J Plant Growth Regul (1988) 7:213–226

Journal of
Plant
Growth
Regulation

Multiple Actions of Abscisic Acid in Senescence of Oat Leaves

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Received August 11, 1987; Accepted March 22, 1988

Abstract. The modifications induced by abscisic acid (ABA) on the senescence of oat leaves in darkness have been studied and are compared with its well-known effects in light. Contrary to the action in light, ABA preserves chlorophyll (Chl) in the dark almost as well as kinetin. Chlorophyll *a* is decolorized more extensively than *b*, and the content of *b* is maintained by ABA almost at its initial level for 4 days. ABA also prevents proteolysis in darkness just as completely as chlorophyll loss, the relationship of both breakdown processes to ABA concentration being strictly log-linear over the range from 1 to 100 μM. In line with this action, ABA inhibits formation of the neutral protease in the dark but not in the light. The data suggest that ABA and kinetin operate to preserve chlorophyll and protein by different mechanisms, since their actions are neither independent nor synergistic but actually interfere with one another. In this connection, protein values given by the Lowry and Bradford methods have been compared. In parallel with the effect on senescence, ABA slowly opens the stomata in the dark. This effect increases with time, and by day 3 the stomata in ABA are as open as in leaves on water in light. Thus all these effects of ABA in darkness are strikingly opposite to those commonly observed on leaves in natural lighting. In addition, ABA powerfully inhibits the formation of ethylene in the dark by the detached oat leaves, and this inhibition also tends to increase with time. Finally, a slight antagonism to ABA's action on senescence is exerted by *p*-coumaric acid in the light but not in the dark.

It is well known that abscisic acid (ABA) causes the stomata of both monocot and dicot leaves to close in light (Mittelheuser and van Steveninck 1969, Jones and Mansfield 1970, Walton 1980). Correspondingly, ABA also promotes senescence; leaves detached and floated on solutions of ABA senesce in light almost as rapidly as they would on water and in darkness (Satler and Thimann

1979). However, little attention has been paid to the responses of leaves to ABA in darkness, although this is of special interest because ABA and darkness each cause both stomatal closure and leaf senescence, so that leaves would be experiencing the simultaneous action of two similar influences. In our earlier work on the relation between stomatal closure and leaf senescence it was noted that leaves floated on ABA solution in darkness did not senesce as far as those on water, the Chl value after 4 days being 60% higher, and the amino nitrogen 56% lower, than in control leaves in the dark on water (Table 1 of Satler and Thimann 1979). At the time the significance of that was not appreciated, but since then it has developed that almost every agent that promotes Chl loss in the light delays it in the dark; these agents include, for instance, three chelating compounds, two polyamines, sodium azide and *n*-pentanol (Satler and Thimann 1983). The fact that these are chemically unrelated compounds strongly suggests that what is critical is not the chemistry of the agent but the nature of the responding system.

We felt it desirable, therefore, to study the response of detached oat leaves to ABA in darkness. The results bring out general features of ABA action, including a delay of senescence, marked opening of the stomata, and strong inhibition of ethylene production, that would not be altogether expected from current understanding of ABA function (see Walton 1980).

Opportunity has also been taken to study the antagonism of certain aromatic acids to the senescence caused by ABA, since an antagonism of these acids to ABA in regard to stomatal aperture (in light) was recently reported by two groups of workers (Laloraya et al. 1986, Rai et al. 1986).

Materials and Methods

Oat seedlings (*Avena sativa* cv. Victory seeds from Svalöv AB International, Svalöv 268 00, Sweden) were grown in vermiculite under continuous fluorescent lights ("daylight" type) giving 35 $\mu E\ m^{-2}\ sec^{-1}$ at plant level. The continuous light was used to avoid photoperiodic complications. From the 7-day-old first leaves, 3-cm segments, each weighing 16–19 mg, were excised 3 mm below the tip (the extreme tip cells being older than the remainder); the additional cut surface introduces a small wound but promotes entry of solutes. After floating on test solutions to which 100 μM crystalline penicillin G was routinely added, either in darkness or in white light at 75 $\mu E\ m^{-2}\ sec^{-1}$, they were extracted with 80% ethanol at 100°C for 10 min, total Chl estimated from absorption at 660 nm and free amino acids by ninhydrin color at 570 nm. In a few cases after 4 days in darkness small amounts of amino nitrogen were detected in the external solution; when this occurred, the amino nitrogen was estimated and added to that of the leaves. Proteins were determined after treatment with 1 N NaOH for 24 h at 25°C with the Lowry procedure by absorption at 540 nm. In several cases comparisons were made with the Bradford procedure, using the same volume (0.5 ml) of the same NaOH solution, and reading absorption at 595 nm. A discussion of the two methods follows (see Murray 1986). Chl *a* and *b* were determined in a few experiments on the ethanol extract by absorption at 665 and 650 nm, using the calculations of Mac-

