

## Growth retardants in relation to the germination of seeds

### III. The synergistic, inhibitory effect of (2-chloroethyl)trimethylammonium chloride and coumarin on germination of kale seeds, and its reversal by kinetin and gibberellic acid

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#### I. INTRODUCTION

There are only a few reports dealing with the effect of the growth retardant, (2-chloroethyl)trimethylammonium chloride, on seed germination (cf. Cathey 1964). According to Wittwer and Tolbert (1960), CCC and related compounds strongly lower the germination power of the light-requiring seeds of lettuce (*Lactuca sativa* L.) cv. Grand Rapids. A marked germination inhibition was noted at a  $10^{-3}$  M allyl-trimethylammonium chloride concentration; this compound counteracted the promotion of germination caused both by red light and gibberellin.

Recently Khan and Tolbert (1965a, 1966a, b) reported that CCC and its analogues in a concentration of  $2.5 \times 10^{-3}$  M had no effect on germination of lettuce cv. Grand Rapids in light. IAA ( $5.7 \times 10^{-6}$  M), other indoles, and coumarin ( $6.8 \times 10^{-4}$  M) completely inhibited germination in these conditions. It is remarkable that CCC reversed inhibition brought about by either IAA or coumarin. In the latter case CCC reversal was controlled by the red-far red photoreversible phytochrome system, since the seeds germinated when the last treatment was red light; CCC did not reverse coumarin inhibition when far red illumination was used last. Since GA, and another growth retardant AMO-1618, were ineffective in counteracting the inhibition of germination caused by coumarin or indoles, the authors concluded that CCC acts at another site than gibberellin biosynthesis.

Michniewicz has found that CCC at as high concentration as 1000 mg/l exerted no effect on germination of tomato and bean imbibed at optimal temperatures. However, this growth retardant positively affected germination at lower temperatures (Michniewicz *et al.* 1965). According to Michniewicz and Chromiński (1966), CCC increases the suction pressure of wheat grains cultivated in conditions of physiological drought, that is in 0.2–0.4 M solutions of  $\text{KNO}_3$ .

In previous papers of this series a seemingly paradoxical concentration effect of DMASA (B995) on germination of the coumarin treated kale seeds has been described (Knypl 1966a, 1967a), and it has been reported that of many species ex-

mined, the seeds of *Brassica* sp. were the most sensitive to the inhibitory action of CCC (Knypl and Słupek 1967). CCC markedly potentiated the activity of coumarin as germination inhibitor; the synergistic, inhibitory effect of CCC and coumarin was reduced by kinetin (Knypl 1967b).

In the present communication some further data on the synergistic, inhibitory interaction of CCC and coumarin, and its reduction by kinetin or gibberellic acid, are presented.

Abbreviations used: CCC, (2-chloroethyl)trimethylammonium chloride; AMO-1618, 4-hydroxyl-5-isopropyl-2-methylphenyl trimethyl ammonium chloride, 1-piperidine carboxylate; DMASA, N-dimethylamino-succinamic acid (called to also B995 or Alar); Phosfon D, 2,4-dichlorobenzyl-tributylphosphonium chloride; GA, gibberellic acid; IAA, indolyl-3-acetic acid; CO, coumarin; K, kinetin.

## II. MATERIAL AND METHOD

The experiments were carried out on seeds of kale, *Brassica eleracea* L. var. *acephala* cv. niski zielony kędzierzawy (i.e. "Little Green Crinkled").

The seeds, in lots of 40, were germinated at  $24.8^{\circ} \pm 0.2^{\circ}$  C in the dark, in 10-cm Petri dishes lined with two discs of Whatman No. 2 paper moistened with 5 ml of an aqueous solution of the substance(s) to be tested. Control series were treated with distilled water. Number of germinated seeds was recorded in diffused electric illumination for 5 days at 24-hr. intervals. Emergence of the radicle was taken as the criterion of germination.

The  $4 \times 10^{-4}$  M solution of gibberellic acid was prepared immediately before use by dissolving a weighed GA sample in warm distilled water, because preliminary tests revealed that kale seeds are very sensitive to ethanol (cf. Discussion). Stock solution of coumarin, prepared by dissolving a 500 mgm sample in 500 ml of boiling distilled water under a reflux condenser, was stored for 2–3 weeks in the dark at room temperature. Stock solutions of kinetin ( $10^{-3}$  M in 0.02 N HCl) and IAA ( $5 \times 10^{-2}$  M) in ethanol, have been kept in a refrigerator; diluted solutions were freshly prepared. Solutions of CCC were prepared immediately before use.

Each test was run in triplicate and repeated 3–4 times. Significance of the results was proved by Student's t-test.

Unless otherwise indicated, the experiments were carried out in summer 1965.

## III. RESULTS

### A. Germination of seeds treated with CCC, coumarin, and CCC in combination with coumarin

The data plotted in Fig. 1 show that CCC concentrations of  $10^{-4}$  M, or less, had no effect on germination. On the contrary, more concentrated solutions of CCC, from  $10^{-3}$  M to  $9 \times 10^{-2}$  M, caused a marked reduction in germination, especially

during the first day of imbibition. Up to a  $4 \times 10^{-2}$  M concentration the inhibition brought about by CCC was transient, because — in dependence on prolonged incubation, the differences between the control and CCC-affected groups had gradually been reduced.

Table 1  
Effect of KCl and NaCl on germination of kale seeds

Treatment	Number of germinated seeds*				
	Days of germination				
	1	2	3	4	5
O	21.6	33.9	36.2	37.2	38.4
KCl, $10^{-2}$ M	21.6	33.3	36.0	36.0	36.8
KCl, $5 \times 10^{-2}$ M	16.0	32.9	34.9	35.6	36.5
KCl, $10^{-1}$ M	9.4	32.5	34.7	35.5	36.5
NaCl, $10^{-2}$ M	19.7	31.5	35.4	36.7	37.0
NaCl, $5 \times 10^{-2}$ M	11.6	30.2	34.7	35.4	36.4
NaCl, $10^{-1}$ M	4.9	29.4	30.2	33.4	34.0

\* 40 seeds = 100 per cent germination. Tests carried out in April.

