Inhibition of chlorophyll disappearance in senescing leaf tissues by coumarin and growth retardants*

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I. INTRODUCTION

The visible symptom of progressing senescence in detached leaves or excised leaf discs, namely yellowing, results from a fall of chlorophyll level, and is correlated with a progressive decrease in the levels of nucleic acids and total proteins. These changes can temporarily be arrested by application of some growth regulators rather specific for a given plant: Kinetin in *Xanthium* (Richmond and Lang 1957; Osborne 1962), *Nicotiana* (Wollgiehn 1961 and 1965; Sugiura et al. 1962), *Avena* (Gunning and Barkley 1963) and *Raphanus* (Burdett and Wareing 1966); auxin in *Prunus* (Osborne 1959; Osborne and Hallaway 1960 and 1964); gibberellic acid in deciduous woody plants (Brian et al. 1959), *Taraxacum* (Fletcher and Osborne 1965 and 1966), *Rumex* (Whyte and Luckwill 1966) and *Tropaeolum* (Beevers 1966); imidazole (Person et al. 1957); certain phenylureas (Bruce et al. 1965), etc.

The hormonal retardation of leaf senescence is associated with the maintenance of the synthesis of RNA and protein (Wollgiehn 1961 and 1965; Sugiura et al. 1962; Osborne 1962; Osborne and Hallaway 1964; Fletcher and Osborne 1966; Burdett and Wareing 1966). On the basis of these findings a hypothesis has been formulated that senescence in leaf cells is associated with the hormonal regulation of protein and nucleic acids synthesis; auxins, cytokinins or gibberellins retard senescence by maintaining the DNA as a functional template for the synthesis of messenger RNA, directing the synthesis of proteins (Osborne 1965).

CCC* and DMASA failed to postpone chlorophyll degradation in the leaf discs of *Solanum tuberosum* 'Aquila' X *S. demissum* (Bruinsma 1966). Nevertheless, there are several lines of indirect evidence that growth retarding chemicals (Cathey 1964; Michniewicz 1964) can possibly be active as the regulators of senescence processes. For example, CCC prolonged life-span of etiolated wheat seedlings (Linser and Farryahi-Aschtiani 1965); CCC and DMASA prolonged the life of cut flowers (Halevy and Wittwer 1966); DMASA delayed maturation and absce-

* The following abbreviations will be used: AMO-1618, 4-hydroxy-5-isopropyl-2-methylphenyltrimethyl ammonium chloride, 1-piperidine carboxylate; CCC, (2-chloroethyl) trimethylammonium chloride; DMASA, N,N-Dimethylaminoacetaminic acid; Phosfon D, 2,4-dichlorobenzyltributyl-phosphonium chloride; GA, gibberellic acid.
sion of the fruit of *Prunus malus* L. (Edgerton and Hoffman 1966), etc. In 1963 Humphries suggested that the shortening of stems by CCC may delay senescence; according to Birecka (1966), CCC delays ageing of lower leaves of oat and wheat.

Plants of many species produce greener leaves when treated with growth retarding chemicals (Cathey 1964). Recently Halevy and Wittwer (1965) reported that CCC and DMASA retard the degradation of chlorophyll in *Phaseolus vulgaris* L. var. Contender. CCC and DMASA retard also senescence in the tissue of lettuce var. Grand Rapids, being inactive in broccoli (Halevy et al. 1966). The deterioration of discoloration of mushrooms (*Agaricus campestris*) was inhibited by DMASA but not by CCC or *N*-benzyladenine (Halevy et al. 1966; Halevy and Wittwer 1966).

DMASA has been reported to decrease the rate of chlorophyll degradation in isolated cells of tobacco (Jyung et al. 1965). According to Ruddat and Troxler (1966), AMO-1618 increased the content of chlorophyll in the leaves of *Steria rebaudiana*. In contrast, AMO-1618 and CCC inhibited the synthesis of chlorophyll in the cotyledons of *Hirschfeldia incana* (Negbi and Rushkin 1966).