Kinney (1941). For this purpose a Seitz spectrophotometer was used because of its narrower band widths.

When proteases were to be determined, 25 leaf segments were ground in 3 ml of ice-cold MES buffer pH 6.0 containing 2 mM sodium metabisulfite and filtered, and 2.5 ml was applied to a column of Sephadex G25 (Pharmacia PD-10). The exudate was discarded, and the proteins were eluted with the first 3.5 ml of the same buffer. The amino acids came out with a subsequent 6 ml of the buffer. The proteins and amino acids were determined on aliquots of these eluates. The acid protease was determined on 0.8 ml of the protein fraction plus 1.7 ml of 200 mM acetate buffer, brought to pH 4.4 and incubated 1 h at 45°C with 2% hemoglobin. The reaction was stopped by adding 0.5 ml of 20% trichloracetic acid. The neutral protease was treated in the same way at pH 6.6. After the hydrolysis the solutions were held 1 h at 0°C and centrifuged at 3200*g* for 10 min, and the liberated amino acids and peptides were determined in the supernatant by absorption at 280 nm. Abscisic acid (Sigma, ± *cis-trans*) solutions were brought to pH 6.9 with NaOH, without added buffer. Aromatic acids were CP. Tetrapeptides were from Sigma.

For ethylene measurements, 20 of the 3-cm subapical segments were floated on water or ABA solution for 20 h in an open dish in light, then placed in a 7-ml vessel with their bases dipping in 0.5 ml water or ABA. After 1, 2, or 3 days in the dark, the vessels were sealed with rubber serum stoppers for 1, 2, or 4 h (to be sure the results were not dependent on the time of sealing), and gas samples were then removed for analysis in a Perkin-Elmer gas chromatograph, using a flame ionization detector and a column of GDX 502 40–60 mesh. When repeated samples were to be taken, an equal volume of air (0.5 ml) was injected to maintain the pressure. The ethylene content was calculated from the peak height, calibrated with an injection of 5 ppm ethylene.

Stomatal aperture of the segments was measured at first with the Li-Cor instrument (Li-Cor Instruments, Lincoln, NE) and later with the more rapid Delta-T automatic porometer (Decagon Devices, Pullman, WA). The segments were lifted off the solution, hastily blotted dry, and inserted adaxial side downward in the head of the instrument within 30 sec. Five readings were taken at once and averaged. Care was taken not to expose the darkened segments to daylight for more than 30 sec. Results with the two instruments showed good agreement (Fig. 5).

Results

A Note on Protein Determinations

The measurement of total protein by a standard procedure is in good part an arbitrary one. When using a reproducible biological material, one can effectively follow changes in the total protein by a standard method, but this reflects little about possible subtler changes in the types of breakdown products. In the present series of researches on foliar senescence, the procedure of Lowry et al. (1951) has been used throughout and has given reproducible results. However, it has often been noted that a relatively large increase in free amino nitrogen is accompanied by only a small decrease in total protein. Since hydrolysis of one 35-kD protein would yield some 270 units of free amino ni-