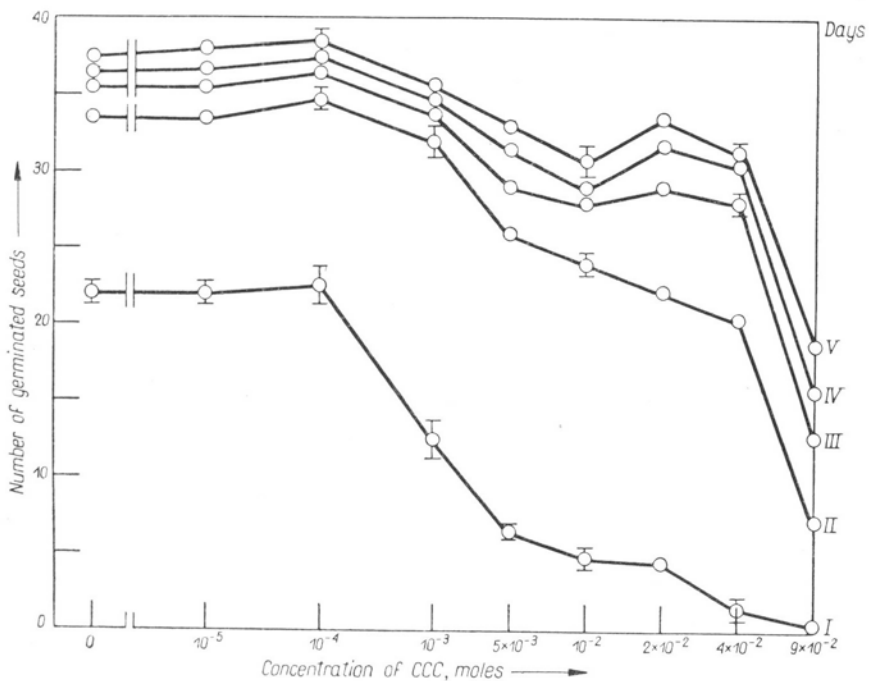


Fig. 1. Course of germination of kale seeds treated with CCC.

Vertical bars denote S.E.  $\times 2$ ; if not noted, S.E.  $\times 2$  is smaller than a circle. Days of germination are noted with Roman numerals. 40 seeds = 100 per cent germination.

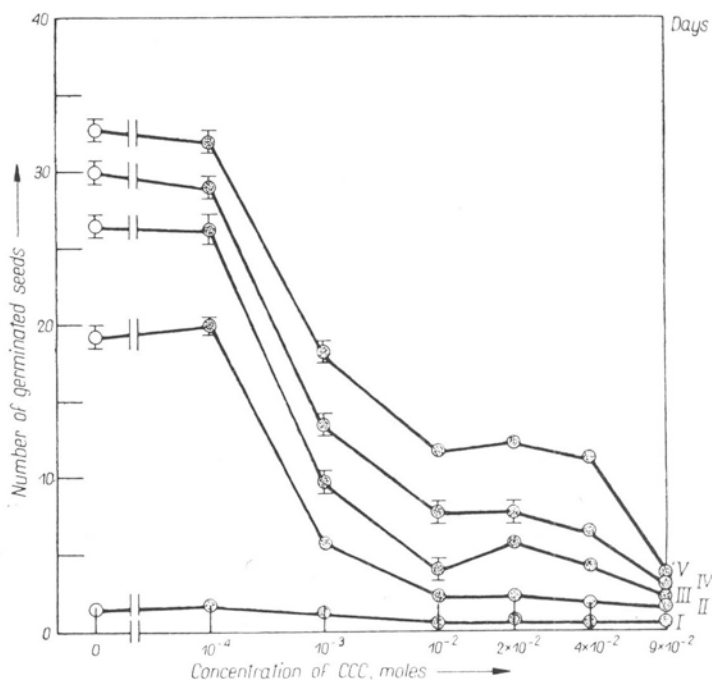


Fig. 2. Effect of CCC on germination of kale seeds treated with 100 mg/l of coumarin.

Group 0 received only coumarin; other groups — coumarin in combination with CCC. Other details as in Fig. 1.

Germination of the kale seeds was only slightly suppressed by  $5 \times 10^{-2}$  M KCl or NaCl (Table 1). It is evident, therefore, that the effect of CCC on germination is specific, not caused by osmotic phenomena.

Coumarin (100 mg/l) had drastically reduced germination of the kale seed during first 24 hours (Table 2). On the second day in this group the germination per-

Table 2  
Effect of coumarin and CCC on germination of kale seeds

Treatment**	Number of germinated seeds* $\pm$ S.E.				
	Days				
	1	2	3	4	5
O	22.2 $\pm$ 0.7	33.6 $\pm$ 0.4	35.4 $\pm$ 0.4	36.5 $\pm$ 0.4	37.2 $\pm$ 0.5
Coumarin	3.3 $\pm$ 0.5	16.3 $\pm$ 0.7	22.2 $\pm$ 0.8	25.0 $\pm$ 0.9	28.0 $\pm$ 0.8
CCC	6.6 $\pm$ 0.3	25.9 $\pm$ 0.4	29.1 $\pm$ 0.6	31.5 $\pm$ 0.6	32.9 $\pm$ 0.5
Coumarin + CCC	0.9 $\pm$ 0.1	3.3 $\pm$ 0.1	4.7 $\pm$ 0.2	6.5 $\pm$ 0.2	7.4 $\pm$ 0.4

\* 40 seeds = 100 per cent germination.

\*\* Concentrations: coumarin, 100 mg/l; CCC,  $5 \times 10^{-3}$  M.

