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Ethylene as a flower-promoting agent in *Lemna*

By

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With 2 Figures

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Summary

SCHARFETTER E., LESEMANN C. and KANDELER R. 1987. Ethylene as a flower-promoting agent in *Lemna*. – Phyton (Austria) 27 (1): 31–37, with 2 figures. – English with German summary.

Within a certain concentration range, the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) promotes flowering in the long-day plant *Lemna gibba* G1 as well as in the short-day plant *Lemna aequinoctialis* 6746, when plants are induced to low or moderate flowering by other agents. The optimum ACC concentration is lower in *L. gibba* (10^{-8}M) than in *L. aequinoctialis* (10^{-7}–10^{-6}M). Higher ACC concentrations are inhibitory in both species.

Keeping the plants on the water surface in a crowded state also has a flower-promoting effect in *L. gibba* and *L. aequinoctialis*. Inspite of the fact that overcrowding induces a transient stimulation of endogenous ethylene formation in both plant species, the contact of neighbour plants seems to act on flower initiation by another, hitherto unknown signal.

Zusammenfassung

SCHARFETTER E., LESEMANN C. und KANDELER R. 1987. Äthylen als blühförderndes Agens bei *Lemna*. – Phyton (Austria) 27 (1): 31–37, mit 2 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Die direkte Vorstufe des Äthylens 1-Aminocyclopropan-1-carboxylsäure (ACC) fördert innerhalb eines bestimmten Konzentrationsbereichs die Blütenbildung sowohl bei der Langtagpflanze *Lemna gibba* G1 als auch bei der Kurztagpflanze *Lemna*

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aequinoctialis 6746, wenn die Pflanzen mit Hilfe anderer Mittel zu einer schwachen bis mäßigen Blüte induziert werden. Die optimale ACC-Konzentration liegt bei *L. gibba* niedriger (10^{-8}M) als bei *L. aequinoctialis* (10^{-7}–10^{-6}M). Höhere ACC-Konzentrationen sind bei beiden Arten blühhemmend.

Werden die Pflanzen auf der Wasseroberfläche in einem dicht-gedrängten Zustand gehalten, hat auch diese Behandlung einen blühfördernden Effekt bei *Lemna gibba* und *L. aequinoctialis*. Trotz der Tatsache, daß ein solches Zusammendrängen der Pflanzen in beiden Arten eine vorübergehende Stimulation der endogenen Äthylen-Bildung induziert, scheint der Nachbarkontakt der Pflanzen auf die Blütenbildung über ein anderes, noch unbekanntes Signal wirksam zu werden.

Introduction

Members of the *Lemnaceae* have been widely used to investigate the physiology of flower induction (see KANDELER 1984, 1985). In this way some substances – including cytokinins, salicylic acid, EDDHA, ferricyanide, and cyclic AMP – were detected, which induce or at least promote flowering in long-day dependent as well as in short-day dependent strains of *Lemnaceae*. Most of the plant growth substances were examined thoroughly, but only one publication deals with an ethylene-releasing substance. PIETERSE (1976) reported that Ethrel (2-chloroethane phosphonic acid) could not induce flowering in the long-day plant *Lemna gibba* G3, when plants were cultivated in a sucrose-containing medium under continuous light. Supplying 10 mg/l EDDHA rendered possible some flowering under these conditions, but additionally given Ethrel diminished the effect of EDDHA. Recently we have shown that also in *Spirodela punctata* O5 EDDHA-induced flowering is inhibited by Ethrel or the ethylene precursor ACC (SCHARFETTER et al. 1986).

In other plants positive as well as negative effects of exogenous ethylene and Ethrel on flower induction were found (see BERNIER & al. 1981). Promotion or inhibition of flowering by ethylene are independent of the photoperiodic reaction type and of the taxonomic position of plants. ZEEVAART (1978) reviewing the effects of phytohormones on flower formation concluded that – in spite of the effects of exogenously supplied ethylene – there is no evidence that endogenous ethylene plays a role in floral induction.

