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On the Fading of an Ephemeral Flower

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Key Term Index: curling reaction, ethylene, protein synthesis, turgor, cell wall modification; *Ipomoea tricolor*.

Summary

The mechanism in the ribs which leads to the curling up of the corolla of *Ipomoea tricolor* was investigated. It seems that two processes are involved in this movement. Due to differential turgor changes the ribs start to roll up. At the same time extracellular enzymes cause a reduction of the tensions in the structure of the cell walls. This modification enables the ribs to continue the curling up process.

Introduction

Professor MOTHES celebrating his jubilee may find it not too tactful that our contribution deals with the fading of a flower. However, the morning glory can also be conceived as a meaningful symbol: Fading, senescence and abscission of flowers is always the prelude to anthesis of a new set of blossoms.

Ephemeral flowers are rewarding objects for the study of developmental processes which normally are rather slow. They are particularly suitable for investigating the various phenomena associated with fading and senescence.

The fading of the corolla of *Ipomoea tricolor* starts in the early afternoon of the day of flowering and is largely completed in the late evening. Externally visible symptoms of fading include the curling up of the corolla (Fig. 1) and color changes from blue to purple. In terms of physiological processes associated with the strictly timed ageing

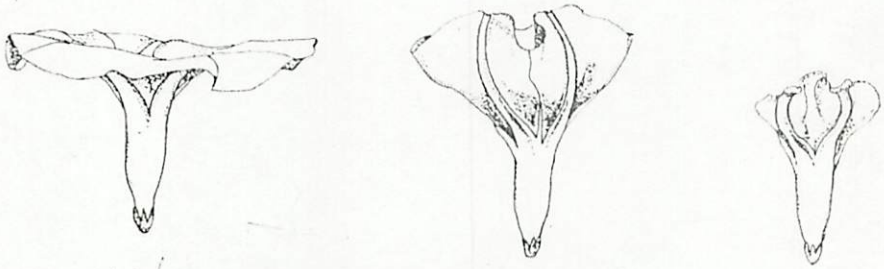

Fig. 1. *Ipomoea tricolor*: Evidently, the ribs are responsible for the curling of the fading corolla.

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the degradation of biopolymers present in the cytoplasm and in the cell walls as well as the export of the breakdown products from the corolla have been studied (Matile and Winkenbach 1970, Wiemken-Gehrig et al. 1974). These processes appear to be highly organized, as is convincingly demonstrated by the fact, that certain hydrolases such as RNase, β-glucosidase and β-galactosidase are synthesized *de novo* whilst the total protein content of the corolla is rapidly declining (Wiemken and Wiemken 1975, Baumgartner et al. 1975). This illustrates the importance of cellular compartmentation of lytic processes.

The surprisingly strict timing of the development of the *Ipomoea* corolla points to the existence of a controlling mechanism. In fact, Kende and Baumgartner (1974) were able to demonstrate that the curling up of the ageing corolla is induced by exogeneous ethylene; moreover, the curling was shown to coincide with a burst of endogeneous ethylene. The data available up to now indicates a rather complex effect of ethylene. Not only the curling up of the corolla but also metabolic processes such as the synthesis of RNase are controlled by ethylene.

Detailed work on the physiology of ageing is rendered more difficult by the heterogeneity of higher plant organs such as the corolla of *Ipomoea*. After Hanson and Kende (1975) detected that isolated segments for ribs (the particular tissue which is responsible for the curling up of the corolla) behave in vitro as they do in vivo, work with a comparatively simple system became possible. In the present paper we report on results from experiments concerning the mechanism of curling up of rib segments.

Material and Methods

Plant material. — *Ipomoea tricolor* (Cav.) was grown in the greenhouse on garden soil. In summer, the plants were kept under natural photoperiodic conditions, whereas in winter, supplementary illumination was provided in a photoperiod of 14 h.