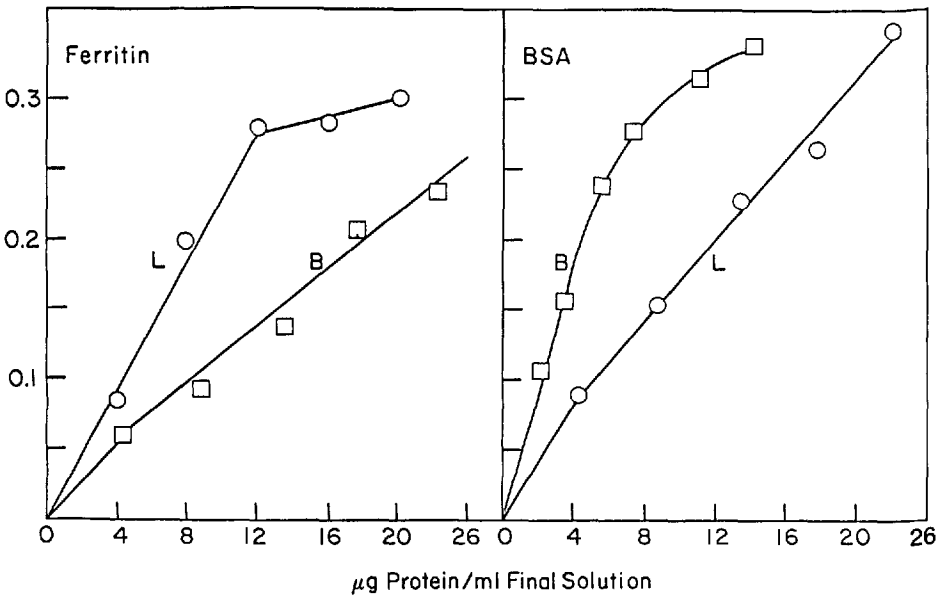

Fig. 1. Calibration curves for pure protein using the Bradford (B) and Lowry (L) procedures. Left, ferritin; right, bovine serum albumin. The reagents, temperatures, etc. were the same for both proteins.

trogen, such a discrepancy is to be expected, and the changes in free amino nitrogen are therefore more sensitive indicators of proteolysis than the small changes in protein. Nevertheless variations in results, and differences in the conclusions to which the different methods lead, have prompted a closer study of the procedures.

The two methods in common use are those of Lowry et al. (1951) and those of Bradford (1976). Although either one can usefully indicate changes in a given material, their responses to pure proteins do not agree. Figure 1 shows calibration curves made with two pure proteins, bovine serum albumin (BSA) and ferritin (Fn). For the same concentration of protein, the Bradford method gives higher readings than the Lowry with BSA, but lower readings than the Lowry for Fn. The ranges of linearity are different also. But at least with these two pure proteins the values do fall within about a factor of 2 from each other, whereas with partial breakdown products, such as peptones, the discrepancies are much larger. Thus the protein content of Bacto-Peptone, using the calibration curve for BSA, is given by the Bradford method as 8.3%, whereas the Lowry method gives more than 32%. For Tryptone the corresponding values were 10.1% and 26.5%. It was concluded that the Lowry procedure responds more strongly to intermediate breakdown products. Accordingly, since pure peptides are now available, the responses of the two methods to two pure tetrapeptides were compared. Table 1 shows that indeed the Lowry method shows apparent "protein," whereas the Bradford method does not. The values are not proportional to concentration, either. It has been claimed that the

Table 1. Apparent protein content of two pure tetrapeptides, read from the BSA calibration curve.

Tetrapeptide	Concentration ($\mu g/ml^{-1}$)	Apparent protein, μg	
		Bradford method	Lowry method
Val-Ala-Ala-Phe	625	1.1	27.6
	375	0	26.4
	125	0	20.4
Pro-Phe-Gly-Lys	625	0	72
	375	0	58
	250	0	42

Lowry method has the disadvantage that it responds to phenolics, but evidently a much more serious disadvantage is its response to simple peptides.

Figure 2 compares the results of the Lowry and Bradford procedures as used on the same cold 1 N NaOH protein extracts of the standard oat leaf segments. To facilitate comparison, the scales for the three curves are not identical. It will be seen that the Chl contents are linearly proportional to the number of leaf segments, but the protein curves are less so, and the Bradford response shows signs of saturation before the Lowry (cf. the ferritin curve in Fig. 1).

The basic difference between the methods is, of course, that the Lowry method responds to specific amino acids (partly phenylalanine and tyrosine) and certain sequences (Murray 1986), whereas the Bradford responds to polypeptides large enough to absorb a dye. Moderate liberation of amino acids or small peptides can therefore take place while leaving intact quite large portions of the molecule that still react with the dye. For this reason the Bradford method in our hands shows smaller increments in response to increasing concentrations of proteins and tends to indicate a smaller extent of proteolysis than the Lowry method. All in all, we conclude that the measurement of the increase in free amino nitrogen gives a somewhat more sensitive indication of proteolysis than the values for residual protein. Indeed, since the leaves in our experiments are detached and in contact only with air and water or buffer, free amino acids can only increase from proteolysis.

