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Control of Senescence in Rumex Leaf Discs by Gibberellic Acid[1]

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Abstract. **The kinetics of chlorophyll and protein decomposition and the effect of gibberellic acid (GA) were examined in senescing leaf discs of *Rumex crispus* and *R. obtusifolius*. Loss of *Rumex* total chlorophyll proceeds at a slow rate for about 2 days followed by a period of rapid logarithmic decline. Chlorophyll *b* is lost at a slightly faster rate than chlorophyll *a* during senescence in discs as well as *in situ*. GA causes a complete cessation of net chlorophyll and protein degradation for several days in *Rumex*, in contrast to the incomplete senescence inhibition generally observed with cytokinins. GA is fully effective even when added at the middle of the logarithmic phase of chlorophyll loss. Senescence inhibition by GA is apparently gradually reversed upon GA removal. The cytokinins, kinetin and 6-benzylaminopurine, were also effective in *Rumex* leaf discs, indicating that the senescence retarding effect was not restricted to the gibberellins.**

The regulation of leaf senescence by cytokinins has received much attention since the 1957 report of Richmond and Lang (12). Retardation by gibberellic acid (GA) of the senescence of excised leaf tissue has been noted only recently (5) and has subsequently been reported in several species (1, 10, 13). Little information is available on the time course of senescence in such systems, with the exception of Fletcher and Osborne's work with *Taraxacum* (6). This report deals with the senescence of *Rumex* leaf discs and presents new information on the kinetics, reversibility, and specificity of the hormone response.

Materials and Methods

Mature leaves were harvested from wild plants of *Rumex crispus* and *R. obtusifolius* growing in Berkeley and Albany, California and from plants grown under controlled conditions from seeds of the wild plants. Seed dormancy was overcome by a brief red light exposure (8). The seedlings were grown in soil under a 16 hr daily photoperiod of 500 to 1000 ft-c white fluorescent light at a constant temperature of 25°. Discs 6 mm in diameter were punched from the leaf blade with a paper punch, randomized, and floated adaxial side up on water or test solutions in plastic Petri dishes. The dishes were then incubated at 30° in darkness.

Samples of the leaf discs taken initially and after periods of incubation were homogenized and used for total protein and chlorophyll determination. Protein was extracted and determined by the method of Lowry *et al.* (9) as previously described (7). Chlorophyll *a* and *b* were determined spectrophotometrically in 96 % (v/v) ethanol solution according to Wintermans and de Mots (14). Data are usually expressed as the percentage of chlorophyll or protein initially present remaining after incubation. The net decrease in tissue chlorophyll and protein content is the measure of senescence used in this work. Experiments were performed at least twice and gave reproducible results. Representative experiments are presented.

Results

The time course of chlorophyll and protein loss is illustrated in figure 1. The loss of chlorophyll always shows a distinct lag phase during which total chlorophyll decreases at a rate of less than 5 % per day. After this time chlorophyll loss proceeds at a rapid pace until more than 80 % of the initial amount is degraded. Chlorophyll loss during this period follows a logarithmic decline. The decay of chlorophyll *a* and *b* follows a similar pattern except for the following: a more rapid drop in chlorophyll *b* during the lag phase; and a less rapid decrease in *b* toward the end of the period of chlorophyll loss. The slightly greater rate of chlorophyll *b* loss during the log phase leads to an increase in the chlorophyll *a*:*b* ratio during senescence (from 3.2–4.6 in this case). A relatively faster chlorophyll *b* decline was also measured in *R. crispus* leaves yellowing on the plant.