Isolated segments of ribs. — Flowers were collected after anthesis and segments of the ribs, 10 mm in length, were excised according to Hanson and Kende (1975). A strip of intercostal tissue, 1 mm in width, remained attached to each side of the rib.

Sets of excised segments of ribs were floated on 5 ml of solution in 25 ml Erlenmeyer flasks stoppered with rubber caps. Appropriate volumes of ethylene were injected into the gas phase via the rubber caps. The incubations were performed at 28° C in the dark.

Measurement of the angles of curling. — The degree of curling was determined by measuring the angle as indicated in Fig. 2 (Hanson and Kende 1975). The straight ribs of the opened corolla correspond to an angle $\alpha = 180°$. After completion of the curling in vitro the isolated ribs exhibit angles up to 400—440°.

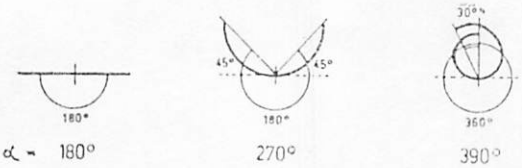
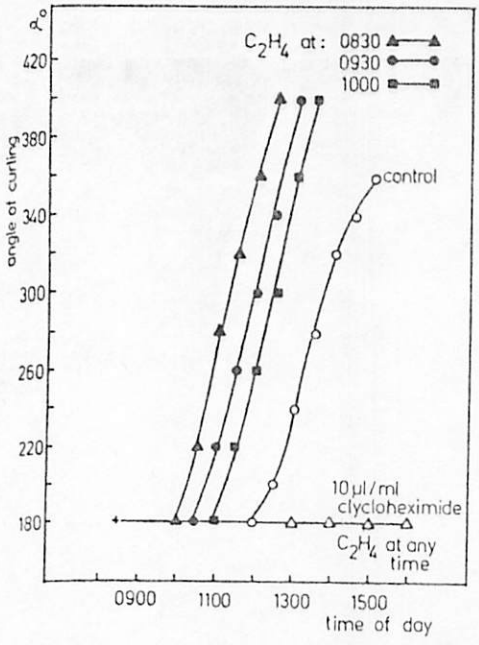


Fig. 2. *Determination of angles of curling in isolated segments of ribs.*

Results and Discussion

Isolated segments of ribs begin to curl up at about the same time of day as the intact corolla begins to fade. In addition, they can be induced to curl up prematurely if treated with 10 ppm ethylene. It appears from Fig. 3 that after the tissue is exposed to ethylene about 90 min elapse before the angle of curling starts to increase rapidly. This corresponds exactly to the fading reaction induzed by ethylene in the intact corolla (KENDE and BAUMGARTNER 1974).



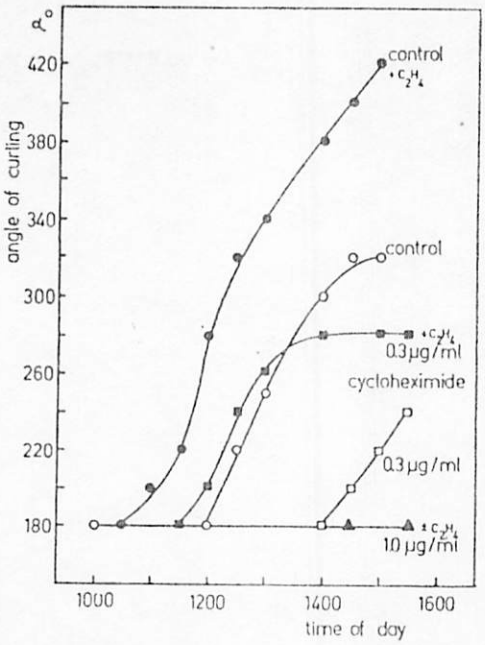


Fig. 3. *Induction of curling by ethylene and its inhibition by cycloheximide in segments of ribs.*

Fig. 4. *Determination of the optimal concentration of cycloheximide for inhibiting the ethylene-induced curling of segments of ribs.*

In order to investigate a possible involvement of protein synthesis in the curling reaction triggered by ethylene the effect of cycloheximide was tested. Surprisingly, this inhibitor of protein synthesis at the level of translation causes a complete inhibition of the curling process if supplied to segments at a concentration of 10 μg/ml (Fig. 3).