The Actions of ABA on Chl and Proteolysis

The Chl contents of the leaf segments after incubation on a series of ABA concentrations for 5 days in light or 3 days in darkness are compared in Fig. 3. The almost log-linear decrease in Chl caused by ABA in light (open circles) is an extension of previous findings of numerous workers (e.g., Mittelheuser and Van Steveninck 1969, Ho et al. 1985). Correspondingly, the leaf segments in darkness (solid circles) show that ABA caused almost a log-linear protection of Chl against the dark-induced decrease. The highest concentration used does not quite maintain the Chl at the same level as in light, but it reaches 82% of that level.

When Chl *a* and *b* were determined separately, it was clear that the protec-

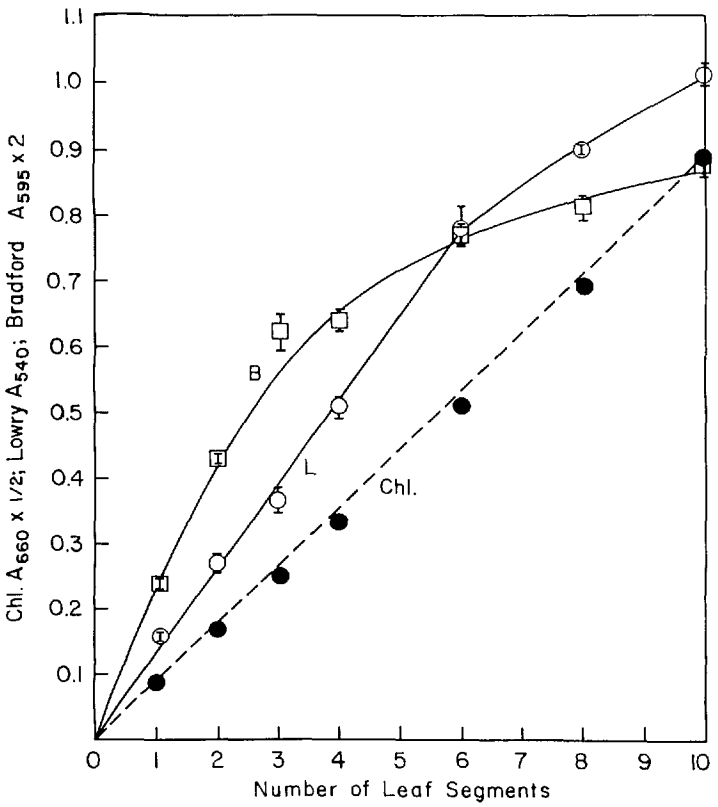

Fig. 2. Protein contents of the cold NaOH extract of oat leaf segments as functions of the number of segments (in 10 ml extract), by Bradford (B) and Lowry (L) procedures. To plot on the same scale and to compare with the Chl content of the 80% ethanol extracts, the ordinates for the Bradford method (corrected for the large blank) have been doubled, and those for the Chl halved.

tion conferred by ABA was different for the two compounds. In Fig. 4 the changes are shown as percent of the initial value. Chl *a* is decomposed more extensively in the 4 days of darkness than Chl *b*, and ABA protects it only partially, even 1 mM bringing it up to only 42% of initial. The same concentration of ABA brings Chl *b* to 100% of its initial value.

The action of ABA in lowering the free amino acids is even more powerful than that on Chl. Even at a level as low as 100 μM, the accumulation of free amino acids in the dark is inhibited by 80%. Figure 5 shows that, as with Chl, the decrease in free amino nitrogen below that of the controls is nearly log-linear with ABA concentration. For Chl the correlation coefficient is 0.99, for amino nitrogen it is −0.97. Indeed, the two curves show a striking symmetry, but this is partly due to the choice of scale, the proteolysis being about twice as sensitive to ABA as the Chl loss. ABA itself, at levels comparable to those being used here, does not interfere with the ninhydrin reaction.