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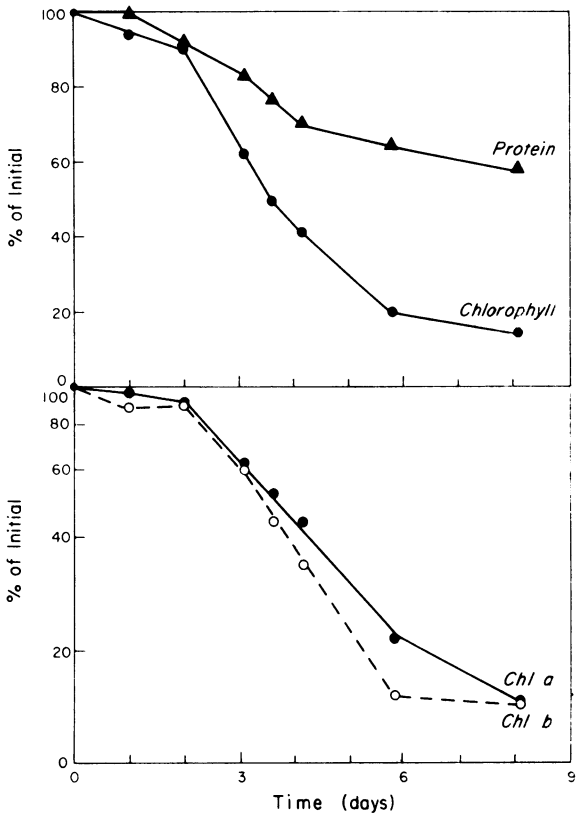

FIG. 1. Time course of senescence of *R. crispus* leaf discs on water. Upper part of the graph, protein and chlorophyll loss on a linear scale. Lower part, percentage loss of chlorophylls *a* and *b* on a logarithmic scale. Incubation time in days on abscissa.

Protein decomposition follows a pattern generally similar to that of chlorophyll except that rapid protein loss often slightly precedes that of chlorophyll (fig 1). The protein loss is not as extensive as that of chlorophyll, however, and begins to level off when only 30 to 40 % of the initial is degraded.

The effect of GA applied to *R. crispus* leaf discs on day zero is shown in figure 2. Chlorophyll loss is completely inhibited by GA for about 5 days. After this time a gradual decrease in chlorophyll begins. This decrease is not overcome by replacing the GA solution every 2 or 3 days. The protein data, although more variable, also indicate an essentially complete senescence inhibition by the GA treatment for several days.

The lag phase-log phase behavior of senescence in control discs presented the interesting possibility that chlorophyll and protein decomposition might be irreversible once the log phase of decline had begun. This was tested by GA addition in the middle of the period of rapid senescence. The results illustrated in figure 3 show a nearly complete senescence inhibition by GA addition at this time. The inhibition of chlorophyll and protein loss under these conditions was rapid and could be measured within 12 hr of GA addition.

The GA-induced senescence retardation was checked for reversibility in the following experiment. Leaf discs were floated on water for 2.5 days, treated 1 day with GA, washed, and further incubated on several changes of water to insure GA removal. The results shown in figure 4 show that the inhibition of chlorophyll loss caused by the 1-day GA treatment is gradually overcome. On day 15.5, chlorophyll loss in the previously GA treated discs is as complete as that attained by water controls. Protein loss is not shown in this figure, but it is as great in the treated discs as in the controls.

