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A Comparative Study of the Effects of Abscisic Acid and New Terpenoid Abscisic Acid Analogues on Plant Physiological Processes

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Abstract. The effects of new terpenoid abscisic acid analogues in comparison to abscisic acid (ABA) on some physiological and biochemical processes of various plant species have been investigated. The analogues exhibited ABA-like effects by inhibiting cell elongation and germination, and by promoting abscission, as well as the accumulation of proline and induction of stomatal closure coupled by a reduction in transpiration. In response to low temperature, the analogue-treated plants showed improved resistance to cold accompanied by a decrease in electrolyte leakage.

Abscisic acid (ABA) has multiple physiological effects on growth and development of plants. ABA is an effective growth inhibitor of various plant species. It reduces *Lemna* growth (Newton 1974, McLaren and Smith 1976) by inhibiting cell division (Albanell et al. 1985) and cell elongation (Doss et al. 1983). It inhibits not only growth but germination as well (Dommes and Northcote 1985, Kepczynski 1986). On the other hand, ABA is an effective promoter of various processes. Its application leads to abscission acceleration (Addicott 1982) which may be useful for facilitating mechanical harvest operations to increase crop yield. There is convincing evidence that ABA creates a protective mechanism toward water stress and other stresses. It plays a role in reducing transpiration by promoting stomatal closure (Dörffling and Tietz 1985, Mansfield 1976). It has also been reported to induce the accumulation of proline (Stewart 1980, Stewart and Voetberg 1985). ABA has been investigated extensively in regard to possible application in agriculture and horticulture. However, its stability in solution and in plants, due to its rapid deactivation by photoisomerization and by metabolism, is too low for agricultural use. It is possible that substitution in the molecule could render it more stable and facilitate its uptake by intact plants. Thus, a number of experiments were undertaken to screen ABA analogues for biological activity (Gale and Hagan 1966, Ogunkanmi et al. 1974). To date, field-study results have not determined these analogues to be potentially useful.

Recently, Grossmann and Jung (1984) described new terpenoid analogues of ABA and investigated their effects. The basic structure of the analogues is similar to that of ABA. However, the dienoic side chain of the ABA molecule is replaced by a conjugated alkene–alkine unit. In addition, the 4′-ketone group in the cyclohexenyl ring is substituted by hydrogen or by a cyclic ketal unit. Likewise, the carboxyl group is replaced by an acetal or ether function.

Experiments showed that the application of these analogues may be useful in improving the resistance of plants to water stress (Jung and Grossmann 1985). The analogues were also found to be capable of increasing resistance of crop plants to chilling and freezing (Flores et al. 1988). A higher survival rate was reported in chilled cucumber seedlings pretreated with the analogues. Because of their potential, further experiments were performed to compare their effectiveness with that of ABA. This paper reports the effects of ABA and the new ABA analogues, coded LAB 144143 and LAB 173711 (Fig. 1), on other plant physiological and biochemical processes, such as germination, growth, abscission, stomatal closure, transpiration, diffusion resistance, chilling tolerance, and proline accumulation in various species.

Materials and Methods

Preparation of Test Solutions

Preparations of the analogues, LAB 144143 and LAB 173711

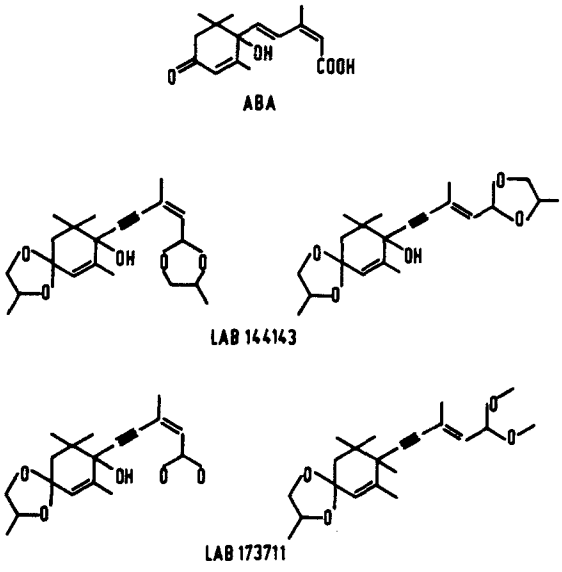

Fig. 1. The Structure of ABA and its isomers of the new terpenoid ABA analogues. After Jung and Grossmann 1985.