Our investigations with inhibitors of ethylene formation (AVG, AOA, Co^{2+}) and ethylene-releasing substances (ACC, Ethrel) in *Spirodela punctata* O5 have led to the conclusion that flower initiation depends on the endogenous ethylene level only after accomplishment of certain other flower-inducing processes (SCHARFETTER & al. 1986). EDDHA as a flower-promoting agent has to be present in the nutrient solution for effectiveness of the above-named substances. Surprisingly both, the inhibitors of ethylene formation and the ethylene-releasing substances, have a flower-inhibiting effect in *S. punctata* supplied with EDDHA. The assumption that an intermediate ethylene level is needed for flower initiation, which can be suppressed or can be exceeded, is corroborated by the results communicated in this paper. In photoperiodically induced *Lemna gibba* G1 flowering is promoted or inhibited by the ethylene precursor ACC depending on the concentration used. Also in *Lemna aequinoctialis* 6746 flowering is promoted only with a certain ACC concentration, when plants are induced to long-day flowering by supply of CCC and ABA.

Material and methods

Lemna gibba L. G1 (long-day plant) and *Lemna aequinoctialis* WELWITSCH 6746 (short-day plant) were precultivated axenically under long-day conditions (16 hours per day light with Osram-HQIL lamps, irradiance 22 Wm^{-2}) in Erlenmeyer flasks with autoclaved medium after PIRSON & SEIDEL (1950), but with iron as Fe(III)-EDTA. Temperature was 26 ± 0.5° C during light, and 22 ± 0.5° C during dark phase. In the experiments with *L. gibba* long-day conditions were used as during preculture. *L. aequinoctialis* obtained continuous light from Osram HQIL lamps with 44 and 22 Wm^{-2} during experiments of Fig. 2 and Tab. 1, respectively (temperature 26 ± 0.5° C). The medium for experimental cultures was sterile-filtered (except for the experiments of table 1). Each experimental group consisted of 2 or 3 replicates. Duration of the experiments was 17 days (Fig. 1), or 10 days (Fig. 2), or 7 days (Tab. 1). All experiments were carried out at least twice.

In *L. gibba* all young fronds of a culture, which still were enclosed in a pouch of their mother frond, were examined for flower primordia under a dissecting microscope and the percentage of flowering fronds was determined. In *L. aequinoctialis* the further developed (visible) fronds of a culture were used instead of non-visible fronds for determination of the flowering percentage.

Abbreviations: ABA, abscisic acid; ACC, 1-aminocyclopropane-1-carboxylic acid; AMP, adenosine 5'-monophosphate; AOA, aminooxyacetic acid; AVG, aminoethoxyvinylglycine; CCC, 2-chloroethyltrimethylammonium chloride; EDDHA, ethylenediamine-di-o-hydroxyphenylacetic acid; EDTA, ethylenediaminetetraacetic acid.

Results and Discussion

When the ethylene precursor ACC is added to the nutrient medium of *Lemna* cultures, ethylene is formed by the plants very rapidly (FÄRBER, unpublished gas-chromatographic determinations). Thus, ACC can be used as an ethylene-releasing agent in experiments with *L. gibba* and *L. aequinoctialis*. Figure 1 shows the results of an experiment, in which the effect of ACC on flowering of *L. gibba* was tested. Plants were held under long-days and, therefore, were photo-induced. Control plants, which were float-

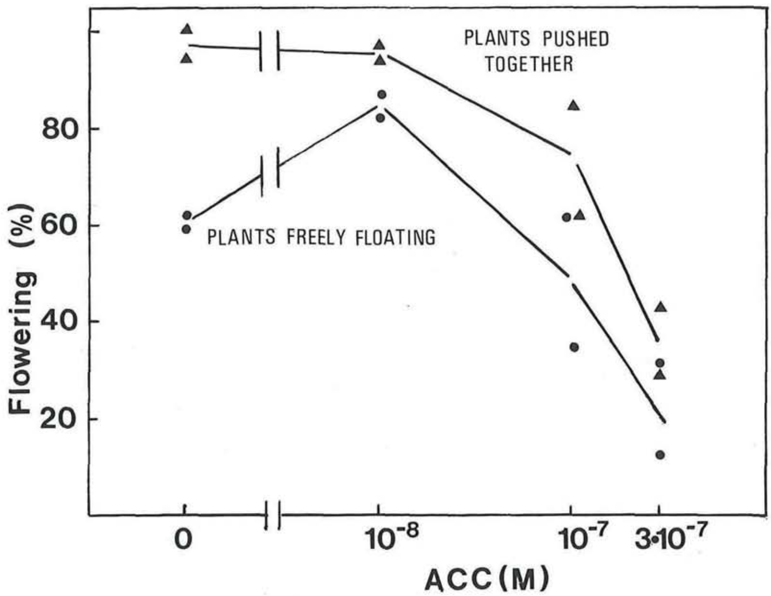

Fig. 1. The effect of ACC and overcrowding on flowering in *Lemna gibba* G1 under long-day conditions. –●–, plants freely floating; –▲–, plants pushed together.