The present experiments were undertaken in order to study the effect of three growth retarding chemicals (CCC, Phosfon D, DMASA) and coumarin on chlorophyll degradation in leaf discs of five plant species, incubated in the dark. Coumarin (Mayer and Poljakoff-Mayber 1961) was among the substances tested because it has been suggested that it can be active as regulator of senescence (Knypl 1965).

II. MATERIAL AND METHODS

1. Plants, and incubation of leaf discs. Five species of plants, *Brassica oleracea* L. var. acephala cv. 'niski zielony kędzierzawy', *Helianthus annuus* L. var. 'Pastewny', *Phaseolus vulgaris* L. var. 'Pinto', *Cichorium intybus* L. var. 'Witloot', and *Zea mays* L. var. 'Golden Bantham', which had grown in the garden for three or four months were used. Fully expanded, upper leaves were harvested at about 10 o'clock a.m., thoroughly washed with tap and distilled water, and blotted with filter paper. 7—or 10—mm discs were punched with a cork borer from the intervein areas of the blades, distributed into groups of 22, 30 or 33, and floated on 5 or 10 ml of distilled water (controls) or aqueous solutions of the substances to be tested, poured into 5.5—cm Petri dishes. Penicillin G (60 I.U. per ml) was added to each solution.

Each disc in a given Petri dish derived from a separate leaf, but in each Petri dish of a given experimental series one disc from a given leaf was placed. That is, fifteen discs were punched from each of twenty leaves if, for example, twenty discs, were to be placed in each of all the fifteen dishes in a given series.

The discs were maintained at 25° C in darkness. After culturing for definite periods, the discs were removed from the media, washed with distilled water, blotted and either extracted with 80 per cent ethanol or dried for four hours at 104°C with subsequent weighing.
Details were as follows:

A. Kale. 7-mm discs were punched from 35—42 cm long leaves, starting 4 cm below the leaf apex; 30 discs were placed in each 5.5-cm Petri dish on 5 ml of the solution to be tested and incubated for 2, 4 or 6 days; 14 discs were used for the determination of chlorophyll content, 14 for the determination of dry matter, and remaining 2 ones were discarded; chlorophyll was extracted with 15 ml of 80 per cent ethanol.

B. Sunflower. 7-mm discs were punched from the middle part of 29—35 cm long leaves, detached from plants beginning to flower; 22 discs were floated on 5 ml of the test solution in 5.5-cm Petri dish, and incubated for 4 or 8 days.

C. Bean. 10-mm discs were punched from the leaves, harvested from plants beginning to flower; 33 discs were floated on 10 ml of the test solution in 10-cm Petri dishes, and cultured for 3, 5 or 8 days.

D. Succory. 10-mm discs were punched from 38—45 long leaves harvested from 4 month old plants, starting 10 cm below the leaf apex; 22 discs were floated on 10 ml of the test solution in 10-cm Petri dishes, and incubated for 4, 6 or 8 days.

E. Maize. 10-mm discs were punched from the middle 25 cm part of the 75—85 cm long leaves, starting 25 cm below the leaf apex and omitting the main vein; 22 discs were floated on 10 ml of a solution of the substance to be tested, poured into a 10-cm Petri dish and incubated for 3, 5 or 7 days.

2. The estimation of chlorophyll content. Leaf discs were ground with quartz sand, CaCO₃ and 80 per cent ethanol in a mortar with a pestle, and filtered. The filtrate was made up to a standard volume of 15 ml with 80 per cent ethanol. Optical density of chlorophyll extracts was measured at 666 m.μ. with a Spekol’Zeiss-Spectral-colorimeter. Optical density of the filtered culture media was also measured at 666 m.μ.

In the foregoing paragraphs the dry matter and the content of chlorophyll will be expressed in milligrams of, and as O.D. at 666 m.μ. of 15-ml ethanol extracts of chlorophyll from: fourteen 7-mm leaf discs of kale; ten 7-mm leaf discs of sunflower; ten 10-mm leaf discs of bean, succory and maize.