(kindly provided by Dr. W. Rademacher, BASF Agricultural Research Center, Limburgerhof, FRG), were dissolved in either 10 ml acetone or 7.2 ml cyclohexanone (Merck, Darmstadt, FRG). These solutions were diluted with distilled water to 1 L to obtain the following concentrations: 10^{-5}, 10^{-4}, and 10^{-3} mol L^{-1}. Emulphor EL (BASF) was added as a wetting agent to a final concentration of 0.18%. (±)-ABA was used for comparison (obtained from Fluka, Basel, Switzerland). The control solution consisted of a mixture of the solvent, wetting agent, and distilled water.

Growth of Lemna Cultures

Ten *Lemna minor* L. fronds (taken from the Botanical Garden, Institut für Allgemeine Botanik, Hamburg, FRG) were grown in plexiglas vials (9.5 × 9.5 × 5.7 cm) containing 50 ml test solution at a concentration of 10^{-5} mol L^{-1}, diluted with Hoagland's nutrient medium in a ratio of 1:1 (vol:vol). The plants were maintained in a growth chamber under a continuous light of 240 μE m^{-2} s^{-1} PAR by Sylvania cool white lamps (VHO/215W, GTE, CAN) at 24°C.

Growth rate, expressed as the percent increase in the number of fronds, was recorded daily for a period of 14 days.

Germination

Seeds of *Helianthus annuus* L. cv. Bismarckianus (Sperling and Co., Lüneburg, FRG), *Oryza sativa* L. cv. IR 42 RYT 3009 (kindly provided by Dr. M. Dingkuhn, IRRI, Philippines), *Lepidium sativum* L. cv. Einfacher Stamm 74 Neu (Saatzucht Hild, Marbach, FRG), *Pisum sativum* L. cv. Kleine Rheinländerin (Hild, Marbach, FRG), and *Cucumis sativus* L. cv. Delikateß Spezialzucht (Hild, Marbach, FRG) were germinated on a layer of filter paper (Schleicher and Schüll) moistened with test solutions (10^{-4} mol L^{-1}) in 15-cm diameter petri dishes. These were incubated in darkness at 25°C. For each treatment, two replicates of 100 seeds were used. Seed germination (the number of seeds from which the radicle had emerged through the seed coat) was monitored daily for a period of 7 days.

Abscission Acceleration Using Explants

The main shoots of vegetative plants of *Coleus rehneltianus* (syn. *pumilis*) Berger (cloned stock kindly provided by Dr. H. Veen, Wageningen, The Netherlands) were used as sources of explants. Explants (1.5 cm in length), devoid of axillary buds, were excised between the basal and apical node. They were aligned in normal orientation to gravity on plexiglas racks mounted over 1% agar in 14.5-cm diameter petri dishes. The cut surface of the petiole section was sealed by a thin agar disc (5.0 × 5.0 × 1.5 mm) containing test solution at a concentration of 10^{-4} mol L^{-1}. The petri dishes were covered and incubated at 27°C. Every 1–2 h, the pulvinar end of the petiole was given a light tap, using a forcep (about 5 g), to determine if separation at the abscission zone had taken place. The bioassay has been previously described in detail by Böttger (1970).

Abscission Acceleration Using Whole Plants

Cuttings from young plants of *Coleus rehneltianus* (syn. *pumilus*) Berger were cultivated in garden soil in the greenhouse at 22°C under natural light conditions. Plants with seven nodes were sprayed twice at 2-week intervals with the test solution (10^{-3} mol L^{-1}). At 2- to 4-day intervals, the leaves on five plants per treatment were assessed for abscission.