Two types of precautionary experiments were carried out. First, to be quite

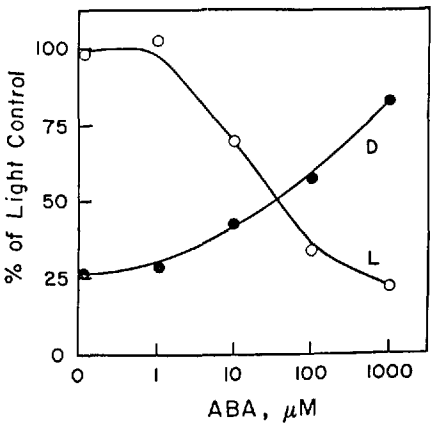

Fig. 3. Chl contents of oat leaf segments floated on water or ABA after 4 days in light (L) or dark (D).

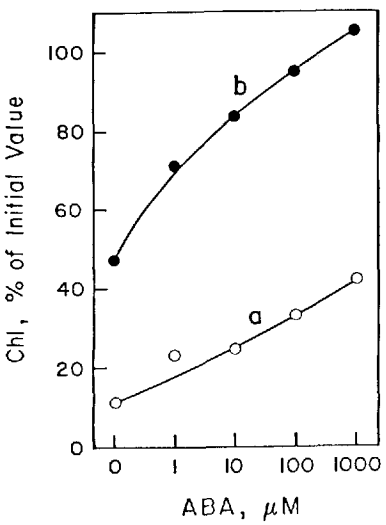

Fig. 4. Chl *a* and *b*, each as percent of initial value, after 4 days in darkness on ABA. Data are the mean of four replications. Control values: Chl *a*, 28.5 ± 2.1 μg/cm²; Chl *b*, 5.9 ± 0.5 μg/cm².

sure that this apparent reversal of ABA's well-known effects (nearly always reported in light or in day-night conditions) could not be due to some change in the sensitivity of the plants or to isomerization of the ABA, the responses in dark and light were compared in the same experiment, using leaves from the same group of seedlings and the same 200-μM solution of ABA. The results (Table 2) leave no doubt; the Chl and protein contents are higher, and the free amino nitrogen much lower, in the dark, but the changes are opposite in the light. When the effects of ABA in the dark on protein were compared by the two protein methods in the same experiment, ABA increased the protein content by 12% (Lowry) or 18% (Bradford).

Prevention of proteolysis could be due either to inhibition of the action of

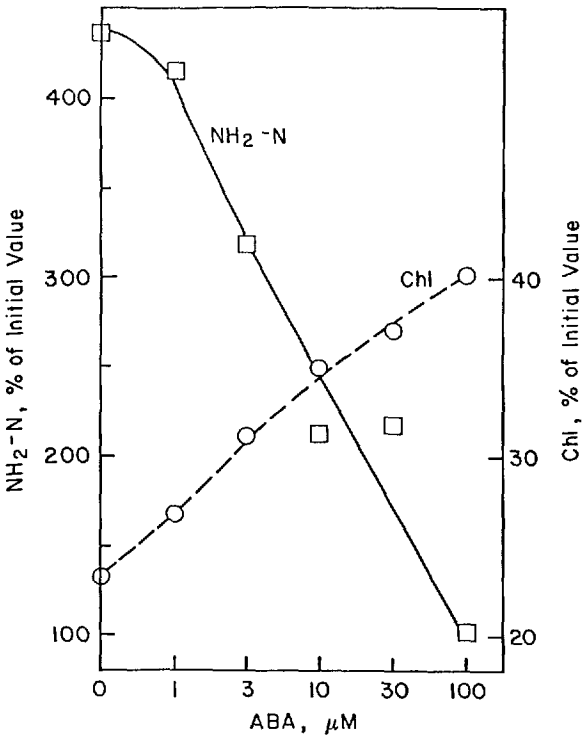

Fig. 5. Amino nitrogen, □, left scale, and Chl, ○, right scale, both as percent of the initial value after floating 4 days on ABA in darkness.