Each treatment was repeated 3—4 times; duplications were carried out within each treatment.

III. RESULTS

1. Kale. The excised leaf discs of kale supplied only with distilled water gradually turned yellow, especially on the 3rd and 4th days of incubation (Fig. 1A). Coumarin at 750 mg/l considerably prevented the loss of chlorophyll, slightly stimulating at the same time the loss of dry matter and the release of brown pigments into the culture media. Six days later, the coumarin-treated leaf discs were dark green and showed any visible toxic symptoms. Coumarin at 500 mg/l also markedly postponed the chlorophyll degradation but — in contrast to 0-250 and 750 mg/l — slightly stimulated the growth of some white mould; after six days the leaf discs in this series were occasionally flexible.
In the presence of Phosfon D the loss of chlorophyll was also markedly delayed (Fig. 1B). In the 6th day the discs supplied with $10^{-3} - 10^{-2}$ M of Phosfon D were uniformly green on the whole surface, free of moulds, and showed no any visible toxic symptoms. In contrast, the discs treated with $10^{-4} - 5 \times 10^{-4}$ M solutions of this growth retardant were dark green at the periphery and had a sharply separated yellow spot in the centre. At lower concentrations of Phosfon D, the central yellow spot was larger and the peripheral green well thinner.

Phosfon D at $10^{-4} - 5 \times 10^{-4}$ M stimulated growth of moulds, stimulated the release of green-brown pigments into the culture medium, and markedly stimulated

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**Fig. 1.** Effect of coumarin and growth retardants on chlorophyll degradation and the loss of dry matter in leaf discs of kale. Treatments: A — coumarin; B — Phosfon D; C — CCC. Number on the right mean days of incubation; S-6, O.D. of the culture media after 6 days.
the loss of dry matter (Fig. 1B). Nevertheless, the treated discs even on the sixth day of incubation were not flexible.

The content of chlorophyll in kale leaf discs cultured for four days in $5 \times 10^{-3} - 5 \times 10^{-2}$ M of CCC was about two times higher in comparison with the water control (Fig. 1C). $5 \times 10^{-2}$ M CCC solution was most effective in arresting the loss of chlorophyll as measured on the 6th day. Nevertheless, this solution of CCC slightly stimulated growth of moulds, and the discs became slightly flexible.

![Graphs showing the effects of coumarin and CCC on chlorophyll degradation and dry matter loss.]

**Fig. 2.** Effects of coumarin (A) and CCC (B) on chlorophyll degradation and the loss of dry matter in sunflower leaf discs. S-8, O.D. of the culture media after 8 days.

As can be seen from Table 1, DMASA was practically inactive in the preservation of chlorophyll breakdown. This compound at $10^{-2}$ M caused the appearance of necrotic lesions.

Kinetin at $5 \times 10^{-5}$ M was as active as $10^{-2}$ M of CCC in the retardation of chlorophyll breakdown. Gibberellic acid was also active in this respect (Table 2). However, in contrast to coumarin and growth retardants, kinetin and GA inhibited the loss in dry matter.

2. Sunflower. *H. annuus* is inconvenient for the senescence tests since during eight days the level of chlorophyll and dry matter in the leaf discs supplied only with water fell to about one half of the original value. Coumarin (Fig. 2A), Phosfon D (Table 3) and CCC (Fig. 2B) arrested the fall of the chlorophyll content. CCC stimulated the loss of dry matter and the release of brown pigments into the culture medium. Coumarin (750 mg/l) stimulated and inhibited the loss of dry matter as measured after 4 and 8 days, respectively. Phosfon D at the highest concentration of $5 \times 10^{-3}$ M inhibited both the loss of dry matter and the release of brown pigments; at the lowest concentration of $5 \times 10^{-4}$ M which mostly arrested the degra-
Table 1

