conditions.

Fe-EDDHA was more potent than EDDHA in inducing flowering in *Wolffia microscopica* (SETH et al. 1970), and ferric citrate induced flowering in *Wolffia microscopica* and *Lemna paucicostata*. On the basis of these results Maheshwari and co-workers concluded that chelating agents somehow facilitate the entry of iron into the plants and that the iron uptake influences flowering in these two duckweeds. A study on the endogenous levels of iron in *Wolffia microscopica* in the presence of Fe-EDDHA and EDDHA supported this hypothesis. Plants provided with Fe-EDDHA had 25–50% more iron than those grown in a medium containing EDTA and a low concentration (4 mg/liter) of ferric citrate (SETH et al. 1970). Experiments with radioactive iron (^{59}Fe) confirmed these observations. Plants grown in EDDHA medium took up 500–700% more iron than those grown in the medium containing EDTA and ferric citrate. However, a study of the endogenous levels of copper in vegetative and flowering plants showed a decrease of the copper level by 15–30% in flowering plants. The conclusions of Maheshwari and co-workers that the modification of flowering by chelating agents is due to an increased availability of iron diverge from those of HILLMAN (1962) who suggested that EDTA influences flowering by chelating excess copper.

In order to test the possible role of iron or copper in induction of flowering in Lemnaceae, studies were initiated in 1970 by Pieterse and his associates at the University of Kentucky. *Lemna gibba* G3 was utilized in the investigations. It was demonstrated that the incorporation of EDDHA and Fe-EDDHA in M medium or 1/3 Hutner's medium resulted in profuse flowering under continuous light (PIETERSE et al. 1970a, b). In the media containing EDDHA, flowering was more pronounced than in those with Fe-EDDHA. Moreover, EDDHA was effective over a wider range of concentrations than Fe-EDDHA (PIETERSE et al. 1970b). EDTA was less potent in inducing flowering than EDDHA and Fe-EDDHA. A decreasing amount of iron was not associated with a decline of flowering in *Lemna gibba* G3 while with increasing copper concentration the flowering was adversely affected. Consequently, it was suggested by PIETERSE et al. (1970b) that the metal which influenced flowering in *Lemna gibba* G3 was most likely copper.

The role of copper in regulating flowering was further substantiated by the investigations of BHALLA & SABHARWAL (1972a) with a strain of *Lemna minor*. When plants were grown in M medium, there was no flowering either under long-day or short-day photoperiodic conditions. However, addition of EDDHA into the medium induced flowering. The levels of endogenous copper in vegetative and flowering plants showed a difference. The level of copper decreased by 45–60% in flowering plants, thereby strongly suggesting a role of copper in flowering of *Lemna minor*.

Recent investigations regarding the induction of flowering in *Lemna gibba* G3 by aspirin (BHALLA & SABHARWAL 1972b) further accentuate the role of copper in flowering. Inclusion of aspirin in the nutrient medium induces flow-

ering in this plant. In all experiments the level of endogenous copper on mg dry weight basis in plants grown on media with and without aspirin showed differences. In both 1/3 Hutner's and M medium the level of copper decreased by 60–75% in plants in aspirin-containing media, thereby confirming the role of copper in the flowering of *Lemna gibba* G3.

The exact mechanism by which chelating agents and copper ions influence flowering in *Lemna gibba* G3, however, remains to be investigated. HILLMAN (1962) hypothesized that copper interferes with the role played by the phytochrome action. Recently a similar assumption was made by EVANS (1971), who proposed that if phytochrome brings about its effect on flowering by changing membrane permeability, as has been suggested by JAFFE (1968), copper might be a co-factor for phytochrome action on membrane potential.

Remarkable influence of heavy metals on the flowering of *Xanthium* has been demonstrated by SALISBURY (1957). When cobaltous ions were applied to the leaves, the critical dark period was extended. This cobalt action was attributed to a complex formation of the metal with a macromolecule (probably protein) which participates in time measurement in the leaf. SALISBURY & EICHHORN (1963) speculated that cobalt may convert the configuration of the macromolecule into an inert form. This view of Salisbury seems applicable to copper action on duckweed flowering. Copper might inactivate a protein involved in the timing mechanism of *Lemna gibba* G3.