Table 2. Chl and free amino nitrogen of oat leaf segments after floating on water or 200 μM ABA for 4 days in light or darkness.[a]

Treatment	Chl	α-Amino nitrogen	Protein
Water, dark	20.1 ± 1.2	362 ± 6	50.9 ± 0.9
ABA, dark	35.5 ± 0.8	113 ± 4	67.7 ± 5.5
Water, light	99.4 ± 5.5	139 ± 5	100 ± 0.8
ABA, light	31.6 ± 4.0	351 ± 12	76.3 ± 2.6

[a] Mean of three experiments; data expressed as percent of initial values.

the proteases or to inhibition of their formation. Oat leaves contain two major proteases, one active in the pH range 4–5 and the other in the neutral range (Drivdahl and Thimann 1977). The acid protease increases first in normal senescence, and the neutral one later; the decrease in the acid enzyme after 3 days in darkness was tentatively ascribed to its having been hydrolyzed by the neutral enzyme.

Table 3 shows the changes in acid and neutral proteases in 3 days as a result of treatment with 300 μM ABA. For comparison with the preceding data, the Chl analyses are included; the responses of these to the ABA are about as shown above, the breakdown of Chl being inhibited by ABA in the dark but

Table 3. Effect of ABA (300 μM) on chlorophyll and protease content after 3 days in light and darkness.[a]

	Chl	Acid protease	Neutral protease
Dark control	23 ± 1.5	290 ± 3	115 ± 25
Dark + ABA	33 ± 0.7	220 ± 4	79 ± 12
Light control	79 ± 0.5	243 ± 31	329 ± 32
Light + ABA	12 ± 1.4	349 ± 97	224 ± 64

Initial values (100%): Chl 2.77 mg/g fresh wt, acid protease 380, neutral protease 272 units.
[a] Each figure the mean of three extractions. Data as percent of initial values.

promoted in the light. It is evident that ABA increases acid protease formation in the light but perhaps decreases it in the dark. In both cases the controls produce 2.5–3 times as much protease as the initial content, which agrees well with our earlier results (Fig. 10 of Martin and Thimann 1972). Changes in the neutral proteases (pH 6) are somewhat variable, but the values in Table 3 are typical. Although the absolute protease content varied from one experiment to another, the changes due to ABA, as percent of the initial values in the same experiment, were quite reproducible.

Since ABA in the dark has effects similar to those of the cytokinins—i.e., protecting both Chl and protein from breakdown—it was of interest to see whether these two hormones are operating through the same mechanism, since if so they should either act independently or even perhaps synergize. In the light, ABA and kinetin are well-recognized antagonists. Accordingly, Table 4 presents data on the Chl and free amino nitrogen of leaf segments treated with ABA and kinetin separately and together. Two concentrations of each are shown. Kinetin at the concentrations used is more effective than ABA (at the concentrations of ABA used) in protecting Chl, the values in each case showing no decrease from the initial. ABA, on the other hand, is the more effective in protecting against proteolysis. (Kinetin is in general not so powerful an inhibitor of proteolysis as it is a preserver of Chl.) The values given by the combination of the two are, for Chl, intermediate between those for the separate hormones; for proteolysis the protection is even less than by either one alone. This means that there is a marked antagonism between the two, which is clearly brought out by the figures for the increase or decrease from the controls.

The Action of ABA on Stomatal Aperture

With other treatments, the protection of Chl and protein from breakdown has been associated with opening of stomata (Satler and Thimann 1979, 1981, 1983). This relation holds, in general, for oat leaves with a wide variety of reagents and treatments, but at least with *Tropaeolum* there are exceptions (Thimann 1985). Accordingly, it was important to determine whether ABA causes opening of stomata in the dark. Figure 6 presents one of five experi-

Table 4. Chl and free amino nitrogen contents of oat leaf segments after 4 days in darkness on ABA, kinetin, or both together.[a]

	Control	ABA (100 μM)	Kinetin (20 μM)	ABA + kinetin (100 + 20 μM)	ABA (200 μM)	Kinetin (30 μM)	ABA + kinetin (200 + 30 μM)
Chlorophyll	31	49	109	63	52	108	68
Increase due to treatments		18	78	32	21	77	37
Amino-N	540	156	268	440	154	275	424
Decrease due to treaments		384	272	100	386	265	116

[a] Data as percent of initial values.

ments. Results obtained with the two different porometers agree very well. All experiments show not only that there is clear opening of stomata but also that the differences between ABA and the dark controls tend to increase with time. By the third day in ABA the stomata are as widely open as on water in light. Stomatal opening by cytokinins also increases with time (data not shown). ABA in light causes the expected closure (see Zeiger 1983). Since the changes in Chl and protein are measured after 3–4 days, they would presumably be associated with a summation of the stomatal differences over the whole period. The agreement with this expectation appears satisfactory.