The effect of DMASA on degradation of chlorophyll in kale leaf discs

<table>
<thead>
<tr>
<th></th>
<th>Time of incubation, days</th>
<th>Concentration of DMASA, M</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>O.D. at 666 mµ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.100</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>.240</td>
<td>.250</td>
</tr>
<tr>
<td>6</td>
<td>.100</td>
<td>.125</td>
</tr>
<tr>
<td>Dry matter, mg.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>21.5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>18.2</td>
<td>18.2</td>
</tr>
<tr>
<td>6</td>
<td>13.2</td>
<td>12.0*</td>
</tr>
<tr>
<td>S—6</td>
<td>.040</td>
<td>.056</td>
</tr>
</tbody>
</table>

* Significantly different from control at P = 0.05;
** Significantly different from control at P = 0.01.
S—6, O.D. at 666 mµ of the culture media after 6 days of incubation.

Table 2

The effects of kinetin and gibberellic acid on the degradation of chlorophyll in kale leaf discs

<table>
<thead>
<tr>
<th></th>
<th>Time of incubation, days</th>
<th>Concentrations, M</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
<td>5×10⁻⁶</td>
</tr>
<tr>
<td>O.D. at 666 mµ</td>
<td>0</td>
<td>1.100</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>.206</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>.085</td>
</tr>
<tr>
<td>Dry matter, mg.</td>
<td>0</td>
<td>21.4</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>18.0</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>13.0</td>
</tr>
<tr>
<td>S—6</td>
<td></td>
<td>.070</td>
</tr>
</tbody>
</table>

* Significantly different from control, at P = 0.01.
S—6, O.D. at 666 mµ of the culture media after 6 days of incubation.

dation of chlorophyll, this compound stimulated both the loss of dry matter (after 4 days) and the release of green-brown pigments into incubational media.

3. Bean. Coumarin at 100 and 250 mg/l accelerated the loss of chlorophyll in ‘Pinto’ bean leaf discs as measured on the 3rd and 5th days of incubation. There was only a slight loss of chlorophyll in the leaf discs treated with 750 mg/l of coumarin (Fig. 3A). At all concentrations tested, coumarin accelerated both the loss of dry matter and the release of green-brown pigments into the surrounding media. On the 8th day, the coumarin (250–750 mg/l) affected discs were flexible, and those treated with 500 mg/l were additionally mouldy.
Fig. 3. Effects of coumarin (A), Phosfon D (B), and CCC (C) on chlorophyll degradation and the loss of dry matter in 'Pinto' bean leaf disc. S-8, same as in Fig. 2.
Fig. 4. Chlorophyll degradation and the loss of dry matter in the leaf discs of succory treated with coumarin (A), Phosfon (D), BCCC (C) and kinetin (D). Other details as in Figs. 1 and 2.
Fig. 5. Effects of coumarin (A), Phosfon D (B) and CCC (C) on chlorophyll degradation and the loss of dry matter in maize leaf discs. S-7, O.D. of the culture media after 7 days of incubation.
4. Succory. At all concentrations tested, coumarin highly retarded the chlorophyll degradation in the succory leaf discs, and stimulated the loss of dry matter (Fig. 44). The discs treated with 100–750 mg/l of coumarin were flexible, and on those incubated in 250–500 mg/l solution of coumarin white mould and brown spots appeared.

Table 3
The effect of Phosfon D on chlorophyll degradation in sunflower leaf discs

<table>
<thead>
<tr>
<th>Time of incubation, days</th>
<th>Concentration of Phosfon D, M</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>O.D. at 666 μm</td>
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</tr>
<tr>
<td>0</td>
<td>0.900</td>
</tr>
<tr>
<td>4</td>
<td>0.650</td>
</tr>
<tr>
<td>8</td>
<td>0.450</td>
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<tr>
<td>Dry matter, mg.</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>14.9</td>
</tr>
<tr>
<td>4</td>
<td>11.9</td>
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<td>8</td>
<td>7.0</td>
</tr>
<tr>
<td>S–8</td>
<td>0.098</td>
</tr>
</tbody>
</table>

* Significantly different from control, P = 0.01.
** Significantly different from control, P = 0.05.
S-8, O.D. at 666 μm of the culture media after 8 days of incubation.