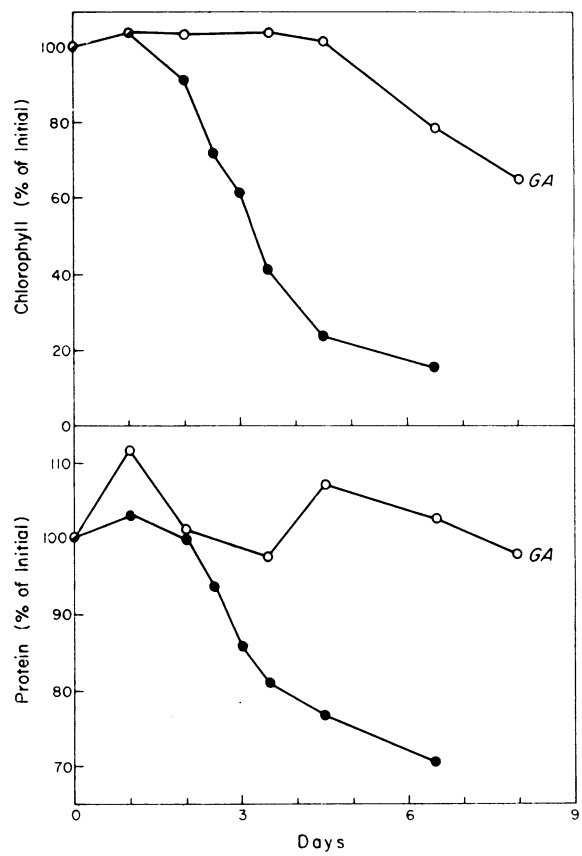

FIG. 2. Retardation of chlorophyll and protein loss in *R. crispus* leaf discs by 10 μM GA. Discs were floated on GA or water from day zero.

The specificity of the retardation of *Rumex* leaf disc senescence by particular growth regulators was tested by addition of the cytokinins, kinetin and 6-benzylaminopurine (BAP). Nearly saturated solutions of kinetin (approx 40 mg/l) caused a substantial retardation of chlorophyll loss. Lower concentrations were in some cases also effective. BAP was effective over the range of concentrations tested, (1.0–500 μM) and at short times of incubation was nearly as effective as GA on chlorophyll loss (table I). BAP and kinetin additions during the logarithmic phase of senescence retarded, but did not completely block, further chlorophyll decomposition.

The behavior of *R. obtusifolius* leaf tissue was similar to that of *R. crispus* with respect to the time course of pigment and protein loss, the effectiveness of GA during the slow and rapid phases of senescence, and the increase of the chlorophyll *a*:*b* ratio during chlorophyll decomposition in discs and *in situ*.

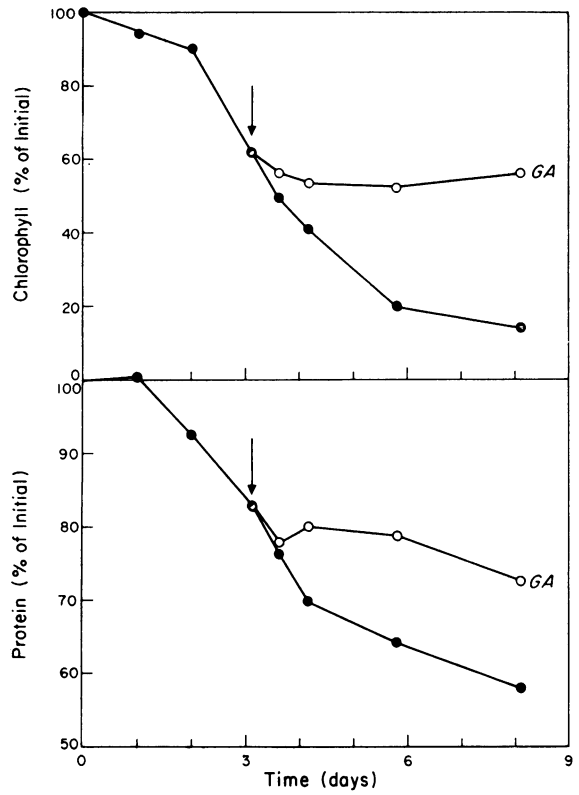

FIG. 3. Effect of mid-course addition of 10 μM GA on *R. crispus* leaf disc senescence. GA was added to 1 set of samples on day 3 (arrow).