The same effect is obtained with a ten times lower concentration, whilst at 0.3 μg/ml of cycloheximide the response of the ribs to exogeneous ethylene as well as the curling up of the control segments are only partially inhibited (Fig. 4).

The inhibitory effect of cycloheximide is perfectly reversible. If the segments are transferred to distilled water after various periods of treatment with cycloheximide a normal ethylene induced curling reaction can be observed. The process of curling seems to depend upon a continuous synthesis of protein. This can be concluded from experiments in which segments were transferred from water to cycloheximide at the onset and in the course of the curling process, respectively (Fig. 5). The rate of curling is re-

duced to zero within only 60 min if cycloheximide is given 30 min after the onset of curling. If the beginning of treatment coincides with the onset of curling the inhibition is complete.

The nature of the proteins which have a function in the process of curling is unknown. Of the enzymes known to be synthesized de novo in the course of fading of the corolla, the glycosidases (Wiemken and Wiemken 1975) which are possibly involved in cell wall lytic processes deserve particular attention. Observations reported by Haxsos and Kende (1975) on the effect of *p*H upon the curling of segments suggest that, indeed, the action of mural enzymes could be an important factor in the curling reaction. Likewise, the dependence of the rate of curling on temperature points to the involvement of enzymes.

Effects of *p*H and ethylene on the curling of segments are shown in Fig. 6. It appears that the exposure of the ribs to citrate-phosphate buffer, *p*H 4.5, has almost the same effect as has the treatment with ethylene. At *p*H 5.5 the onset of curling is markedly delayed with regard to the ethylene-treated control, but still premature as compared with the untreated control. A conspicuous effect of *p*H on the rate of curling appears also from the data compiled in Fig. 6. Since the tissue is severely damaged at *p*H values below 4.5, the analysis of *p*H effects is restricted to the range above *p*H 4.5. It has yielded ample evidence that the effect of buffer solutions depends on the buffer con-