Table 4
Effects of DMASA and gibberellic acid on chlorophyll degradation in succory leaf discs

<table>
<thead>
<tr>
<th>Time of incubation, days</th>
<th>Concentrations, M</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
</tr>
<tr>
<td>O.D. at 666 μm</td>
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</tr>
<tr>
<td>0</td>
<td>1.350</td>
</tr>
<tr>
<td>4</td>
<td>.690</td>
</tr>
<tr>
<td>6</td>
<td>.345</td>
</tr>
<tr>
<td>Dry matter, mg.</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>23.3</td>
</tr>
<tr>
<td>4</td>
<td>19.5</td>
</tr>
<tr>
<td>6</td>
<td>13.1</td>
</tr>
<tr>
<td>S–6</td>
<td>.080</td>
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</tbody>
</table>

* Significantly different from control, P = 0.01.
** Significantly different from control, P = 0.05.
S-6, O.D. at 666 μm of the culture media after 6 days of incubation.

Similar symptoms were produced by Phosfon D at 10^-4–5x10^-4 M: The leaf discs were brown, flexible and mouldy. The discs treated with the highest dose of Phosfon D, 5x10^-3 M, were dark green, turgid and free of moulds (Fig. 4B).

CCC retarded significantly the chlorophyll degradation only at 10^-2–5x10^-2 M (Fig. 4C). In 10^-2 M concentration this compound did not induce any visible toxic symptoms; the leaf discs treated with 5x10^-2 M CCC were brown and flexible.
DMASA at the optimal concentration of $5 \times 10^{-3}$ M caused no any toxic symptoms. At $10^{-2}$ M DMASA accelerated the loss in dry matter and induced flexibility of the discs; at this concentration DMASA was mostly active as the agent preventing a fall in the chlorophyll content (Table 4).

GA at $10^{-4}$ M also retarded the degradation of chlorophyll (Table 4). Most active in this respect was $10^{-5} - 5 \times 10^{-5}$ M kinetin (Fig. 4D). In these kinetin solutions the leaf discs of succory were green even in the eighth day of incubation.

5. Maize. Coumarin, Phosfon D and CCC considerably reduced the loss of chlorophyll in excised maize leaf discs incubated in the dark for 5 or 7 days (Figs 5A–5C), and did not induce any visible toxic symptoms. Phosfon D treated maize discs were yellow in the centre and green at the periphery, free of moulds.

Table 5
The effect of kinetin on degradation of chlorophyll in maize leaf discs

<table>
<thead>
<tr>
<th>Time of incubation, days</th>
<th>0</th>
<th>$10^{-6}$</th>
<th>$10^{-5}$</th>
<th>$10^{-4}$</th>
</tr>
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<tbody>
<tr>
<td>O.D. at 666 m$^\mu$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.100</td>
<td>.154</td>
<td>.160</td>
<td>.190</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry matter, mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>33.6</td>
<td>24.9</td>
<td>23.1</td>
<td>24.5</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-7</td>
<td>.125</td>
<td>.135</td>
<td>.110</td>
<td>.125</td>
</tr>
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</table>

* Significantly different from control at $P = 0.01$.
S-7, O.D. at 666 m$^\mu$ of the culture media after 7 days of incubation.

Kinetin was slightly effective as the agent postponing the breakdown of chlorophyll in excised leaf discs of maize (Table 5).