Direct Effect of ABA and ABA Analogues on Stomatal Closure

Seeds of *Commelina communis* L. (kindly provided by Dr. T. Mansfield, Lancaster, UK) were sown in standard soil and grown in the greenhouse. Abaxial epidermal strips were peeled from the lamina of the youngest fully expanded leaves and floated cuticle side down on 10 ml of the test solution (10^{-4} mol L^{-1}) in 5-cm diameter petri dishes. They were incubated in a growth chamber at 25°C under a continuous light of 240 μE m^{-2} s^{-1} PAR by Sylvania cool white lamps (VHO/215W) for 1 or 3 h. At the end of the incubation period, two strips for each treatment and incubation period were mounted on a microscope slide, and the pore diameters of 10 randomly chosen stomata were measure on each epidermal strip. Stomatal pore width was measured under a light microscope (Zeiss, FRG) equipped with a vernier eye piece (magnification ×400).

Measurement of Transpiration Using a CO_2–H_2O Porometer

Seeds of *Hordeum vulgare* L. cv. Claudia (Saatzucht Dr. Carstens, Bad Schwartau, FRG) were sown in garden soil in 17 × 18 cm pots and germinated in the greenhouse. Three-week-old seedlings were transferred to a growth chamber maintained at 22°/18°C day/night with a 16-h photoperiod and 60% relative humidity. The leaves were sprayed until runoff of the test solutions (10^{-4} mol L^{-1}) occurred. The width of the middle leaf lamina

was measured before it was clamped in a cuvette. CO_2- and H_2O-gas exchange were then measured using a porometer (H. Walz Meß- und Regel-technik, Effeltrich, FRG). The data obtained were used together with photon flux density and air flow data to calculate transpiration and diffusion resistance.

Electrolyte Leakage of Chilled Tissues

Six-day-old seedlings of *Zea mays* L. cv. Früher Goldprinz (Sperling and Co.) were raised in a growth chamber at 30°/20°C with a 16-h photoperiod at 245 $\mu E\ m^{-2}\ s^{-1}$ PAR from Sylvania cool white lamps and 80% relative humidity. They were sprayed with the test solutions (10^{-3} mol L^{-1}) and 24 h later the seedlings were transferred to 1.5°C and maintained for 5 days. The response to the chilling temperature was estimated by determining the leakage of electrolytes into deionized water from the cells of detached primary leaves. Five fully expanded leaves were excised at random and cut into 3-cm segments. Five mid-lamina segments in brown glass vials were fully immersed in 50 ml of deionized water, infiltrated under vacuum at 5 mbar for 1 h, and kept at 7°C for up to 20 h. At the end of the incubation period, the leakage of electrolytes was assessed by measuring the electrical conductivity of the suspending medium with a LF530 Conductance Meter (WTW, Weilheim, FRG), which was previously calibrated with deionized water.

Proline Accumulation

Samples for analysis consisted of fully expanded primary leaves of 13-day-old *Hordeum vulgare* L. cv. Claudia plants (Saatzucht Dr. Carsten) grown in garden soil in the greenhouse. Ten leaves per treatment per incubation period were excised from the plants. The cut ends of the leaves were immersed in 50 ml of the test solutions (10^{-4} mol L^{-1}). The leaves, maintained at 21° and 16°C under continuous light, were allowed to take up the solution through the cut ends for 1, 2, 3, 4, 5, 6, 7, 8, and 24 h. At the end of each incubation period, leaves were frozen in liquid nitrogen for subsequent proline determination via the method of Bates et al. (1973).

Results

Growth of Lemna *Cultures*

ABA and the analogues delayed the rate of multiplication of *Lemna* fronds (Fig. 2). Up to day 12, the growth rate of ABA- and analogue-treated plants was very slow. Between 12 and 14 days, however, the growth rate increased in the analogue-treated plants. On the other hand, the multiplication rate in control-untreated cultures increased progressively attaining a 175% increase after 14 days compared with only 60% in LAB 144143- and 56% in LAB 173711-treated cultures. ABA showed the greatest inhibitory effect with an increase in plant number of 20%. The analogues showed a similar pattern, but slightly weaker activity than ABA. This was most evident 14 days after application.