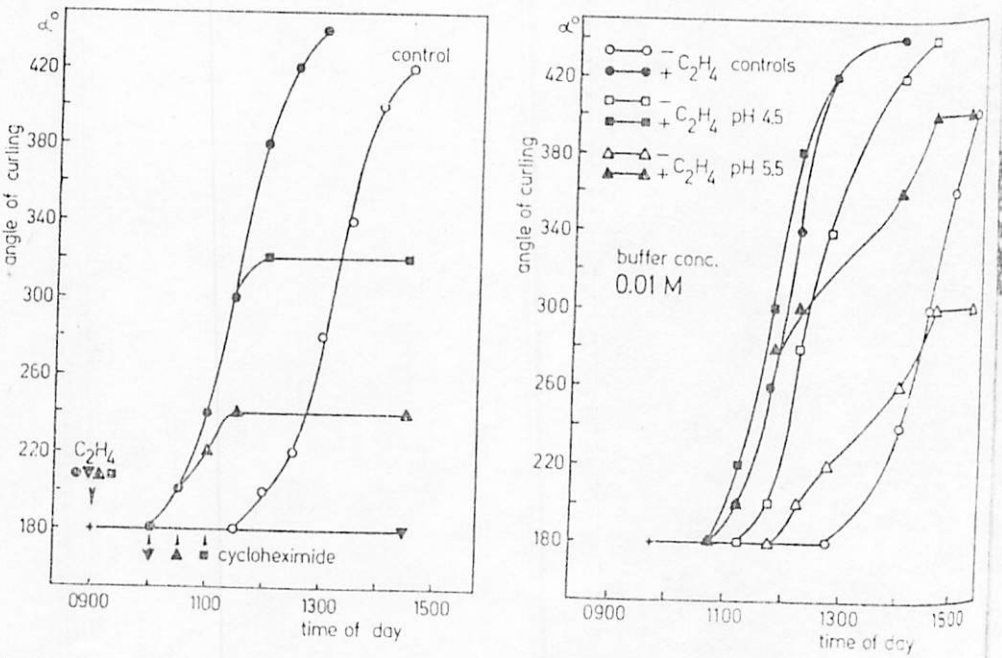


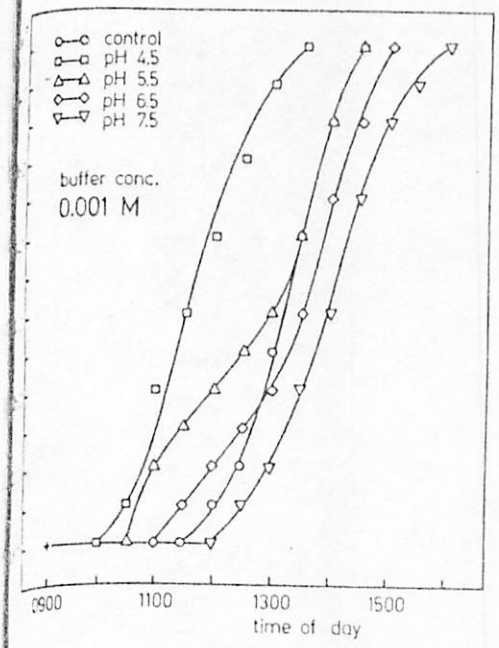
Fig. 5. *Effect of cycloheximide on segments of ribs induced with ethylene to curl up.* The segments were treated with 10 ppm of ethylene throughout the experiment and transferred to solutions containing 1 µg/ml of cycloheximide at various stages of curling.

Fig. 6. *Curling of segments of ribs in the presence and absence of exogeneous ethylene as influenced by the pH of the buffer (0.01 M citrate-phosphate) on which they are floated.*

centration in addition to pH. In the presence of 0.01 M buffer the curling proceeds to the normal final angle of 400—440° only at the optimal pH of 4.5. At pH 6.5 and 7.5 the curling is incomplete in the presence, and does not even begin in the absence of ethylene. If, however, the buffer concentration is reduced to 1 mM complete curlings can be observed over the entire range of pH values tested (Fig. 7). Under these conditions the pH determines only the onset and (pH 5.5 and 6.5) the initial rate of curling.

Hanson and Kende (1975) investigated the ethylene-induced efflux of micromolecules and ions from isolated segments of ribs. Since acid buffer solutions have similar effects on the curling process as has the treatment with ethylene, it may be concluded that this ethylene-induced efflux results in changes of pH in the mural space which, in turn, causes the change of activities of mural enzymes. In the presence of buffer solutions the changes of the actual mural pH and the curling reaction should depend on the buffer capacity. Our results obtained with two buffer concentrations are compatible with this idea. Moreover, recent results obtained by Lüscher (unpublished) demonstrate that the segments are capable of adjusting the pH of the solution on which they are floating.

The ethylene-inducible efflux of micromolecules was interpreted by Hanson and Kende (1975) in terms of turgor changes which are ultimately responsible for the curving and curling of the corolla ribs. In fact, turgor changes are involved in this