If chelating agents affect flowering via the formation of a complex with copper ions, this may be direct or indirect. For example, an uptake of copper by a chelating agent from an enzyme involved in flowering would be a direct effect. This implies that the chelating agent has a higher stability constant for copper than does the hypothetical enzyme. Alternatively, direct activation or inactivation of an enzyme may also be established through the formation of a metal bridge as was suggested by WALLACE (1962). These hypotheses are, however, purely speculative as it is not known with certainty if chelating agents penetrate plant cells (WALLACE 1962).

The effect would be indirect when the chelating agent influences flowering by keeping the concentration of the free copper in the nutrient medium at a low level. In this case the chelating agent can bring about its effect without entering the plant cells.

The incorporation of radioactive isotopes may throw some more light on the problem of the uptake of chelating agents by plant cells. WALLACE (1962), working with bush beans, demonstrated that ^{14}C EDDHA entered the xylem vessels of the plants. He was, however, not sure whether the uptake had taken place via root cells or whether the chelating agent had directly entered the xylem vessels via broken roots. *Lemna gibba* G3, with only one root, seems to be more suitable for this kind of experiment than bush beans.

The effect of chelating agents and copper on flowering has so far only been observed in the family Lemnaceae. However, more research on possible influences of chelating agents and copper on flowering in other families is needed. Chelating compounds have been shown to exert growth in low concentrations

in various plants (BURSTRÖM 1963). These effects were similar to those shown by auxins in increasing shoot and decreasing root growth.

The Lemnaceae flower very rarely in nature, their multiplication is mostly vegetative by the formation of new fronds in the pockets. Seed formation does not seem necessary for overwintering as during the fall season many species produce special dormant structures, turions. However, under certain conditions flowering and seed formation may still be advantageous for the plants. Seeds are, for example, more resistant to drought than turions or normal vegetative fronds. Possibly, flowering in nature is only induced under certain conditions, for example a certain change in the mineral composition of the water. This might occur under the influence of natural chelating agents. Future investigations on a possible relation between water composition and flowering of a duckweed should prove useful in understanding the mechanism of flowering in nature.

It is clear that the study on flowering in the Lemnaceae is still at an initial phase and that many aspects remain to be investigated.

3. THE EFFECT OF GROWTH REGULATORS ON FLOWERING IN LEMNACEAE

Cytokinins have an effect similar to EDDHA in both *Wolffia microscopica* and *Lemna paucicostata* (MAHESHWARI & VENKATARAMAN 1966; MAHESHWARI et al. 1967; GUPTA & MAHESHWARI 1969). These growth regulators enhanced flowering in *Wolffia microscopica* under short days and induced flowering under long days in Bonner-Devirian medium which contains EDTA (VENKATARAMAN et al. 1970).

In *Lemna paucicostata* flowering was induced under long-day conditions when cytokinin was added to Bonner-Devirian medium containing high amount (5×10^{-4} M) of ferric citrate (GUPTA & MAHESHWARI 1970b). A combined treatment of EDDHA and cytokinin made it possible to bring about flowering in *Lemna paucicostata* under long days at low levels (5×10^{-5} M) of iron. Zeatin and 6-benzyladenine proved to be the most potent in inducing flowering in these two plants.

GA_3 (gibberellic acid) as well as IAA (indole-3-acetic acid) inhibited flowering in *Lemna paucicostata* (GUPTA & MAHESHWARI 1970b). Flowering of *Lemna perpusilla* is also strongly inhibited by gibberellic acid (HILLMAN 1960). On the other hand, OOTA (1965) reported that GA_3 enhanced flowering in *Lemna gibba* G3.

4. GIBBOSITY IN LEMNA GIBBA

LINNAEUS (1753) described *Lemna gibba* in his Species Plantarum as "Lemna foliis sessilibus subtus hemisphaericis", i.e., *Lemna* with sessile leaves which are hemispherical at the lower side. The specific epithet *gibba* referred to this gibbous character of the fronds.

HEGELMAIER (1868), however, observed flat forms of *Lemna gibba*. These flat

forms were very similar to *Lemna minor* and could be distinguished from the latter by the larger air chambers. VAN HOREN (1869), too, reported the occurrence of flat forms. He suggested that running water, a relatively high light intensity and a relatively high temperature are essential factors for the development of gibbous forms.

