ding to the intracellular compartment of the axon (Figure 2). The period during the hypoosmotic stress is 125 min compared with 160 min as the control.

During the hyperosmotic stress, on the contrary, there is an immediate shrinkage of the axon and no volume regulation can be observed even after 6 h of incubation in the hypertonic saline (Figure 1). In this case, no significant modification of the alanine efflux can be recorded (Figure 2).

It appears therefore that an increase in alanine efflux is associated with the rapid volume readjustment occurring after the application of a hypoosmotic stress. A same modification of efflux with various other organic molecules such as urea or propylene-glycol. On the other hand, a similar change in the efflux of 2-amino-isobutyric acid has been observed on bundles of muscle fibers isolated from Callinectes and submitted to a hypoosmotic stress<sup>11</sup>. Moreover, an increase in the permeability to potassium has been recently demonstrated on duck erythrocytes during the volume readjustment following the application of a non-hemolytic hypoosmotic stress<sup>12</sup>. An increased release of amino acids has also been observed on the isolated mussel heart under hypoosmotic stress conditions 18. Although modifications of the extracellular space can partly account for the changes in efflux, it appears thus that an increase in the permeability of the cellular membrane is implicated in this phenomenon.

The increase in the alanine efflux we observe is not modified when the axons are submitted to the hypoosmotic stress in salines where NaCl has been replaced by LiCl or choline chloride or in calcium free salines; although in this last condition, there is a general increase in the alanine efflux. The increase in efflux is however completely suppressed when the axons are dropped in a saline, the ionic composition of which is identical to the ionic composition of the hypotonic saline but which is kept isosmotic to the control saline by addition of sucrose. It is therefore concluded that the increase in efflux is induced by the swelling of the tissue produced by the modification of the osmotic pressure 'per se'.

Thus, in hypoosmotic media, Callinectes axons, after an initial phase of swelling, revert towards their original vo-

lume at least partly by virtue of a loss of cellular osmotic effectors. This loss is a consequence of a temporary increase in efflux which requires changes in membrane characteristics. These changes in efflux seem to be induced by the modification of the osmotic pressure 'per se' and not by the alteration, in the incubating saline, of the concentration of a specific ionic species.

That such a mechanism of isosmotic intracellular regulation can be at play during the adaptation in vivo of a euryhaline species to diluted media is still unknown. This possibility is now under investigation <sup>14, 15</sup>.

Résumé. Au cours du choc hypoosmotique, le volume d'un axone isolé d'une patte de crabe euryhalin Callinectes sapidus est rapidement régularisé. En même temps, on observe une augmentation du flux sortant de l'alanine marquée. Cette augmentation paraît être due à une modification de la membrane axonale, produite par la variation de la pression osmotique dans le milieu d'incubation.

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Laboratory of Marine Membrane Physiology, Duke University Marine Laboratory, Beaufort (North Carolina 28516, USA), 6 December 1971.

- <sup>11</sup> H. Gainer, personnal communication, in preparation.
- 12 F. M. Kregenow, J. gen. Physiol., in press (1971).
- <sup>13</sup> S. K. Pierce and M. J. Greenberg, Am. Zool. 10, 518 (1970).
- 14 This work has been aided by a grant No. HE 12157 from NIMH and a 'Crédit aux Chercheurs' from the Fonds National de la Recherche Scientifique to R.G.
- <sup>15</sup> Acknowledgments: We are grateful to Dr. D. C. Tosteson, Chairman of the Pharmacology and Physiology Department of Duke University, Durham, North Carolina for the interest he has taken in this research. We also wish to thank Dr. J. D. Costlow, Director of the Duke University Marine Laboratory, Beaufort, N. C., for the hospitality we have received at the station.
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## Chemical Promotion of Pistillate Flower Formation in Cucurbita

Flower sex expression in the cucumber proceeds through a nodal sequence in the appearance of flower types <sup>1</sup>. The early nodes formed produce only staminate flowers, followed by a monoecious phase and finally successive nodes of pistillate flowers. This pattern generally occurs even though all floral buds pass through a common bisexual phase during development <sup>2,3</sup>.