IV. DISCUSSION

The experiments described here revealed that coumarin and growth retarding chemicals are active as agents retarding chlorophyll degradation in senescent leaf discs of five plant species. Generally speaking, coumarin and Phosfon D are more active than kinetin or Ga; CCC is as active as kinetine in kale and markedly more active in maize; whereas DMASA is the least active of the other compounds tested. In three species, *Helianthus annuus*, *Phaseolus vulgaris* and *Cichorium intybus*, the arrest of chlorophyll degradation is accompanied by the appearance of severe symptoms of an over-all intoxication of the tissues, manifested by loss in dry matter, increased release of brown pigments into the incubation medium, the appearance of brown lesions on the discs (succory, bean), and growth of moulds. It is of interest that in 'Pinto' bean, coumarin and Phosfon D initially stimulated the loss of chlorophyll.
In the other two species, *Brassica oleracea* and *Zea mays*, coumarin and growth retardants did not induce any visible toxic symptoms in maize, whereas in kale only 500 mg/l of coumarin caused a slight flexibility of the leaf discs and stimulated the growth of moulds. In kale, Phosfon D did not induce any flexibility, though $10^{-4}-5 \times 10^{-4}$ M concentration markedly stimulated the loss of dry matter and the release of brown pigments into the surrounding medium. It is striking also that the leaf discs of kale, maize and bean treated with Phosfon D were yellow in the central part and green at the periphery. This fact suggests that some interaction may occur between this growth retardant and wound hormones.

It seems that the loss of green colour and the loss of dry matter are independent processes. The former depends on the synthesis of RNA and proteins, whereas the latter is dependent on the respiratory activity of the tissue.

Coumarin markedly stimulates the oxygen uptake in sunflower seedlings *(Knypl 1964)*. At 250—500 mg/l it stimulates also oxygen uptake in the leaf tissue of sunflower, bean, kale, potato, tobacco, tomato, and others *(Knypl 1967a)*; at higher concentrations (750—1000 mg/l) it inhibits the uptake of oxygen.

The experiments carried out with the leaf tissue of kale revealed that Phosfon D in $5 \times 10^{-4}$ M and $5 \times 10^{-3}$M concentrations stimulates and inhibits respectively the O$_2$ uptake as measured after 24 and 48 hours of incubation *(Knypl and Rennert 1967)*. It seems, therefore, that the seemingly paradoxical concentration effects of coumarin and Phosfon D on the loss of dry matter in the leaf discs of kale and other species tested here, are dependent on the stimulated respiration at lower and inhibited O$_2$ uptake at higher concentrations of these compounds.

The nature of the retarding effect of coumarin and growth retardants on chlorophyll degradation in leaf discs of five plant species tested here remains obscure: is necessary to test how these compounds affect the synthesis of proteins and nucleic acids. Preliminary experiments carried out with kale revealed that coumarin, Phosfon D and CCC arrest the fall in level of crude proteins; CCC is as effective as kinetin, whereas coumarin and Phosfon D are considerably more effective than kinetin in this respect *(Knypl 1967b)*. These data suggest that coumarin, Phosfon D and CCC can be active as regulators of senescence, in kale and possibly in maize at least. However, the possibility that these compounds inhibit the activity of enzymes, catalysing the degradation of chlorophyll, or chlorophyll-protein complexes, cannot be excluded a priori.

The question of the nature of the increased retention of green colour in leaf discs of bean, sunflower and succory treated with coumarin and Phosfon D remains unanswered, since it is accompanied by a visible poisoning of the tissues.