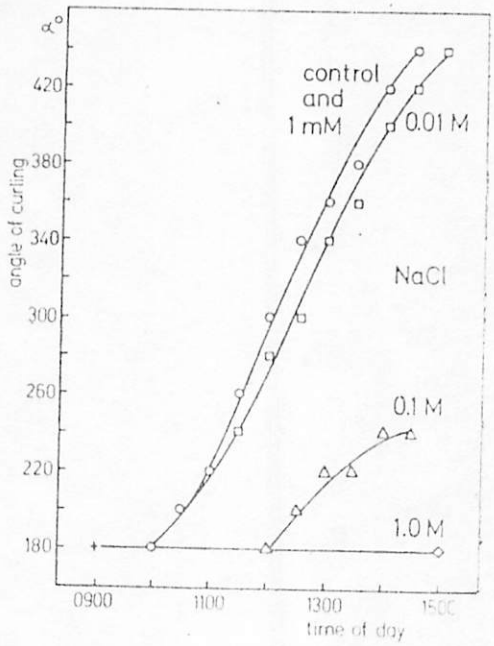


Fig. 7. *Dependence of the curling up of segments of ribs on the pH of the buffer (0.001 M citrate-phosphate) on which they are floated.*

Fig. 8. *Effect of NaCl on the curling of segments of ribs.*
The solutions for floating the tissue contained citrate-phosphate buffer (0.001 M, pH 4.5 and various concentrations of NaCl as indicated.

process. A strong inhibition and delay of curling occurs if 1 mM buffer pH 4.5 is supplemented with 0.1 M NaCl. A tenfold lower concentration of NaCl has only a small effect, whereas in the presence of 1 M NaCl the curling is completely blocked (Fig. 8).

Experiments in which the involvement of turgor changes and cell wall modifications in the curling reaction were further investigated were performed with osmotica such as glucose and sorbitol. If curled up segments ($\alpha = 360°$) are exposed to 1 M glucose the resulting reduction of turgor causes the reversion of curling (Fig. 9). However, the re-

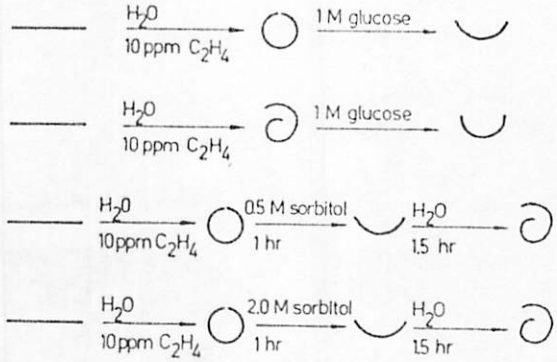


Fig. 9. *Effect of glucose on curled up segments of ribs.*
Effect of a temporary exposure to sorbitol of curled up segments of ribs showing the complete reversibility of the osmotic effect on curling.

version is incomplete; it proceeds only to an angle of about 250° and then comes to a stop. Even in the presence of 2 M sorbitol the probably complete compensation of turgor does not result in a complete reversion of curving of the segments. This finding demonstrates that the curling is associated with changes in the physical properties of the cell wall skeleton of the tissue. These changes are responsible for the shape of the tissue in the absence of turgor. Another means of demonstrating the occurrence of cell wall changes is the killing of the cells. This was done by extracting segments at various stages of curling with hot 80% ethanol. The result is presented in Fig. 10. It appears that extraction has a similar effect as has the exposure to osmotically active solutions. In all cases a reversion of curling for about 20—60° was observed. Obviously, this limit-

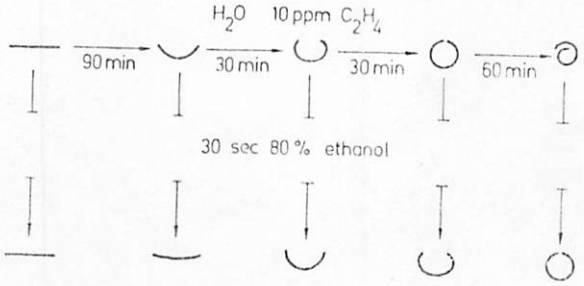


Fig. 10. *Changes of the degree of curvature upon the extraction of segments of ribs with hot 80% ethanol.*
The extraction was performed at various stages of curling as indicated.

ed degree of reversion represents the elastic component of the cell wall structure which turgor pressure transposes into curving. Another means of demonstrating the involvement of turgor is the cancelling of membrane semipermeability by exposing the segments to detergents. In the presence of Triton-×-100 or sodium dodecylsulfate (0.1%) the curling is completely inhibited.