Flower sex expression can be modified by environmental<sup>4,5</sup> and chemical<sup>5,7</sup> factors. The staminate phase is favored by gibberellins<sup>5,6</sup> and the pistillate phase by auxins<sup>7</sup>, growth retardants<sup>8</sup>, and some unsaturated hydrocarbons<sup>9,10</sup>. Recently, much interest has been directed to the role of ethylene<sup>11–13</sup>. This report describes the marked promotion of pistillate flower formation with 1,1,5,5-tetramethyl-3-dimethylaminodithiobiuret (MATB) on nodes that normally would produce only staminate flowers and the reversal of this effect with gibberellin A<sub>3</sub>(GA<sub>3</sub>).

Materials and methods. Cucumber (Cucumis sativus L. cv. National Pickling) were cultured in a greenhouse at a 14-h photoperiod and minimum day and night temperatures of 25 and 21 °C, respectively. Foliar sprays of MATB

(0, 1.4 and  $7.0\times10^{-4}$ , 1.4 and  $2.8\times10^{-3}M$ ) were applied when the first true leaf was approximately 5 cm in diameter. The interaction of MATB  $(7.0\times10^{-4}M)$  and GA<sub>3</sub>  $(1.4\times10^{-3}M)$  at saturating doses was also established.

- <sup>1</sup> T. M. Currence, Proc. Am. Soc. Hort. Sci. 29, 477 (1932).
- <sup>2</sup> E. Galun, Y. Jung and A. Lang, Devl Biol. 6, 370 (1963).
- <sup>3</sup> D. Atsmon and E. Galun, Phytomorphology 10, 113 (1960).
- <sup>4</sup> J. Heslop-Harrison, Biol. Rev. 32, 38 (1957).
- $^5$  M. J. Bukovac and S. H. Wittwer, Adv. Chem. Ser. 28, 80 (1961).
- <sup>6</sup> C. E. Peterson and L. D. Anhder, Science 131, 1673 (1960).
- <sup>7</sup> F. Laibach and F. J. Kribben, Ber. dt bot. Ges. 62, 53 (1950).
- <sup>8</sup> W. D. MITCHELL and S. H. WITTWER, Science 136, 880 (1962).
- <sup>9</sup> F. G. MININA and J. G. TYLKINA, Compt. r. Acad. Sci., USSR 55, 165 (1947).
- <sup>10</sup> F. J. Mehanik, Sady Ogorody 10, 13 (1958) [Hort. Abst. 29, 1426 (1959)].
- <sup>11</sup> A. L. McMurray and C. H. Miller, Science 162, 1397 (1968).
- <sup>12</sup> R.W. Robinson, S. Shannon and M. D. de la Guardia, BioScience 19, 141 (1969).
- <sup>13</sup> J. Rudick, A. H. Halevy and N. Kedar, Planta 86, 69 (1969).

Table I. Effect of MATB on pistillate flower formation and shoot elongation in C. sativus

Measurement	0	Molar concentration a			
		1.4×10 <sup>-4</sup>	$7.0 \times 10^{-4}$	$1.4 \times 10^{-3}$	$2.8 \times 10^{-8}$
Node of first pistillate flower	9.0a	4.9b	3.9b	3.4b	3.9b
Days from seeding to first pistillate flower	43 a	39 ab	37 b	37 b	39 ab
Number of pistillate flowers on first 10 nodes	2.0 a	7.5 b	10.9b	9.2b	9.1 b
Height at the 10th node (cm)	58 a	48 b	43 bc	36 c	25 d
Final height of plant (cm)	132 a	114 ab	112 ab	115 ab	87b

<sup>&</sup>lt;sup>a</sup> Means followed by different letters are significantly different at P 0.05.