Plants grown under the influence of growth retarding chemicals, such as CCC *(Tolbert 1960)*, Phosfon D *(Preston and Link 1958)*, DMASA *(Riddell et al. 1962)* or AMO-1618 *(Ruddat and Troxler 1966)* are dark green. Direct determinations revealed that CCC increases the chlorophyll content, both per leaf and per unit area, in tobacco *(Humphries 1963)* and *Lolium temulentum L.* plants *(Stoddart 1965)*; similar results have been reported for *Stevia* treated with AMO-1618 *(Ruddat and Troxler 1966)*. In tomato, CCC decreased the content of chlorophyll per
leaf, but increased it per weight unit (Birecka and Żebrowski 1966). These data suggest that CCC possibly does not directly affect the synthesis of chlorophyll in the leaves, although it has been reported that CCC and AMO-1618 inhibit the synthesis of chlorophyll in the cotyledons of Hirschfeldia seedlings (Negbi and Rushkin 1966). The data presented in this report and in other papers (Halevy and Wittwer 1965; Halevy et al. 1966) suggest, however, that CCC and other growth retardants, especially AMO-1681 (Ruddat and Troxler 1966) and DMASA (Jyung et al. 1965) may affect the degradation of chlorophyll.

Many thanks are due to Dr. E. Mandl (Villa d'Outremont, Vevey, Suisse) for a sample of DMASA; to Mr. E. Magasanik (Industrial Chemical and Dye Co., Inc., New York) for a sample of Phosfon D (90 per cent formulation, produced by Virginia and Carolina Chemical Co.); and to Mr. J. Roberts (Messrs. L. Light and Co., Ltd., Poyle Colnbrook) for a sample of gibberellic acid.

SUMMARY

Coumarin, Phosfon D and CCC were active in preservation of chlorophyll in senescing leaf discs of five plant species. In sunflower, succory and bean the arrest of chlorophyll degradation was accompanied with the appearance of symptoms of an overall intoxication of the tissues. Kinetin and GA prevented a fall in the level of chlorophyll in succory, inducing no any symptoms of poisoning.

Coumarin, Phosfon D and CCC at optimal concentrations were markedly more effective than kinetin in retarding the loss of chlorophyll in maize. In kale, coumarin and Phosfon D were more effective, and CCC was as effective as kinetin in retarding senescence.

Of the two species tested, kale and succory, DMASA retarded the degradation of chlorophyll only in succory.

It is suggested that coumarin and growth retarding chemicals can be active as the regulators of senescence processes in plants.

(Entered: 21.11.1966.)

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REFERENCES


Osborne D. J., 1962, Effect of kinetin on protein and nucleic acid metabolism of *Xanthium* leaves during senescence, Plant Physiol. 37; 595—602.


Inhibition of chlorophyll disappearance


Zahamowanie rozpadu chlorofilu przez kumarynę i retardanty wzrostu w starzających się tkankach liściowych

Streszczenie

Kumaryna, Phosfon D i CCC hamują rozpad chlorofilu w krążkach liściowych, hodowanych w cienności. W przypadku trzech gatunków — słonecznika, fasoli i cykori, zahamowanie rozpadu chlorofilu związane jest z wystąpieniem objawów ogólnego zatrucia tkanki. Kinetyna i kwas gibberelinowy hamując rozpad chlorofilu u cykori, nie wywołują objawów zatrucia; przeciwnie, kinetyna i GA hamują spadek suchej masy i wydzielenie zielonobrązowych barwników do płynu inkubacyjnego.

Kumaryna, Phosfon D i CCC nie wywołują objawów zatrucia u kukurydzy i jarmużu. Wymiennie trzy substancje są znacznie bardziej aktywne niż kinetyna jako czynniki zabiegające rozpadowi chlorofilu w krążkach liściowych kukurydzy. W przypadku jarmużu, kumaryna i Phosfon D są znacznie bardziej aktywne niż kinetyna, a ta ostatnia jest tak samo aktywna jak CCC w zahamowaniu rozpadu chlorofilu.

DMASA hamuje rozpad chlorofilu w krążkach liściowych cykori, a nie wpływa na ten proces u jarmużu.

Wydaje się, że retardanty wzrostu mogą być czynne jako regulatorzy procesów starzenia się roślin. Doświadczenia opisane w innej pracy (Knyp 1967b) wykazują, że kumaryna i retardanty wzrostu, hamując rozpad chlorofilu w krążkach liściowych jarmużu, hamują również rozpad białek i kwasów nukleinowych.