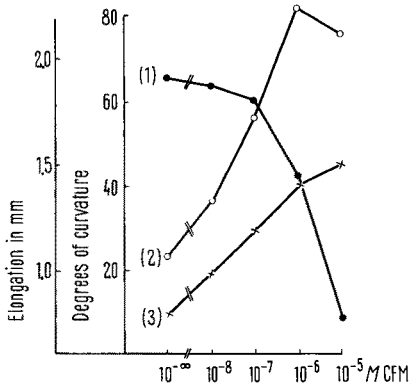
± CFM (dark, 27 °C). The Figure shows the curves received 4 h later: $10^{-6}M$ CFM induces a clear inhibition of geotropism.



Influence of chloroflurenol-methyl (CFM): (1) Geotropism of wheat coleoptile tips (degrees of curvature); 7 experiments (59–66 tips/concentration). (2) Elongation of wheat coleoptile tips (mm); 7 experiments (70 tips/concentration). (3) Elongation of wheat coleoptile sections (mm); 11 experiments (110 sections/concentration).

Elongation: The wheat coleoptiles were grown as in the experiments on geotropism. For the tests we used either 5 mm long tips (tip test), or 3 mm long sections cut 2 mm below the tips (section test). They were incubated in small test dishes with 1 ml of NITSCH buffer ± CFM. The Figure shows the elongation received after 6 h: The same CFM concentrations which depress the geotropic reaction, very clearly stimulate the elongation of tips and of sections.

Consequently, the inhibition of tropisms by morphactins is not mediated by a growth depression. Morphactins seem to influence tropisms in a more specific manner.

Zusammenfassung. Die als Wachstumsinhibitoren bekannten Morphaktine beeinflussen den Geotropismus nicht durch eine Wachstumsdepression: Konzentrationen, die den Geotropismus von *Triticum*-Coleoptilen hemmen, fördern deren Streckungswachstum.

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Abscisin II-Kinetin Interaction in Leaf Senescence

Recently, there has been renewed interest in the problem of ageing and senescence of intact as well as isolated plant parts[1]. It has been shown that the functional life span of leaf discs of various plants can be greatly extended experimentally by a variety of growth substances including auxins, gibberellins and kinins[2]. This hormonal retardation of leaf senescence is linked with maintenance or the enhancement of protein and RNA synthesis, and is often controlled through maintenance of DNA as a functional template for DNA-dependant RNA synthesis[3]. In contrast, a naturally occurring abscission accelerating substance, abscisin II[4], has recently been characterized as a senescence-hastening factor for leaf discs of many plants[5]. Because of its important role in induction and maintenance of dormancy, abscission and onset of senescence, abscisin II has been termed as a 'phytotranquillizer'[6]. It seemed of interest, therefore, to test the interaction between kinetin and abscisin II for the process of leaf senescence; the results are presented here.

Eight-millimeter leaf discs were punched from the mature leaves (33 days old) of *Arabidopsis thaliana* En-2. Lots of 20 leaf discs were floated onto petri dishes containing 5 ml of distilled water or aqueous solutions of the test substances. After incubation at 25 °C in dark for the desired time (Table), the chlorophyll of 10 discs was extracted separately with 10 ml solution of 80% acetone and the optical density of the extract was measured at 665 nm.

The results presented in the Table indicate that, for leaf discs of *Arabidopsis* also, abscisin II proved a potent accelerator of senescence. Within 24 h the leaf discs that were floated on 5 ppm solution of abscisin II had already lost 3 times more chlorophyll in comparison with the control, and 5 times more in comparison with those reared in kinetin (5 ppm). After 48 h of incubation these symptoms seemed even more accentuated and the discs treated

Effect of abscisin II (AB-II) and kinetin (K) on chlorophyll degradation[a]

Treatment	Optical density after	
	24 h	48 h
Water	0.120	0.072
AB-II 1 ppm	0.085	0.034
AB-II 2 ppm	0.070	0.025
AB-II 5 ppm	0.040	0.013
K 1 ppm	0.155	0.096
K 2 ppm	0.185	0.157
K 5 ppm	0.205	0.195
AB-II 2 ppm + K 1 ppm	0.135	0.086
AB-II 2 ppm + K 2 ppm	0.160	0.100
AB-II 2 ppm + K 5 ppm	0.185	0.165

[a] Values represent optical density of 10 ml solution extract of 10 leaf discs. Initial O.D., 0.210.