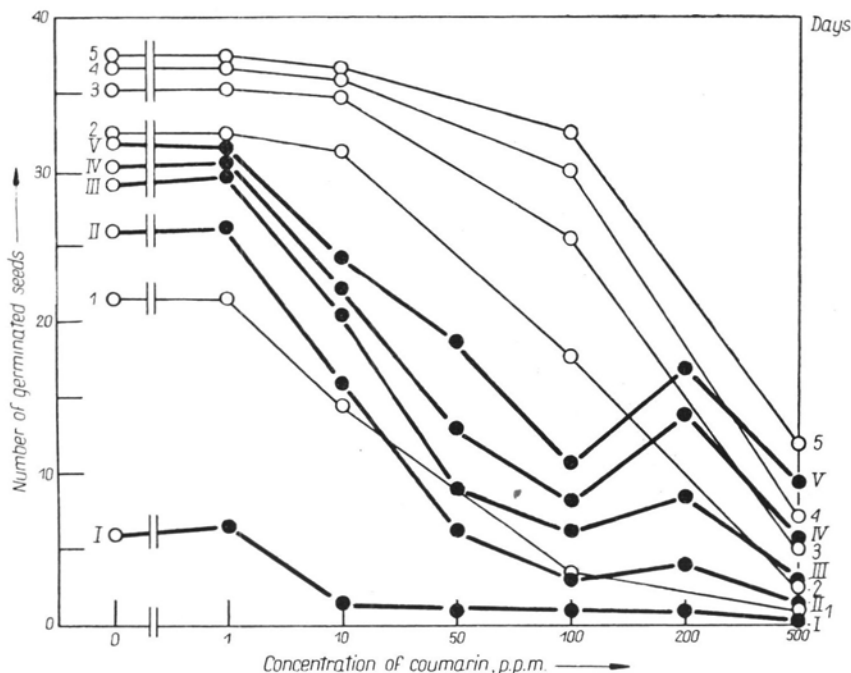


Fig. 3. Effect of coumarin on germination of kale seeds treated with  $5 \times 10^{-3}$  M CCC.

Thin lines, denoted with Arabic numerals: Germination of seeds treated with 0–500 mg/l of coumarin. Thick lines denoted with Roman numerals: Germination of seeds treated with CCC in combination with coumarin (black circles) or only with CCC (open circles). Experiments were carried out in March–April.

40 seeds = 100 per cent germination

centage was about 50, but subsequently the net difference in comparison with the control progressively diminished and on the fifth day the number of germinated seeds in the treated sample was about 25 per cent lower than in the control. Coumarin in this concentration, like CCC, did not permanently inhibit germination (*cf.* Figs. 1 and 2).

Addition of  $10^{-4}$  M CCC to coumarin had no significant effect on germination. In contrast, higher doses of the growth retardant in combination with coumarin led to a considerable, permanent inhibition of germination during the entire period of incubation (Fig. 2). For instance, in the sample treated with  $5 \times 10^{-3}$  M CCC and 100 mg/l of coumarin, only seven seeds germinated on the fifth day, that is about 20 per cent (Table 2).

CCC ( $5 \times 10^{-3}$  M) also markedly decreased germination in the group treated with 10, 50, 200, and 500 mg/l of coumarin (Fig. 3). Germination in the group treated with CCC in combination with 1 mg/l of coumarin was similar as in the CCC treated group. Coumarin in this concentration had no effect on germination.

It is worth noting that germination in the group treated with 200 mg/l of coumarin in combination with  $5 \times 10^{-3}$  M CCC was significantly ( $P = 0.05$ ) higher than

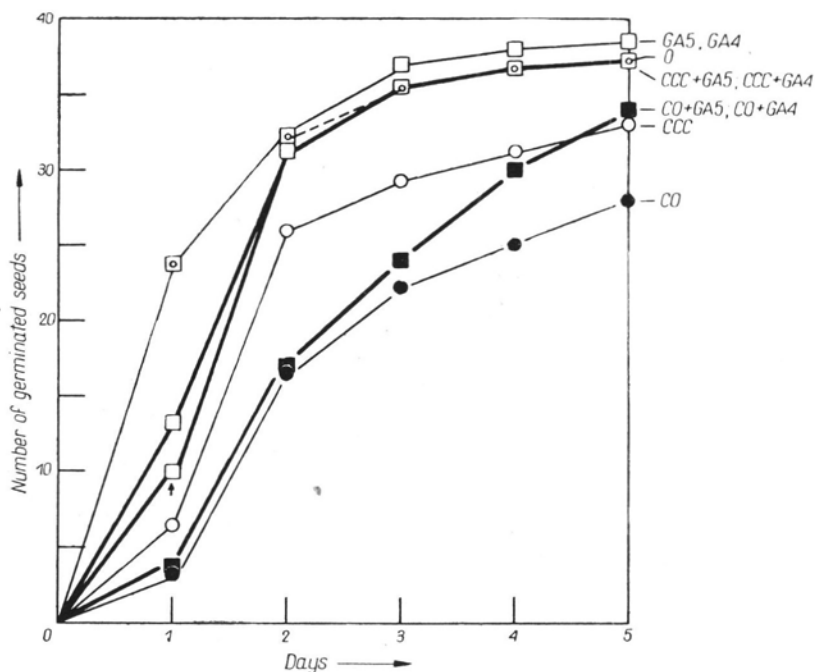


Fig. 4. Effect of gibberellic acid on germination of kale seeds treated either with CCC or coumarin. 0 — Distilled water; CO — coumarin, 100 mg/l; CCC — CCC  $5 \times 10^{-3}$  M; GA5 — GA,  $10^{-5}$  M; GA4 — GA,  $10^{-4}$  M. "+" indicates combinations (thick lines) of the substances with the same final concentration as in the case of single solutions (thin lines); for example, CO+GA5 = coumarin, 100 mg/l, and GA,  $10^{-5}$  M. Small black arrow indicates germination in the group CCC+GA5 after 24 hours.

in the groups subjected to CCC and 100 mg/l of coumarin (Fig. 3); the difference is most evident after 5 days.

Comparison of the data for germination of the 100 mg/l coumarin-treated seeds in summer (Table 2, and Fig. 2) and in spring (Fig. 3) reveals that the sensitivity of kale seed to coumarin is not constant throughout the year. This is a similar phenomenon as that described for the seeds of *Lepidium sativum* L. (Libbert 1961).