GUPPY (1895) observed that gibbous plants gave rise to thin flat forms at the end of the summer. The appearance of the flat forms was accompanied by the death of a large number of gibbous plants. He concluded that the flat plants were special overwintering forms or turions. However, many of the gibbous plants survived the winter and continued to bud off flat new fronds except during extremely cold weather. Gibbous fronds were not produced until the weather became warmer. Guppy thought that for the development of gibbosity the plants require an average daily maximum temperature of 21 °C. After cool summers the gibbous fronds did not produce flat fronds but survived until the next spring.

MASON (1957) noticed that both forms flowered profusely, but fruiting was more frequent in the gibbous form. He suggested that the non-gibbous form might be a sterile hybrid.

HILLMAN (1962) reported that EDTA accentuated the gibbous character of the fronds of *Lemna gibba* G3. This was generally associated with flowering and he suggested that gibbosity may precede flowering. However, he did not study the problem specifically.

DAUBS (1965) stated, in his monograph on the Lemnaceae, that while there is some obvious variation in the degree of gibbosity, there is no continuous gradation between the gibbous and the flat forms. He noticed that even the very young fronds of the gibbous forms showed this characteristic to a marked degree. He suggested that intermediate forms are either *Lemna obscura* or *Lemna disperma*. These two species have been described as being smaller than *Lemna gibba* and also convex at the lower surface. Further, he doubted that the flat plants were overwintering forms since they grew and reproduced.

Doubts about the conclusion of Mason that the flat plants are sterile hybrids were raised by observations of DE SLOOVER (1966) who found a profusely flowering and fruiting population of flat forms of *Lemna gibba*. DE LANGE & SEGAL (1968) proposed that gibbous forms are produced under optimal growth conditions, while only flat forms are found in sub-optimal conditions. DEN HARTOG (1968), on the other hand, considered the evidence for the conclusions of de Lange and Segal insufficient. He stated that, although some flat forms clearly represent modifications of *Lemna gibba*, it is doubtful whether this is true in all cases.

PIETERSE et al. (1970c) observed that flat plants of *Lemna gibba* G3 became gibbous in the presence of different chelating agents. EDDHA and Fe-EDDHA were the most effective in inducing gibbous character of the fronds, while EDTA induced only slight gibbosity. These results clearly demonstrated that, as far as strain G3 is concerned, both flat and gibbous forms belong to the same taxon and represent merely morphological modifications under different nutritional

conditions. Moreover, their results showed that intermediate forms of *Lemna gibba* do exist. Consequently, it may be extremely difficult to distinguish these forms from *Lemna disperma* and *Lemna obscura* and possibly the latter species merely represent forms of *Lemna gibba*.

Under long-day conditions EDDHA and Fe-EDDHA, and to a less extent EDTA, induced both gibbosity and flowering in *Lemna gibba* G3 (PIETERSE et al. 1970b). Under short-day conditions the plants also became gibbous in the presence of these chelates but did not flower (PIETERSE 1972). This suggests that gibbosity is not associated with flowering as was suggested by HILLMAN (1962).

5. TURION FORMATION IN LEMNACEAE

The turions in the Lemnaceae are modified fronds which sink to the bottom of a pond or a ditch and remain submerged during the period of dormancy. The turions of *Spirodela polyrrhiza* were first described by HOFFMANN (1840). They are dark green or purple, and smaller and thicker than normal fronds (JACOBS 1947; HENSSEN 1954). The air spaces in these turions are reduced or absent and the cells are heavily loaded with starch grains (HEGELMAIER 1868; GUPPY 1895; JACOBS 1947). Some species of *Lemna* produce similar turions which are less modified than those of *Spirodela polyrrhiza* (VAN HOREN 1869; THOMPSON 1898; HICKS 1937; LANDOLT 1957). *Wolffia* turions are usually similar to normal fronds but contain a large amount of starch (HEGELMAIER 1868; LANDOLT 1957). PIETERSE et al. (1970d) described the formation of turions in *Wolffiella floridana* in vitro. These turions are shorter and wider than the normal vegetative fronds. They show reduced air chambers and an increased amount of starch in the cells.