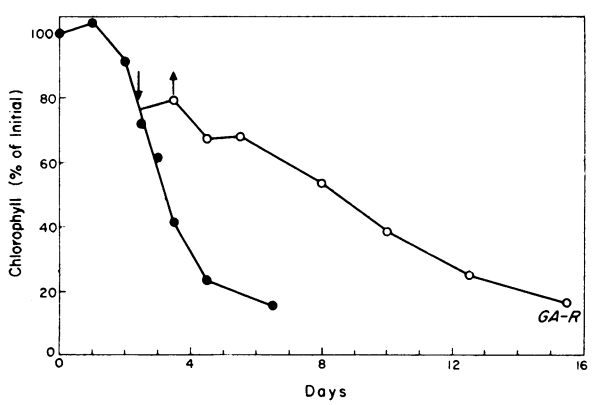

FIG. 4. Apparent reversal of the GA-induced retardation of chlorophyll loss in *R. crispus* leaf discs. Discs were incubated 2.5 days on water and then transferred to 10 μM GA (↓). After 1 day the GA solution was removed, (↑), the discs were washed, and subsequently incubated on water. The water containing the GA-treated discs was changed 4 times; 4, 24, 48, and 72 hr after GA removal on day 3.5. The treated discs (GA-R) are compared with controls floated on water from day zero.

Table I. *Effect of 500 μM 6-Benzylaminopurine (BAP) Addition on Day Zero on the Senescence of* R. crispus *Leaf Discs*

Incubation time	Chlorophyll[1]		Protein[1]	
	Control	BAP	Control	BAP
days				
0	100	100	100	100
2	90	103	91	99
3	69	101	87	93
5	27	88	65	92

[1] % Of initial remaining after incubation.

Discussion

The time course data indicated that chlorophyll and protein decomposition approximately parallel each other in *Rumex* leaf discs senescing on water. The observed logarithmic decline of chlorophyll content after the initial lag period is consistent with a mechanism which involves a constant rate of degradation acting on a fixed amount of chlorophyll which is not replenished by further chlorophyll synthesis. The faster rate of chlorophyll *b* loss relative to chlorophyll *a* during senescence in *Rumex* tissue is apparently unique and is in contrast to that found in leaf senescence of most species examined (4, 15). It would be interesting to compare the distribution of this phenomenon with the occurrence of senescence retardation by GA since the latter also appears to be found only in a limited number of species (5).

The nature of changes leading to the abrupt acceleration of chlorophyll decomposition which begins after the lag phase is obscure. The effectiveness of GA treatment even when chlorophyll and protein loss is halfway complete (fig 3) shows that the tissue is not irreversibly committed to decomposition during the initial lag period. This experiment also presents a leaf senescence system which shows a very rapid response of tissue total protein and chlorophyll levels to hormone treatment. Such a rapid effect should prove useful in separating primary from secondary biochemical events in future attempts to resolve the mode of GA action in leaf senescence.

Whyte and Luckwill (13) reported that *Rumex obtusifolius* leaf disc senescence retardation was specific to the gibberellins. Their inability to obtain senescence inhibition with kinetin may have been due to the use of an insufficiently concentrated solution. In the present study kinetin and especially 6-benzylaminopurine markedly retarded senescence in *Rumex* leaf discs. It thus appears that the senescence of leaf discs which respond to gibberellin can in most cases also be retarded by cytokinins (2, 5, 10).

The completeness of senescence inhibition by GA found in this study is striking. Beevers and Guernsey (2) reported an effect on chlorophyll loss in *Nasturtium* nearly this strong. Protein loss was, however, not completely blocked by GA in their

experiments. Inhibitions of net protein and chlorophyll loss in leaf discs which respond to cytokinins on the other hand are never complete (*e.g.* 3, 11, 12). The eventual decline seen in GA treated *Rumex* discs (fig 2) may be caused by depletion of some cellular metabolite(s) necessary to support GA action. Such a phenomenon could also lead to the apparent reversibility of the GA imposed senescence inhibition shown in figure 4. One could test this possibility by a second GA application during the period of reversal. If the apparent reversal is truly due to a washing of GA out of the cells or a consumption of GA molecules at their site of action, then this must be a rather slow process.

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