Further analyses were carried out only with the application of 100 mg/l coumarin and  $5 \times 10^{-3}$  M CCC.

#### B. Effect of gibberellic acid on germination of kale seeds treated with CCC and coumarin

The visible symptoms of the action of CCC on intact plants are opposite to those induced by GA, and are reversed by GA (*cf.* Wittwer and Tolbert 1960; Cathey 1964; Knypl 1966a). It has been revealed that CCC, from the physiological point of view, competes with the GA system (Lockhart 1962) perhaps by inhibiting the biosynthesis of gibberellins (Ninnemann *et al.* 1964; Harada and Lang 1965).

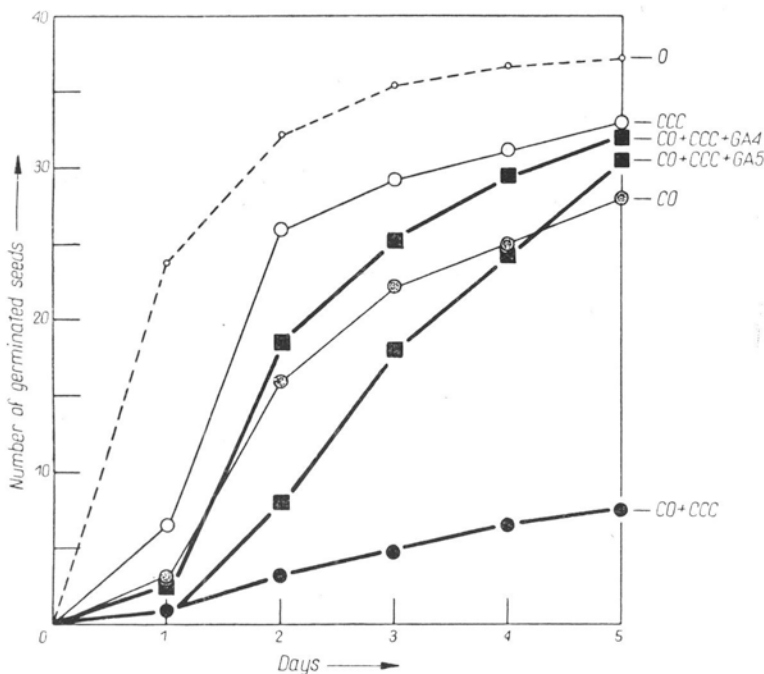


Fig. 5. Effect of gibberellic acid on germination of kale seeds pretreated with CCC in combination with coumarin.

Details as in Fig. 4.

As seen from the data plotted in Fig. 4, gibberellic acid in  $10^{-5}$  M and  $10^{-4}$  M had no effect on the germination of kale seeds. When applied with CCC, it first reduced and finally completely reversed the inhibitory effect of this compound: After two days of imbibition the germination percentage in the CCC plus GA treated sample was the same as in the water control.

GA initially did not affect germination in the groups treated with coumarin. Nevertheless, it increased germination in this sample as measured on fourth and fifth days of incubation (Fig. 4). On the basis of these data it might be assumed that CCC affects germination by influencing the GA system in the seed; on the contrary, coumarin does not directly affect the GA system.

Gibberellic acid markedly reduced the synergistic, inhibitory effect of CCC plus coumarin. After two days of soaking in the group coumarin+CCC+GA (the latter  $10^{-4}$  M), markedly more seeds germinated than in the sample treated only with coumarin, and on the fifth day the number of germinated seeds in the former group was nearly equal to that in the CCC-alone group (Fig. 5, curves CO+CCC+GA4, CO and CCC, respectively). GA in a smaller dose ( $10^{-5}$  M) was also effective in this respect, although during the initial 24 hours it did not increase germination of the coumarin+CCC imbibed seeds. (Fig. 5, curves CO+CCC+GA5, and CO+CCC, respectively).

### C. Effect of kinetin on germination of kale seeds treated with CCC and coumarin

Kinetin ( $10^{-7}$ – $10^{-4}$  M) had no effect on germination when given alone; at  $5 \times 10^{-4}$  M it inhibited germination (Fig. 6).

Kinetin in a concentration of  $10^{-4}$  M was as active as GA ( $10^{-4}$  M) in reversing the symptoms of CCC action (Fig. 7, curve CCC+K4). A higher dose ( $5 \times 10^{-4}$  M) of kinetin was inhibitory as measured on the 2nd to 5th day of incubation; in spite

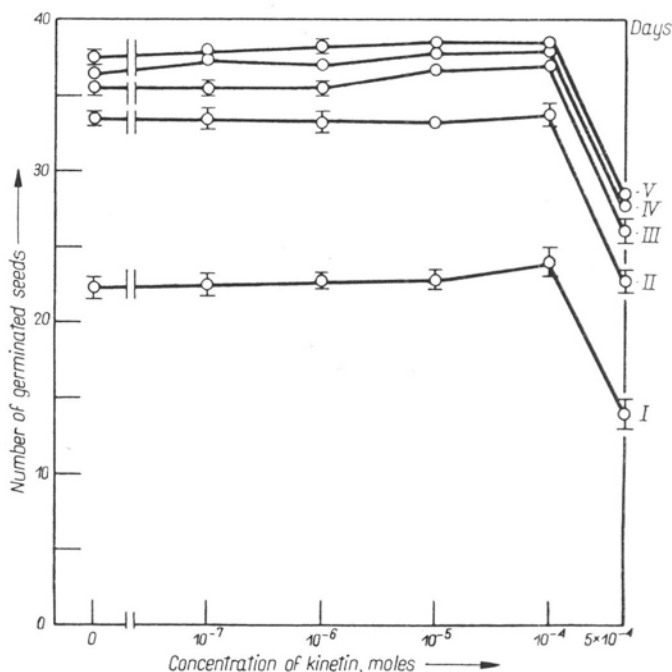


Fig. 6. Course of germination of kale seeds treated with kinetin.