Table II. Reversal of MATB effect on pistillate flower formation in C. sativus with GA<sub>3</sub>

Measurement	Treatment a				
	Control	MATB	$GA_3$	$MATB+GA_3$	
Node of first pistillate flower	9.0	3.9	24.3	22.3	
Number of pistillate flowers on first 10 nodes	2.0	10.9	0.0	0.2	
Days from seeding to first pistillate flower	43	37	64	63	

 $<sup>^{\</sup>rm a}{\rm MATB}$  at  $7.0\times 10^{-4}M$  and  ${\rm GA_3}$  at  $1.4\times 10^{-8}M.$ 

The effect of MATB was determined on other C. sativus cultivars (Marketmore, Wisconsin SMR 58) and on Cu-curbita maxima Duchesne (cv NK 560). Data on appearance and distribution of pistillate flowers and plant growth were recorded. Ethylene evolution from control and MATB-treated ( $10^{-3}$  and  $10^{-4}M$ ) cucumber plants was monitored by GLC (Varian Aerograph 1700, 1.4 mm  $\times$  76 cm activated aluminium oxide column) by assaying the atmosphere of a closed system containing the plants and scrubbed of  $\mathrm{CO}_2$ .

Results. MATB induced pistillate flowers to form on nodes that normally would have produced only staminate flowers. The node number at which the first pistillate flower formed was reduced by half, the number of pistillate flowers produced on the first 10 nodes was increased by 3- to 5-fold and days from seeding to anthesis was significantly reduced (Table I). Associated with the enhancement of the pistillate phase was a suppression of shoot extension in the early development of the plant, which became less pronounced with time (Table I). Staminate flowers continued to be produced but were not recorded. GA<sub>3</sub> delayed the appearance of the pistillate phase and completely reversed the effect of MATB on pistillate flower formation (Table II). There was no evidence that MATB at  $10^{-4}$  or  $10^{-3}M$  induced ethylene evolution in treated plants over a 72-h period following treatment. A similar enhancement of pistillate flower formation was found in the slicing type, cv Marketmore, and in C. maxima.

Discussion. These data illustrate a marked promotion of the pistillate phase of monoecious plants by a substituted thiobiuret. Earlier reports <sup>12, 13</sup> on chemical enhancement of pistillate flower formation have implicated ethylene directly or indirectly. In this study there was no evidence of the involvement of ethylene since evolution rates for MATB-treated plants were not different from control plants during a 72-h post-treatment period. Although no

direct evidence is available MATB may influence pistillate flower formation through endogenous GA. This hypothesis is supported by data showing that the MATB effect can be reversed by exogenous application of GA and from the findings of Atsmon et al.<sup>14</sup> that low levels of endogenous GA-like substances were associated with femaleness in the cucumber. The effect of ethylene on sex expression may also be via endogenous GA, since some functions attributed to ethylene are opposite to those of GA and exogenous applications of GA antagonize the ethylene effect on flower sex expression in cucumbers <sup>12</sup>.

MATB-enhanced femaleness of monoecious plants may have practical implications by causing earlier and concentrated fruiting desirable for machine harvesting <sup>16</sup>.

Zusammenfassung. Blattspritzungen mit 1,1,5,5,-Tetramethyl-3-dimethyl Amino-dithiobiuret (MATB) induzierten die Bildung weiblicher Blüten bei Gurken (C. sativa) und Kürbis (C. maxima). Diese Wirkung von MATB wurde von Gibberellin  $A_3$  vollständig aufgehoben. Es wurden keine Anzeichen für die Abgabe von Äthylen bei den mit MATB behandelten Pflanzen beobachtet.

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Department of Horticulture, Michigan State University, East Lansing (Michigan 48823, USA), 22 November 1971.

<sup>&</sup>lt;sup>14</sup> D. Atsmon, A. Lang and E. N. Light, Pl. Physiol. 43, 806 (1968).

<sup>&</sup>lt;sup>15</sup> P. C. Scott and A. C. Leopold, Pl. Physiol. 42, 1021 (1967).

<sup>&</sup>lt;sup>16</sup> Michigan Agricultural Experiment Station Journal, Article No. 5704.