Changes in the physical properties of cell walls in the course of curling can also be deduced from the following experiments. The successful osmotic reversion of curling depends on the period during which the segments are allowed to stay in the rolled up condition. Fully curled up segments ($\alpha = 400-440°$) were incubated in water for periods up to 2 h and then transferred to 1 M glucose. The results depicted in Fig. 11 demonstrate that the degree of osmotic reversion is markedly reduced when the segments remain in the fully curled state for 1 h or longer. In other words, the elastic component of cell wall structure disappears gradually when the movement of the tissue has been completed.

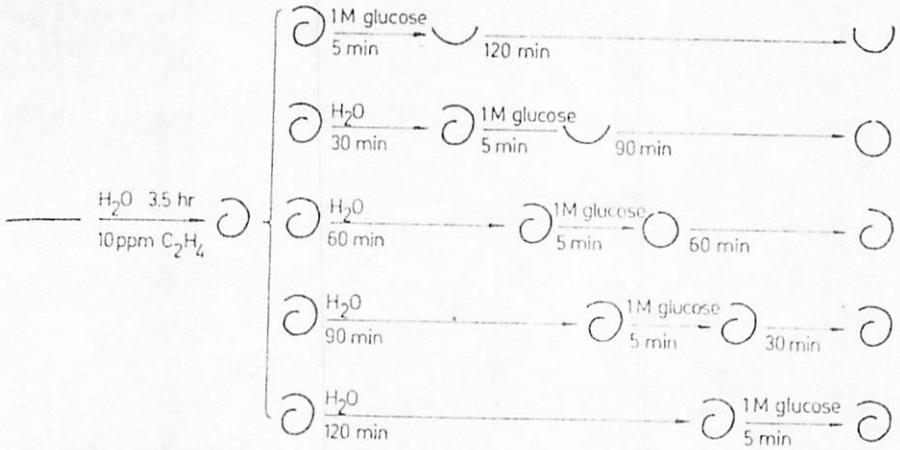


Fig. 11. *Stabilization of the final shape of curled up segments of ribs.*
The tissue was allowed to stay in the curled up state for various periods of time and then subjected to an osmotic treatment.

Recent work done by Richter (unpublished) suggests that only a fraction of the tissue used in the above experiments is responsible for the curling. Excised segments of the ridges to half the depth of the leaf exhibit the same curling reaction as observed with whole ribs. The histological examination of longitudinal sections of these ridges show a compact tissue composed of long, spindle shaped cells. Curling must, therefore, be the consequence of differential growth of these cells on the inner and outer side of the ridges. We postulate that the initial curving of the tissue is caused by differential turgor changes which result in the elastic extension of cells on the outer side (ventral side of the leaf) of the ridges. Further extension requires the enhancement of cell wall plasticity which possibly involves the action of cell wall lytic enzymes. Continuous adjustment of the turgor and of cell wall modifications seem to be necessary for the com-

plete curling. At the end of this process the elasticity of the cell walls is reduced so that the final shape of the tissue is stabilized.

Ethylene appears to induce a complex of metabolic processes associated with fading of the *Ipomoea* corolla. Apart from turgor changes which are possibly associated with changes of permeability properties of the tonoplasts (Hanson and Kende, 1975) the synthesis of enzymes is controlled by this hormone. It is likely that the postulated cell wall modifications involved in the curling of ribs comprehend both degradation and synthesis of cell wall constituents. In any case the fading of the ephemeral *Ipomoea* appears to be far from being the uncontrolled decay of an organ. It appears as the final phase of development which is as highly organized and strictly controlled as is the development of the flower primordia leading to the beautiful flower.

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