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with abscisin II turned almost completely yellow and indicated very little chlorophyll. On the other hand, the leaf discs treated with kinetin appeared perfectly green and fresh. Kinetin was also successful in reversing the senescence-accelerating effect of abscisin II. This is indicated by the fact that the leaf discs reared in abscisin II (2 ppm) in presence of kinetin (5 ppm) showed as much as 8 times more chlorophyll than those floated on 2 ppm solution of abscisin II alone. Not only in leaf discs but even when whole leaves were used, similar results were obtained.

Recently, OSBORNE[3] observed that pretreatment of leaf discs with abscisin II reduced the subsequent incorporation of (^{14}C)leucine into protein. On the contrary, earlier results[2] have clearly shown that kinetin delays leaf senescence by maintaining or enhancing protein synthesis. Thus, in view of the above results, it would appear that kinetin probably reverses the senescence-accelerating effect of abscisin II by exerting its influence on protein and RNA synthesis. However, whether these results indicate genuine interaction between abscisin II and kinetin, or simply represent essentially independent effects which the plant is 'adding' or 'subtracting', is not clear at present, and remains to be decided on the basis of further experimental evidence[7,8].

Zusammenfassung. Abscisin II, eine natürlich vorkommende, die Abtrennung beschleunigende Substanz, erhöhte das Altern von Blattscheiben von *Arabidopsis* sehr. Dieser Effekt von Abscisin II blieb jedoch beinahe vollständig unterdrückt, wenn Kinetin gleichzeitig zugefügt wurde.

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Fluorescein Staining of Human Lymphocytes Induced by *Echis colorata* Venom

Echis colorata (EC) venom was shown to render guinea-pig lymphocytes susceptible to fluorescein-staining in vivo and in vitro[1]. The present study is concerned with a similar action of the venom on human lymphocytes in vitro. Leucocytes were separated from venous blood collected from healthy donors over $^1/_9$ volume 0.115 *M* Na_2 EDTA, pH 7.4, using polyvinylpyrrolidone and ammonium chloride[2]. The leucocytes were spun down for 3 min at 1000 rpm and the concentrated suspension of unwashed cells was used in the experiments. EC venom, stored in freeze-dried form, and 2 of its chromatographically separated fractions[3] – procoagulant fraction devoid of protease (gelatinase) and esterase (substrate *N*-benzoyl L-arginine ethyl ester HCl, NBC), and protease (gelatinase) fraction devoid of procoagulant but containing esterase, were used. The venom procoagulant induces thrombin formation in native as well as in EDTA-plasma[4]. The fractions were applied in concentrations having biological activity similar to that of the whole venom. Serum was prepared from spontaneously clotted blood incubated at 37 °C for 1 h, and defibrinated plasma from EDTA-plasma clotted with thrombin (2 U/ml) subsequently inactivated by similar incubation. Fluorescein (Fluorescite) was obtained from Moore Kirk Laboratories, Inc., Worcester, Mass., and bovine thrombin (Thrombin, Topical) from Parke, Davis and Co., Michigan, USA. Fluorescence was observed with a Philips-Mercury-High Pressure Lamp CS 150 in smears prepared from experimental mixtures incubated at 37 °C for 1 h, as described previously[1].

In the standard experimental mixture containing 0.1 ml leucocyte suspension (1.5×10^8 leucocytes/ml), 0.015 ml 5% fluorescein-saline solution, 0.1 ml EC venom saline solution (1000 µg dry weight, protein content 600 µg/ml), 0.1 ml thrombin-saline solution (200 U/ml), and serum up to a final volume of 1.6 ml, all lymphocytes became fluorescent. EC venom or thrombin alone did not induce fluorescence (results summarized in the Table). Inactivation of thrombin by incubation with human serum prior to addition to the EC venom-containing system prohibited lymphocyte fluorescence. Neither trypsin (twice crystallized, Worthington Biochemical Corporation, Freehold, N.J.) nor papain (twice crystallized, Sigma Chemical Company, St. Louis, Miss.) when added, instead of thrombin, in final amounts of 200 µg and 500 µg, respectively, caused fluorescence in the presence of EC venom.

Lymphocyte fluorescence induced by *Echis colorata* (EC) venom

	Medium	Lymphocyte fluorescence
ECV[a] + thrombin	serum	+
ECV	serum	–
Thrombin	serum	–
ECV + trypsin	serum	–
ECV + papain	serum	–
ECV	defibrinated plasma	+
EC procoagulant	defibrinated plasma	–
EC protease	defibrinated plasma	–
EC procoagulant + EC protease	defibrinated plasma	+

For experimental mixture see text. [a] ECV, whole venom.

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