Details as in Fig. 1

of this fact, it initially markedly reduced CCC inhibition (Fig. 7, curve CCC+K5 $\times$ 4). The curves representing the course of germination in  $5 \times 10^{-4}$  M kinetin, and CCC+kinetin ( $5 \times 10^{-4}$  M) are almost identical.

Kinetin at the optimal concentration of  $10^{-4}$  M markedly reduced the inhibitory effect of coumarin on the germination of kale seeds (Fig. 8, curve CO+K4); at lower concentration it also was effective in this respect (Fig. 8, curve CO+K5). A  $5 \times 10^{-4}$  M kinetin solution decreased germination as measured on the 2nd to 5th day of incubation; however, it markedly reduced coumarin inhibition after first 24 hours (Fig. 8, curve CO+K5 $\times$ 4) despite the fact that this dose of kinetin alone significantly reduced germination as compared with the water control (Fig. 8, curves K5 $\times$ 4, and 0).

Kinetin  $10^{-4}$  M was slightly more effective than GA ( $10^{-4}$  M) in overcoming



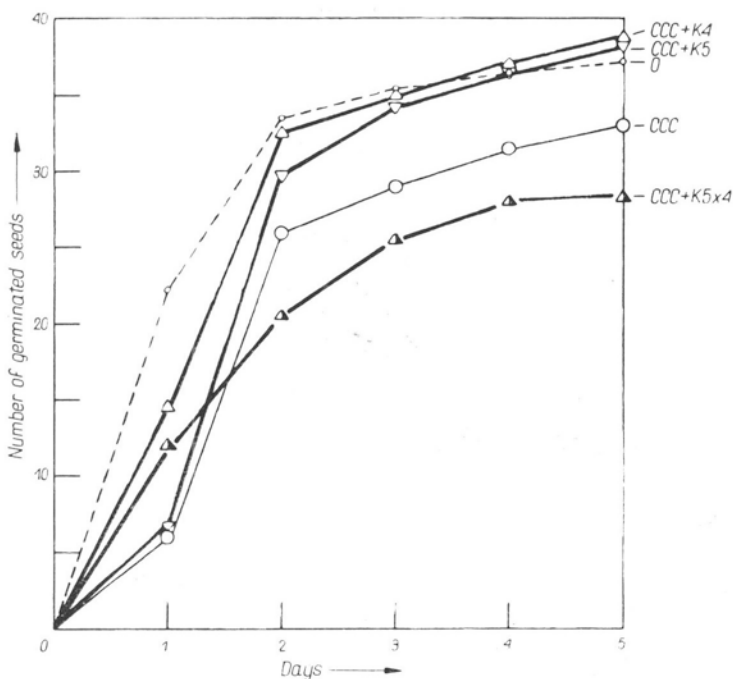


Fig. 7. Effect of kinetin on germination of kale seeds treated with CCC.

0 — Distilled water; CCC— $CCC\ 5 \times 10^{-3}$  M; K5 — kinetin,  $10^{-5}$  M; K4—kinetin,  $10^{-4}$  M; K5x4 — kinetin,  $5 \times 10^{-4}$  M. "+" indicates combinations of CCC and kinetin with the same final concentration as in the case of single solutions; e.g. CCC+K5x4 = CCC,  $5 \times 10^{-3}$  M, and kinetin,  $5 \times 10^{-4}$  M. Germination in the group treated with kinetin alone is presented in Fig. 6.

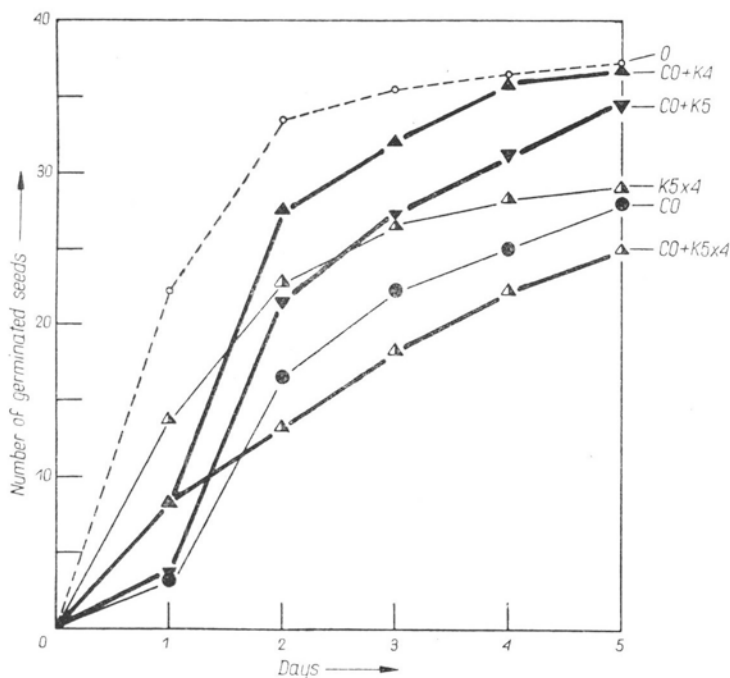


Fig. 8. Effect of kinetin on germination of kale seeds treated with coumarin.

CO — coumarin, 100 mg/l; other details as in Fig. 7; "+" indicates combinations of coumarin with kinetin.