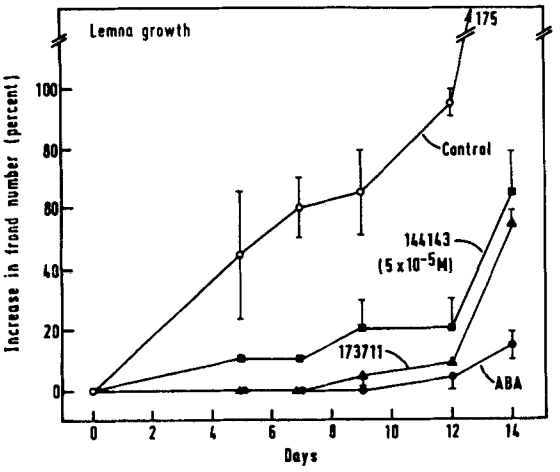

Fig. 2. Inhibition of *Lemna* growth as affected by 10^{-5} mol L^{-1} ABA, LAB 144143, and LAB 173711. Data are means ± SE of two parallel measurements.

Germination

ABA and the analogues not only delayed the time of initiation and completion of seed germination but reduced the rate of germination as well. This is apparent in seeds of garden cress, sunflower, and rice (Fig. 3). Differences in the activities of ABA and the analogues were small. In garden cress, ABA and the analogues showed a greater effect on the inhibition of seed germination 2 days after application. Thereafter, the effect was not pronounced. In sunflower achenes and rice caryopses, seed germination was strongly inhibited and delayed by the test solutions. Only about 5–10% of sunflower achenes incubated in solutions of ABA and the analogues germinated, compared to about 70% for the control after 7 days. For rice caryopses, only about 20–50% germinated compared to almost 100% in the control (Fig. 3). For pea and cucumber seeds, ABA and the analogues did not show a significant effect on the inhibition of seed germination (data not shown).

Abscission Acceleration Using Explants

Explants treated with ABA showed enhanced abscission and attained 100% abscission before the third day (Fig. 4). This was followed a few hours later by explants treated with LAB 144143. Explants treated with LAB 173711 attained 100% abscission after 4 days. In contrast, untreated controls showed slight abscission and exhibited only 27% petiole-fall by the fourth day.

Abscission Acceleration Using Whole Plants

A longer period was required for leaves of *Coleus*

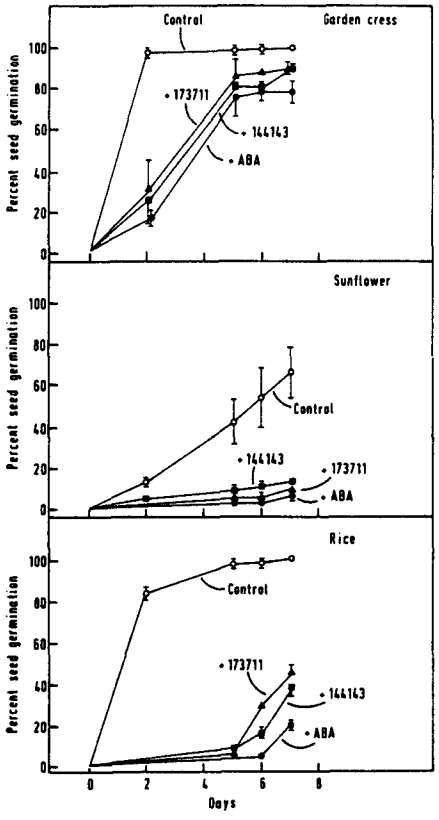

Fig. 3. Germination of garden cress seeds, sunflower achenes, and rice caryopses incubated in 10^{-4} mol L^{-1} solution of ABA, LAB 144143, and LAB 173711 at 25°C in darkness. Data are means ± SE of two parallel experiments with 100 seeds each.