It has been reported that action of ABA on stomatal closure in light is, like some of its other actions, antagonized by some aromatic acids (Laloraya et al. 1986, Rai et al. 1986). To see whether this effect is reflected in similar action on the senescence of oat leaves, a number of experiments have been carried out. Table 5 summarizes five series of experiments with *p*-coumaric acid (neutralized), in both white light and darkness. First, ABA increases the breakdown of both Chl and protein in light, as expected. The *p*-coumaric acid does significantly protect the protein from breakdown, lowering the free amino acid and raising the protein, but its effect on the Chl is barely significant. In particular, in all five experiments the value for free amino acids lay about midway between that of ABA and the control. Thus there is clearly some antagonism to ABA in light. The data with ABA in the dark, however, show no clear effect. Similar experiments with *trans*-cinnamic acid gave comparable results (data not shown). It can be concluded that the reported action on stomatal aperture in light is indeed reflected in a slight antagonism in senescence, but that the antagonism is not appreciably exerted in darkness. Evidently *Avena* is not as sensitive to *p*-coumaric acid as the leaves used by other workers.

Interaction Between ABA and the Ethylene-Forming System

It was during a study of ethylene production, in quite another connection, that the inhibiting action of ABA was first observed. Since the leaves were kept in darkness, and ABA did not inhibit CO_2 production, the effect could not be due to inducing CO_2 deficiency. The inhibition took place mainly in the first 2 days after the leaves were detached and darkened; on the third day the ethylene production by the controls had not changed, but ABA at 100 μM no longer inhibited, and the inhibition by 200 μM had decreased. Table 6 shows representative data. The average hourly production decreases with increasing time after sealing, but the percentage inhibition by ABA actually tends to increase, reaching a maximum of over 80% in a vessel sealed for 4 h. This increased inhibition could hardly be due to increasing internal ABA concentration, since the leaves had been in contact with the ABA for 20 h before the first measurement. A more detailed study of the complex relations between ethylene, CO_2, and ABA is being undertaken.

Discussion

Three aspects of the results call for comment. The action of ABA on senes-

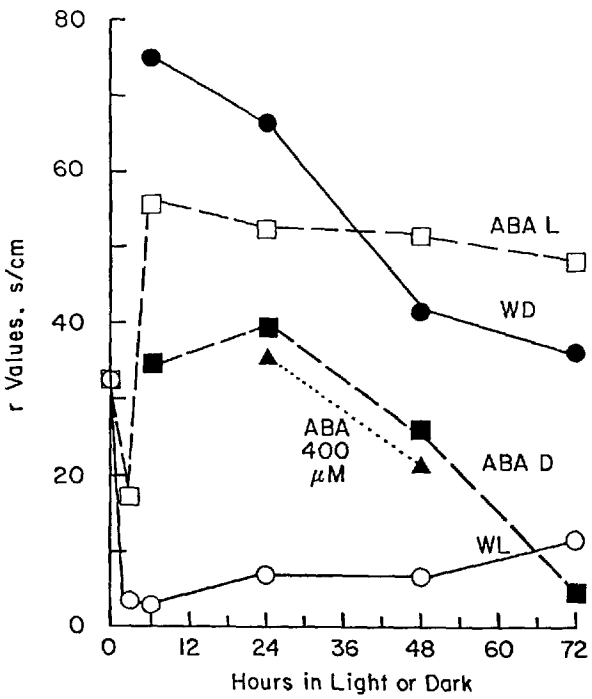

Fig. 6. Stomatal resistance, *r*, in sec/cm, as function of time on water or ABA in light (L) or dark (D) on water (W) or abscisic acid (ABA), 200 μM. Continuous lines, water; dashed lines, ABA. The dotted line shows an experiment with ABA 400 μM and was obtained with the Li-Cor instrument.

cence, which is now shown to be in opposite directions in light and darkness, involves both Chl and protein. The three chelating agents, sodium azide and the aliphatic alcohols, in contrast, show light-dark reversal of their effects on Chl, but they inhibit proteolysis in *both* light and darkness (Satler and Thimann 1983). It follows that the mode of action of ABA at the biochemical level is different from those of all the above reagents. Actually it is also not quite the overall opposite of that of the cytokinins, for the cytokinins open stomata and protect both Chl and protein from breakdown in *both* light and darkness. Methyl jasmonate (MJ), on the other hand, shows no light reversal at low concentrations, but more recent experiments than those of Satler and Thimann (1983) now show that it behaves like ABA at 100 μM and above. Thus after 48 h in the dark, stomata were partially opened by MJ at 100 μM, and after 72 h they were more strongly opened by 20 μM.