ing freely on the medium (not pushed together), exhibited a flowering percentage of about 60%. ACC at 10^{-8}M promotes flowering under such conditions, whereas 3 × 10^{-7}M ACC is inhibitory. Also in *L. aequinoctialis* promoting and inhibiting effects of ACC on flowering can be demonstrated (Fig., 2), when plants are induced to some flowering under long-days by supplying 10^{-6}M CCC and 2 × 10^{-9}M ABA (Kandeler & Hügel 1973). The optimum ACC concentration for flower promotion in *L. aequinoctialis* varies depending on the physiological state of plants (compare the two experiments given in Fig. 2), but in every case the optimum concentration lies distinctly higher in *L. aequinoctialis* (10^{-7}–10^{-6}M ACC) than in *L. gibba* (10^{-8}M ACC). This result is interesting, because it agrees with the fact that frond senescence has opposite effects on flowering in the two species under long-day conditions (Kandeler & al. 1974). Successive daughter fronds produced from one senescing mother frond have a decreasing ability for flowering in *L. gibba* G1, but an increasing ability for flower initiation in *L. aequinoctialis* 6746. Senescence, on the other hand, is correlated with higher ethylene formation in many plants (Noodén & Leopold 1978).

Figure 1 contains in addition the results from experimental groups, in which not the volume, but the usable surface of medium was restricted for plants. The plants were held in a crowded state by glass cylinder inserted

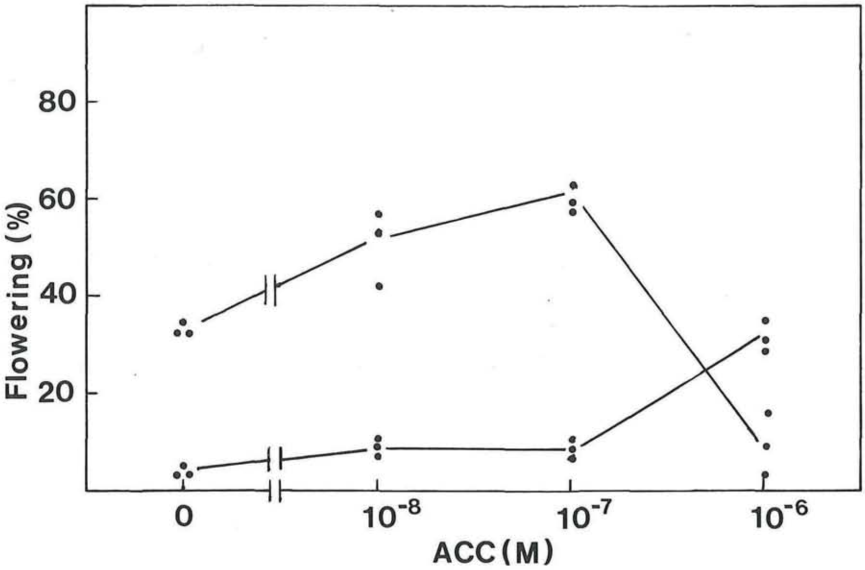

Fig. 2. The effect of ACC on flowering in *Lemna aequinoctialis* 6746 under long-day conditions. Data from two independent experiments are given. Plants were induced to some long-day flowering by addition of 10^{-6}M CCC and 2×10^{-9}M ABA to the nutrient solution.

into the culture vessel from above and containing perforations for medium and gas exchange. Such a crowded state of *Lemna* plants influences the endogenous ethylene formation (FÄRBER & al. 1986). In *L. gibba* G1 ethylene evolution increases slowly, reaching a peak 18 hours after pushing the plants together and then decreases. In *L. aequinoctialis* 6746 ethylene increases immediately after crowding with a peak two hours later and then returns to low levels. The data given in Fig. 1 show that overcrowding has a flower-promoting influence in *L. gibba* in control plants as well as in ACC

Table 1

The effect of overcrowding on flowering under long day in *Lemna aequinoctialis* 6746. F: plants freely floating; P: plants pushed together. Values of two replications are given in each experiment.