Detailed investigations on the formation of turions in *Spirodela polyrrhiza* in vitro were done by JACOBS (1947), HENSSEN (1954), and CZOPEK (1959, 1963, and 1964). Jacobs studied both growth and turion formation under many combinations of controlled temperature, light intensity and light duration. He concluded that turions were produced under a condition which would maintain photosynthesis at levels in excess of carbohydrate utilization for growth and respiration. Thus, increased CO_2 levels strongly favoured turion formation. Turions of *Spirodela polyrrhiza* never produced daughter turions; at least two vegetative generations were to intervene between new turion formation.

HENSSEN (1954) reported that *Spirodela polyrrhiza* formed turions under conditions of moderate mineral deficiency or by addition of various sugars. Sucrose caused turion formation in both light and dark, but glucose was effective only in light. Fructose and maltose also caused turion formation in light while the effect of these two sugars was not tested in dark. Henssen followed starch formation, amylase activity and pH changes, but was unable to establish any relation between these factors and turion formation.

CZOPEK (1959) observed morphological differences between turions formed in light and in dark and reported a correlation between turion formation and dense crowding of vegetative fronds on the surface of the nutrient medium.

Moreover, she found that exhaustion of one (or more) mineral compounds in the nutrient medium induced turions. In accordance with the results of HENSSEN (1954), it was also observed by CZOPEK (1959) that sucrose induced turions in *Spirodela polyrrhiza*.

PERRY (1968) demonstrated that turion formation in *Spirodela polyrrhiza* can be induced by manipulation of light intensity, photoperiod, night temperature, day temperature, and concentration of nitrate in the medium. Different clones of *Spirodela polyrrhiza* from Vermont (U.S.A.), Puerto Rico, and Buenos Aires (Argentina) were tested for turion formation. The clones of *Spirodela polyrrhiza* from Buenos Aires and Puerto Rico did not form turions under any of the experimental conditions.

PERRY & BYRNE (1969) reported the formation of turions in *Spirodela polyrrhiza* within 10 days under non-inductive environmental conditions by abscisic acid in concentrations as low as 0.01 μg/liter. However, one clone from Puerto Rico did not form turions in response to any concentration of abscisic acid tested. STEWART (1969) independently arrived at conclusions similar to those of Perry and Byrne. He reported the induction of turions by 0.15 μg/l abscisic acid in *Spirodela polyrrhiza*.

VAN STADEN & BORMAN (1969) studied the effect of different concentrations of abscisic acid on *Spirodela oligorrhiza*. Abscisic acid arrested growth at concentrations down to 10 μg/l, but turion formation was not reported. Also, in *Lemna minor* striking growth inhibitions by abscisic acid were observed without the induction of turions (VAN OVERBEEK & MASON 1968). In addition, PIETERSE et al. (1970d) reported that the fronds of *Wolffiella floridana* became smaller in the presence of abscisic acid, but no turion formation was observed.

It has been demonstrated that gibberellic acid enhances the germination of turions of *Spirodela polyrrhiza* (LACOR 1969). This is in general agreement with earlier work where gibberellic acid has been shown to affect dormancy in higher plants (BRIAN 1966). *Wolffiella floridana* produced turions in 1/3 Hutner's medium containing 3% sucrose (PIETERSE et al. 1970d). At 1% the plants remained floating while at a sucrose concentration of 2% a mixture of floating plants and turions was observed. At 5% the plants became yellow and growth was adversely affected.

Endogenous gibberellins from floating plants and turions of *Wolffiella floridana* were extracted and partially purified by PIETERSE et al. (1971). Gibberellin-like activity was detected in two zones of the chromatogram corresponding to Rf 0–0.1 and Rf 0.4–0.5 by dwarf pea bioassay. The active compound(s) in the two zones have been referred to as SMF, i.e. slow moving factor(s), and FMF, i.e. fast moving factor(s). There were quantitative differences in the gibberellin-like substances of floating plants and turions. The floating plants contained more of SMF but less of FMF as compared to the turions. The chromatographic behavior of SMF was similar to gibberellin A_1 and A_3 while the identity of FMF was uncertain. Possibly SMF prevented the excessive starch accumulation in floating plants through the induction of hydrolytic enzymes.

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