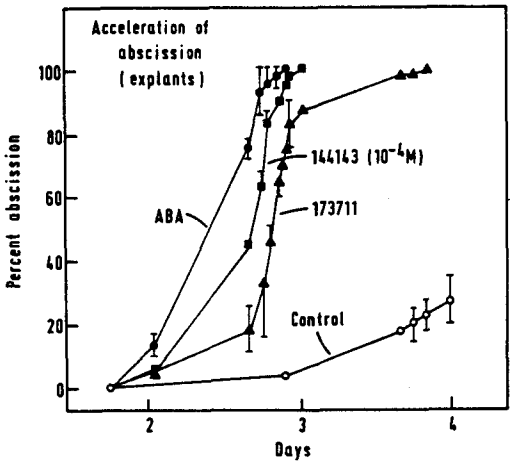

Fig. 4. ABA and the analogues as abscission accelerators in *Coleus* explants. For the bioassay, 10^{-4} mol L^{-1} solutions of ABA, LAB 144143, and LAB 173711 were used. Data are means ± SE of two parallel experiments with 20 explants each.

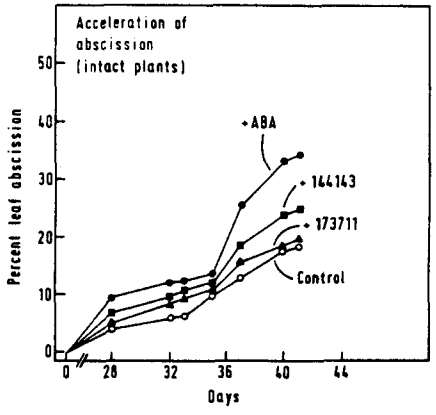

Fig. 5. Effect of ABA, LAB 144143, and LAB 173711 on abscission of intact *Coleus* plants. The plants were sprayed twice with a concentration of 10^{-3} mol L^{-1} of the test solutions. Data are means ± SE of five measurements.

plants to abscise compared to the explants. A gradual rise in the percentage of leaf abscission after treatment was exhibited (Fig. 5), but the differences between the treatments with the analogues and the control remained small. LAB 144143 proved to be more active than LAB 173711. ABA had the highest activity.

Effect of Abscisic Acid and Synthetic ABA Analogs on Stomatal Closure

The results in Fig. 6 show that the application of ABA analogues caused a marked decrease in stomatal pore width. Incubation of epidermal strips in ABA, LAB 144143, and LAB 173711 for 3 h resulted in almost 20-, 5-, and 2-fold decreases in pore width, respectively.

Transpiration and Diffusion Resistance

Application of ABA and the analogues as a spray to intact barley leaves resulted in a reduction in the rate of transpiration (Fig. 7). LAB 144143 and LAB 173711 showed an ABA-like inhibitory effect. LAB 144143, however, was more effective in reducing transpiration than LAB 173711 and showed nearly a similar effect as ABA. Transpiration rates of LAB 144143- and ABA-treated plants were apparently lower than those of the control. In contrast, diffusion resistance was apparently higher in the ABA- and analogue-treated plants compared to the control, which indicates the ability of treated plants to more effectively close their stomates than control plants. Thus, this may increase the resistance of plants to water stress conditions.

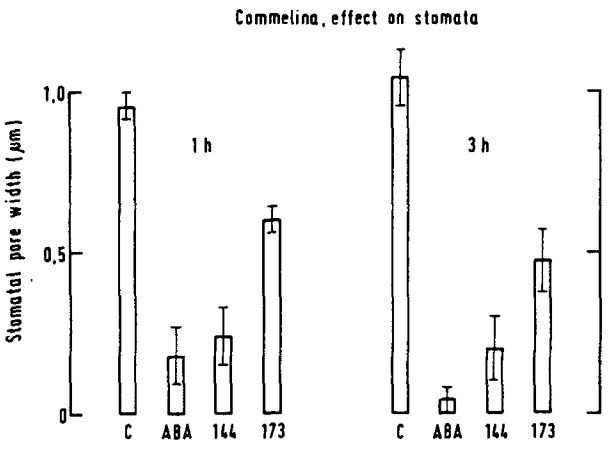

Fig. 6. Stomatal pore width measurements on detached abaxial epidermis of *Commelina communis* L. after incubation for 1 and 3 h in 10^{-4} mol L^{-1} solutions of ABA, LAB 144143, and LAB 173711. Data are means ± SE of 20 measurements.