Conditions		Flowering (%)			
		experiment A		experiment B	
Control	F	0,	0	0,	0
Sucrose (1%) + $CuSO_4$ (2μM)	F	16,	30	37,	35
Sucrose (1%) + $CuSO_4$ (2μM)	P	41,	37	46,	47

treated plants. From this result it must be concluded that overcrowding is acting on *Lemna* flowering primarily not by changing the endogenous ethylene formation but rather by another unknown signal. If the crowded state acted through an increase in ethylene formation, the inhibition of flowering by 10^{-7}M and 3×10^{-7}M ACC should be greater in crowded than in free floating plants.

The effect of crowding on flowering has been investigated also in *L. aequinoctialis* (Table 1). In these experiments moderate long-day flowering of control plants was induced by the combined effect of sucrose (1%) and 2 µM $CuSO_4$ (Schuster 1968). Overcrowding of fronds managed in the same way as in *L. gibba*, has a small, but distinct flower-promoting effect. The physiological basis of signal transmission between neighbouring fronds is unknown at the present and needs further investigation.

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References

Bernier G., Kinet J.-M. & Sachs R. M. 1981. The physiology of flowering, Vol. II. – CRC Press, Boca Raton, Florida.

Färber E., Königshofer H. & Kandeler R. 1986. Ethylene production and overcrowding in *Lemnaceae*. – J. Plant Physiol. 1924: 379–384.

Kandeler R. 1984. Flowering in the *Lemna* system. – Phyton (Austria) 24: 113–124.

– 1985. *Lemnaceae*. – In: Halevy A. H. (Ed.), Handbook of flowering Vol. III, p. 251–279. – CRC Press, Boca Raton, Florida.

– & Hügel B. 1973. Blütenbildung bei *Lemna paucicostata* 6746 durch kombinierte Anwendung von Abscisinsäure und CCC. – Plant Cell Physiol. 14: 515–520.

– – & Rottenburg Th. 1974. Gegensätzliche Wirkung der Sproßalterung auf die Blütenbildung bei *Lemna paucicostata* und *Lemna gibba*. – Biochem. Physiol. Pflanzen 165: 331–336.

Noodén L. D. & Leopold A. C. 1978. Phytohormones and the endogenous regulation of senescence and abscission. – In: Letham D. S., Goodwin P. B. & Higgins T. J. V. (Eds), Phytohormones and related compounds. Vol. II, p. 329–369. – Elsevier/North Holland, Amsterdam.

Pieterse A. H. 1976. Specific interactions in the physiology of flowering and gibbosity of *Lemna gibba* G3. – Plant Cell Physiol. 17: 713–720.

Pirson A. & Seidel F. 1950. Zell- und stoffwechselphysiologische Untersuchungen an der Wurzel von *Lemna minor* L. unter besonderer Berücksichtigung von Kalium- und Kalziummangel. – Planta 38: 431–473.

Scharfetter E., Färber E. & Kandeler R. 1986. Involvement of ethylene in the effect of EDDHA on flowering and vegetative development in *Spirodela punctata*. – Veröff. Geobot. Inst. ETH, Stiftung Rübel, Zürich 87: 168–177.

SCHUSTER M 1968. Die Bedeutung von Starklicht und Kupfer für die phytochromgesteuerte Morphogenese von *Lemna perpusilla*. – Doctoral thesis, University Würzburg, West Germany.

ZEEVAART J. A. D. 1978. Phytohormones and flower formation. – In: LETHAM D. S., GOODWIN P. B. & HIGGINS T. J. V. (Eds), Phytohormones and related compounds: Vol. II, p. 291–327. – Elsevier/North Holland, Amsterdam.

Recensio

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Die herkömmliche Pflanzenzüchtung wird auf die Dauer der Forderung, für die ständig wachsende Menschheit ausreichend Nahrungsmittel zu produzieren, allein nicht nachkommen können. Die Biotechnologie erreicht heute ein Stadium, in dem verbesserte oder neuartige Kulturpflanzen hergestellt werden können, sie bildet damit eine wertvolle Ergänzung zu der bereits einige Generationen alten Züchtungspraxis. Davon erhofft man sich, den Anforderungen der Zukunft besser dienen zu können. Die Möglichkeiten zur Manipulation im cellulären und subcellulären Bereich