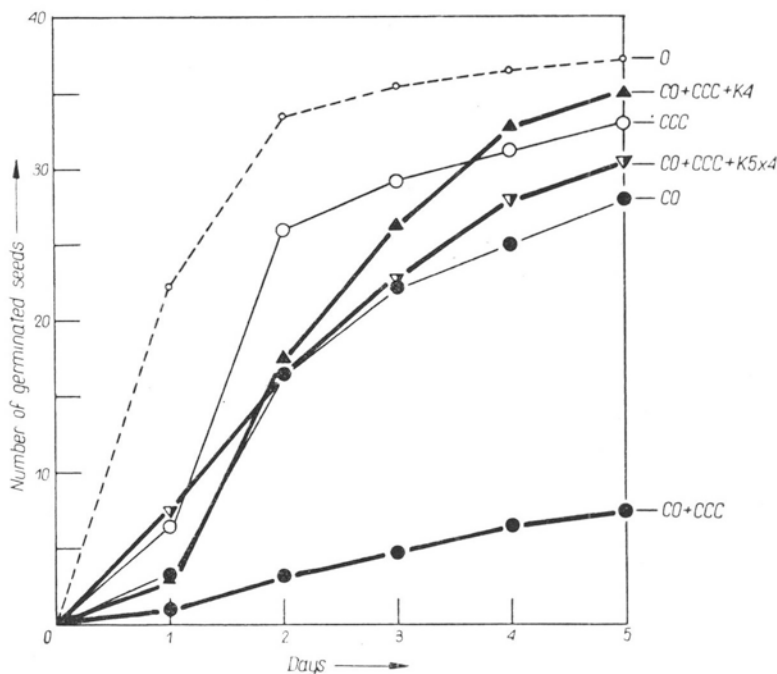


Fig. 9. Effect of kinetin on germination of kale seeds treated with CCC in combination with coumarin.

0 — Distilled water; CO — coumarin, 100 mg/l; CCC —  $5 \times 10^{-3}$  M; CO+CCC+K4 — coumarin, 100 mg/l, plus CCC,  $5 \times 10^{-3}$  M, plus kinetin,  $10^{-4}$  M; CO+CCC+K5x4 — coumarin, 100 mg/l, plus CCC,  $5 \times 10^{-3}$  M, plus kinetin  $5 \times 10^{-4}$  M; CO+CCC, coumarin, 100 mg/l, plus CCC,  $5 \times 10^{-3}$  M.

the synergistic, inhibitory effect of CCC combined with coumarin (Fig. 9, curve CO+CCC+K4, and Fig. 5, curve CO+CCC+G4). It is remarkable that a supra-optimal dose of kinetin,  $5 \times 10^{-4}$  M, was the most effective in this respect as measured after 24 hours of imbibition.

#### D. Effect of IAA on germination of kale seeds treated with CCC and coumarin

Indolyl-3-acetic acid overcame the inhibitory effect of CCC on growth of *Avena* coleoptile sections (Kuraishi and Muir 1963), *Avena* primary leaf sections (Cleland 1965), sunflower hypocotyl segments (Knypl 1964, 1966b), and stem segments of Alaska peas with growth retarded by CCC (Kuraishi and Muir 1963). Direct analysis revealed that the diffusible auxin from stem apices of pea plants whose growth was retarded by CCC was only one-seventh the amount of diffusible auxin from normal plants (Kuraishi and Muir 1963). These data suggest that CCC may act, in some cases at least, by interfering with the auxin metabolism (*cf.* Knypl 1966a). In accordance with this assumption is the finding that CCC affects the IAA oxidase activity in cucumber seedlings (Halevy 1963), and that it reverses the inhibitory effect of IAA on germination and growth of lettuce (Khan and Tolbert 1966b).

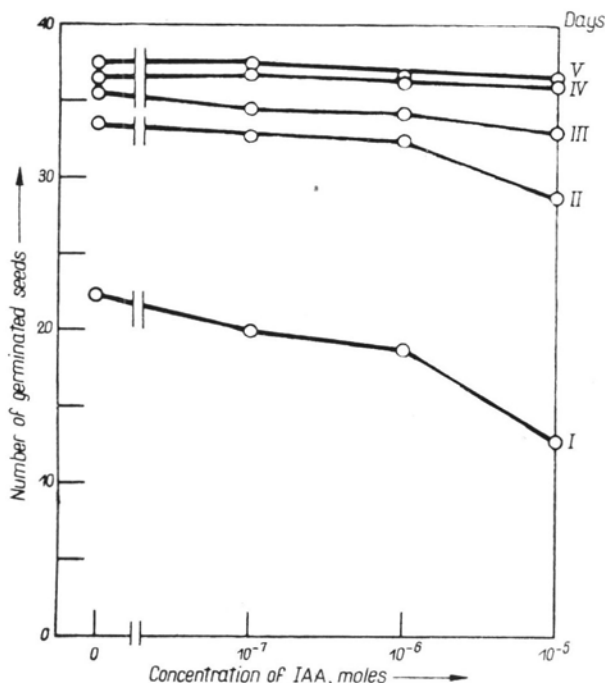


Fig. 10. Course of germination of kale seeds treated with indolyl-3-acetic acid.

Details as in Fig. 1.

As it is seen from the data of Fig. 10, IAA in a  $10^{-7}$ – $10^{-6}$  M concentration had no effect on germination of kale seeds. At  $10^{-5}$  M it inhibited germination.

IAA ( $10^{-6}$  M) in combination with coumarin markedly reduced germination as compared with the coumarin-alone group (Fig. 11, curves CO+IAA, and CO, respectively). IAA significantly reduced germination when applied in combination with CCC (Fig. 11, curve CCC+IAA), and insignificantly increased germination in combination with CCC plus coumarin (Fig. 11, curve CO+CCC+IAA).

#### IV. DISCUSSION

The study revealed that CCC retards germination of kale seeds (Fig. 1), and that a synergistic, inhibitory interaction occurs between CCC and coumarin (Fig. 2 and Fig. 3). This fact is of special interest in view of a recent report that CCC in a  $2.53 \times 10^{-3}$  M concentration completely overcomes the inhibitory effect of coumarin (100 mg/l) on germination of the photosensitive lettuce cv. Grand Rapids seeds incubated in white light or exposed to red light (Khan and Tolberd 1965a, 1966a). In the quoted experiments CCC reversed also coumarin inhibition of growth of lettuce rootlets (Khan and Tolbert 1966a). In other tests CCC reduced the coumarin stimulated growth of sunflower hypocotyl segments (Knypl 1964). Thus, depending on a test object, CCC may enhance (e.g. kale seeds) or reduce (e.g. lettuce seeds, sunflower hypocotyl sections) the effects brought about by coumarin.