The ability of ABA to promote the loss of Chl and protein in light apparently casts it in the role of enzyme stimulant under these conditions, although it can be considered as an inhibitor of senescence-promoting enzymes in the dark. ABA has stimulating action in certain other functions too. For instance, it induces formation of two new proteins in barley aleurone (Ho et al. 1985) and of two new forms of adenosine methyl transferase (Dogbo and Cameron 1986); it also substitutes for darkness in the opening of *Pharbitis* flowers (Takimoto 1987). Thus, although ABA is often considered as an inhibitor of growth, it is able to stimulate some growth processes. As a parallel but opposite case, the inhibition of senescence by cytokinins, together with their known inhibition of

Table 5. Partial reversal of ABA effects on senescence of oat leaf segments by *p*-coumaric acid[a]

Treatment	After 4 days in light			After 4 days in darkness		
	Chl	α-Amino nitrogen	Protein (Lowry)	Chl	α-Amino nitrogen	Protein (Lowry)
ABA 200 μM	24.1 ± 0.7	260 ± 12.2	57 ± 2.0	35.2 ± 1.9	176 ± 11.7	42.0 ± 2.1
ABA 200 + Coum 100 μM	25.7 ± 0.9	224 ± 5.3	59 ± 2.3	34.5 ± 0.4	164 ± 12.5	40.3 ± 8.1
Control	67.6 ± 2.0	179 ± 1.9	71 ± 2.3	21.1 ± 0.3	352 ± 9.5	36.0 ± 6.6

[a] Mean of five experiments. Data as percent of initial values.

Table 6. Effect of air access.[a]

Treatment	Chlorophyll	Amino nitrogen	Protein
Covered			
Control	22	500	27
ABA 100 μM	33	92	49
ABA 200 μM	42	79	57
Open			
Control	28	428	27
ABA 100 μM	44	136	54
ABA 200 μM	62	103	53

[a] Data as percent of initial values after 4 days in darkness.

lateral root formation (Wightman et al. 1980), could cast the cytokinins in the role of inhibitors. True hormones evidently need to have both types of effect, on different systems, in order to serve the plant effectively.

We considered the possibility that the opening of the stomata by ABA could be due to its greatly increasing the sensitivity of the guard cells to CO_2. It is known that very high CO_2 concentrations, 10% or above, do tend to open the stomata in darkness (Jones and Mansfield 1970, Klockare and Falk 1981). It can be calculated that if the Petri dishes are tightly closed and contain ten 3-cm segments, then after 4 days in darkness the CO_2 content of the air will be some 66 times the normal, or about 2%. However, the action in the opposite sense, namely stomatal closure by ABA in light, did not depend on CO_2, since Jones and Mansfield (1970) found it was not reversed in CO_2-free air, which makes that explanation less likely. Also, in our own experiments, daily opening of the Petri dishes did not affect the results, nor did leaving them continuously open or closed (Table 6). (It is usual for the stomata of floating leaves in the dark to open slowly day by day.) This possible explanation can therefore be discarded.

The opposite actions of ABA in light and darkness invite speculation as to the environmental value of this reversal to the plant. For under dry conditions, stomata close and ABA accumulates in the leaves, so that in the daytime senescence would be promoted. At night, however, when the relative humidity rises, causing at least partial opening of the stomata, this not only antagonizes the incipient senescence in itself but is intensified by the action of the residual

ABA in protecting against senescence. The net effect will be a much more active dark reversal of the changes occurring in the light. In other words, the reaction of the leaves to ABA in the dark must improve the ability of the plant to sustain temporary water stress. The inhibition of ethylene formation would tend, though less actively, to operate in the same direction.

Acknowledgments. Supported by grants PCM 850-1051 from the National Science Foundation and 134-84 from the North Atlantic Treaty Organization. We thank Dr. Daphne Osborne for constructive suggestions.

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