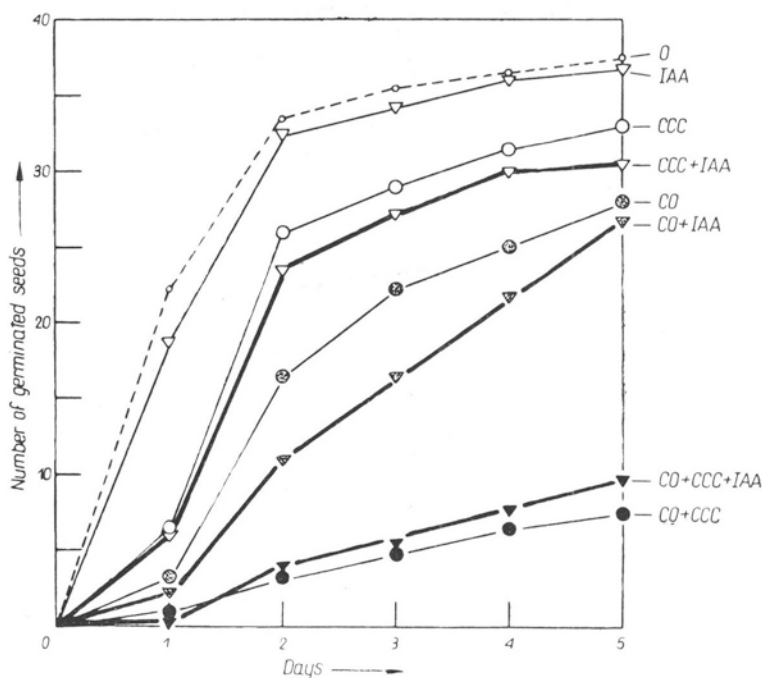


Fig. 11. Effect of indolyl-3-acetic acid on germination of kale seeds treated with CCC and coumarin applied alone or in combination.

0 — Distilled water; CO — coumarin, 100 mg/l; CCC — CCC  $5 \times 10^{-3}$  M; IAA — IAA,  $10^{-6}$  M. "+" indicates combinations of the substances with same final concentration as in the case of single solutions; e.g., CO+CCC+IAA = coumarin, 100 mg/l, plus CCC,  $5 \times 10^{-3}$  M, plus IAA,  $10^{-6}$  M.

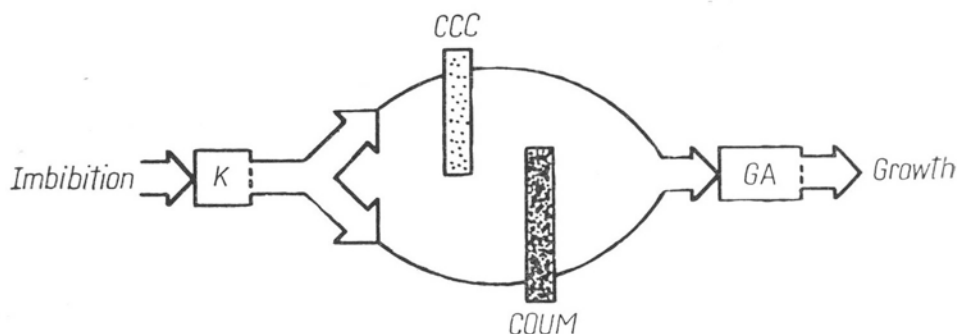


Fig. 12. Possible sites of action of CCC and coumarin in the germinating seed of kale.

K — Kinetin controlled alternative metabolic pathways; CCC, presumed position of the CCC-induced metabolic block (biosynthesis of gibberellins?); COUM, presumable position of the coumarin-induced metabolic block in a phase of activation; GA, the gibberellin stimulated phase of early growth, i.e. protrusion the root tip through the seed-coat.

The data of Figs 4 and 7 show that the marked short-lived inhibitory effect of CCC on germination is probably not a result of CCC-blockage of gibberellin biosynthesis (cf. Ninnemann et al. 1964, Harada and Lang 1965) because kinetin was as effective as GA in reversing this effect. Since GA up to the third day of soaking did not effect the germination percentage of the coumarin affected seeds (Fig. 4), reversing at same time the synergistic, inhibitory effect of CCC plus coumarin, it may be concluded that (1) in the coumarin-alone treated seed the GA system is not affected, and (2) that in the seed treated with a combined solution of coumarin and CCC the GA system is affected, presumably indirectly.

In the contrast to gibberellic acid, kinetin at the optimal concentration of  $10^{-4}$  M markedly reduced the inhibitory effect of coumarin, and at the supra-optimal concentration of  $5 \times 10^{-4}$  M it was initially the most effective agent in reversing the synergistic inhibitory effect of CCC plus coumarin. It is worth noting that germination in the series treated with a combination of coumarin+CCC+kinetin (the latter  $5 \times 10^{-4}$  M) was significantly ( $P = 0.002$ ) higher than in the group treated with coumarin+kinetin ( $5 \times 10^{-4}$  M) (cf. Fig. 9, curve CO+CCC+K5 $\times$ 4, and Fig. 8, curve CO+K5 $\times$ 4, respectively). Interaction experiments in seed germination where essentially the all-or-none response among the members of a population is established, are extremely difficult to interpret and one cannot determine, without direct chemical analysis, whether there is an interaction at all, or whether the seed simply responds to the difference between a promotive and an inhibitory effect. Nevertheless, it seems clear that kinetin in the seed of kale acts as an antagonist, from the physiological point of view, of both coumarin and CCC, since kinetin alone either had no effect on germination or inhibited this process, reversing or reducing at the same time the coumarin and CCC inhibitions. Kinetin in combination with red light reversed the coumarin inhibition of germination of lettuce seeds (Khan and Tolbert 1965b), decreased the inhibitory effect of coumarin on the growth of the first leaf of maize (Knypl 1966c), and counteracted the coumarin promoted growth of sunflower hypocotyl sections (Knypl 1966b). On the other hand,  $10^{-6}$  M kinetin reduced to some degree the retardation of growth brought about by CCC in sunflower hypocotyl sections (Knypl 1966b), and in a supraoptimal dose — inhibitory for the control — it reduced the CCC inhibition of growth of *Nicotiana tabacum* L. callus tissue cultured in vitro (Rennert and Knypl 1967). All these data support the validity of the assumption that kinetin may be regarded as the physiological antagonist of coumarin, and in some cases, of CCC.

It seems that the antagonistic interaction of kinetin with coumarin and CCC in the experiments reported here is restricted to germination proper, and possibly does not comprise the early phases of growth, since  $10^{-4}$  M kinetin inhibited growth of the rootlets (data not shown). It may be suggested that CCC and coumarin act on alternative metabolic pathways, leading to germination. These metabolic pathways, of an unknown nature, are probably directly controlled by kinetin. Blockage of any one of them by CCC or coumarin applied alone results only in retardation or transient inhibition of germination. On the contrary, blockage of both these pathways by CCC and coumarin applied in a combined solution, results in permanent

inhibition of germination and in disturbance of the GA system necessary for the initiation of growth, that is for protrusion of the root tip through the seed-coat. Therefore, both kinetin and gibberellic acid overcome the synergistic, inhibitory effect of CCC and coumarin, but kinetin acts here as "anti-coumarin" and possibly "anti-CCC" by stimulating the processes inhibited by coumarin and CCC, while externally applied GA substitutes endogenous gibberellins, the synthesis of which was disturbed by CCC and coumarin inhibition of some preceding metabolic links (cf. Fig. 12). Thus,  $10^{-4}$  M kinetin would act as a stimulator of a phase of activation in imbibed seeds (cf. Evenari 1957) and as an inhibitor of growth, GA as a stimulator of a phase of early growth, coumarin and CCC as inhibitors of the phase of activation. Coumarin does not directly affect the GA system in the kale seed (Fig. 4), and in the seed of lettuce (Mayer 1959, Khan and Tolbert 1965a). If we assume that kinetin directly or indirectly stimulates the synthesis of gibberellins, it may be suggested that CCC delays germination by inhibiting the synthesis of gibberellins; however, it is more probable that the primary site of action of CCC in the germinating seed of kale is beyond the GA system (cf. also Khan and Tolbert 1966 a, b).

Khan and Tolbert (1966 b) reported that CCC reversed the inhibitory effect of IAA and other indoles on germination of lettuce seed cv. Grand Rapids. In the present experiments IAA potentiated the inhibitory activity of coumarin, being without effect on the activity of CCC. It seems, therefore, that in the case of kale seed CCC does not act as the antagonist of auxin.

It may be of interest to note that in preliminary tests GA did not reduce the effects brought about by CCC or CCC plus coumarin. In those tests proper dilutions of GA were prepared from ethanolic GA stock solution with evaporation of ethanol on a boiling water bath. Further experiments revealed that seeds of kale are very sensitive to ethanol, and that trace amounts of ethanol present in GA solutions prevented or markedly reduced the physiological activity of GA. For this reason, in the analyses here described GA was dissolved in warm distilled water immediately before use.

A synergistic inhibitory interaction, and its reversal by kinetin, were shown to occur also between coumarin and other growth retardants, Phosfon D and DMSA (B995).

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## SUMMARY

1. CCC delays germination of kale seeds. This effect is initially reduced and finally completely reversed by GA or kinetin, but not by IAA.

2. Inhibition of germination by coumarin is overcome by kinetin, but not by GA or IAA. A positive effect of GA is noted only after 4–5 days of incubation. IAA potentiates the inhibitory activity of coumarin.

3. A synergistic, inhibitory interaction occurs between coumarin and CCC. Coumarin plus CCC inhibition of germination is reduced by GA and kinetin, but not by IAA.

4. It is suggested that kinetin—a stimulator, coumarin and CCC—inhibitors, act on a phase of activation. It is possible that coumarin and CCC block alternative metabolic pathways leading to germination in the seed of kale. These pathways are directly controlled by kinetin. GA reverses the synergistic inhibitory effect of coumarin and CCC by substituting endogenous gibberellins—necessary for early phases of growth, the synthesis of which was blocked by coumarin- and CCC-inhibition of the preceding steps in metabolism of the germinating seed.

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### *Retardanty wzrostu i kiełkowanie nasion*

III. Synergistyczne hamujące działanie chlorku (2-chloroetylu) trójmetyloamoniowego i kumaryny na kiełkowanie nasion jarmużu, *Brassica oleracea* L. var. *acephala*, i jego odwrócenie przez kinetynę i kwas giberelinowy

### STRESZCZENIE

1. CCC opóźnia kiełkowanie nasion jarmużu. Opóźnienie to redukuje GA lub kinetyna, natomiast IAA nie wywiera żadnego działania.

2. Hamujące działanie kumaryny na kiełkowanie nasion obniża kinetyna a wzmacnia IAA. GA początkowo pozostaje bez wpływu, a pewne dodatnie działanie notuje się dopiero po 4—5 dniach hodowli.

3. Kumaryna i CCC działają synergistycznie hamująco na kiełkowanie nasion. Efekt ten obniża GA lub kinetyna, natomiast IAA pozostaje bez wpływu.

4. Przypuszcza się, że kinetyna (stymulator) oraz kumaryna i CCC (inhibitory) działają na fazę aktywacji kiełkujących nasion jarmużu. Jest możliwe, że kumaryna i CCC blokują alternatywne cykle reakcji, wiodących do kiełkowania. Oba te cykle pozostają pod bezpośrednią kontrolą kinetyny, która je wzbuja lub przyspiesza. Tak więc kinetyna, z fizjologicznego punktu widzenia, jest prawdopodobnie rzeczywistym antagonistą kumaryny i CCC. Natomiast giberelina odwraca synergistyczne, hamujące działanie CCC i kumaryny prawdopodobnie dlatego, że zastępuje endogenne gibereliny — niezbędne do zapoczątkowania wzrostu korzenia; do zablokowania syntezy endogennych giberelin dochodzi wskutek zahamowania przez kumarynę i CCC wcześniejszych reakcji metabolicznych w kiełkującym nasieniu. Jest to hipoteza robocza (ryc. 12), domagająca się sprawdzenia eksperymentalnego.