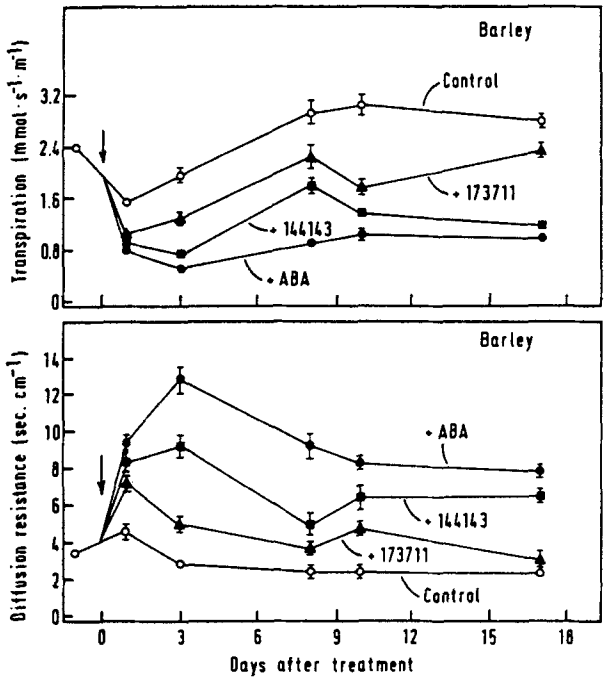

Fig. 7. Changes in leaf transpiration and diffusion resistance of intact leaves of *Hordeum vulgare* L. with time, at 22°/18°C with a 16-h photoperiod, as a response to foliar spray of 10^{-4} mol L^{-1} ABA, LAB 144143, and LAB 173711. Data are means ± SE of 10 measurements. The vertical arrow indicates application of the compounds.

Electrolyte Leakage of Chilled Tissues

The effect of ABA and the analogues on membrane stability in maize in response to low, nonfreezing temperatures is shown in Table 1. As shown, chilling-induced electrolyte leakage is less pronounced in ABA- and analogue-treated leaves compared to control, when plants were chilled at 1.5°C for 5 days. The increase in leakage in untreated control plants reached a value of 97.6 ± 34.5 µs cm^{-1}. This was accompanied by apparent visual injury, such as necrosis and wilting of leaves. Application of ABA and LAB 144143 resulted in about threefold decrease in electrolyte loss compared to control. Treatment of LAB 173711 resulted in a 1.6-fold decrease. The results indicate that ABA and the analogues maintain membrane stability in chilled maize leaves and thus minimize leakage of electrolytes. LAB 144143 proved to be as effective as ABA in preventing electrolyte loss.

Table 1. Leakage of electrolytes from leaves of *Zea mays* sprayed with 10^{-3} mol L^{-1} ABA, LAB 144143, and LAB 173711 and chilled at 1.5°C for 5 days.

Treatment	Electrolyte leakage (µs cm^{-1})
Control	97.6 ± 34.5
ABA	34.6 ± 8.3
LAB 144143	39.6 ± 10.2
LAB 173711	60.3 ± 14.4

Data are given as means ± SE of six measurements. C, control.

Proline Accumulation

Figure 8 shows the effect of ABA and the analogues on proline accumulation as a function of time. The level of proline remained constant within the 24-h period in the untreated control. In contrast, the levels gradually increased in ABA- and analogue-treated leaves. This induction was more pronounced after 7 h of incubation and continued to increase drastically. The analogues proved to be as effective as ABA. Incubation in LAB 144143, LAB 173711, and ABA solutions resulted in a 10.6-, 8.0-, and 5.8-fold increase, respectively, in leaf proline levels compared to the control 8 h after application.

Discussion

In all of the processes investigated our results show that plants treated with the ABA analogues LAB 144143 and LAB 173711 behaved in a manner similar to those treated with ABA. Bioassay evaluations and kinetic studies showed that application of the compounds resulted in growth inhibition due to a decrease in cell elongation and/or a delay in the

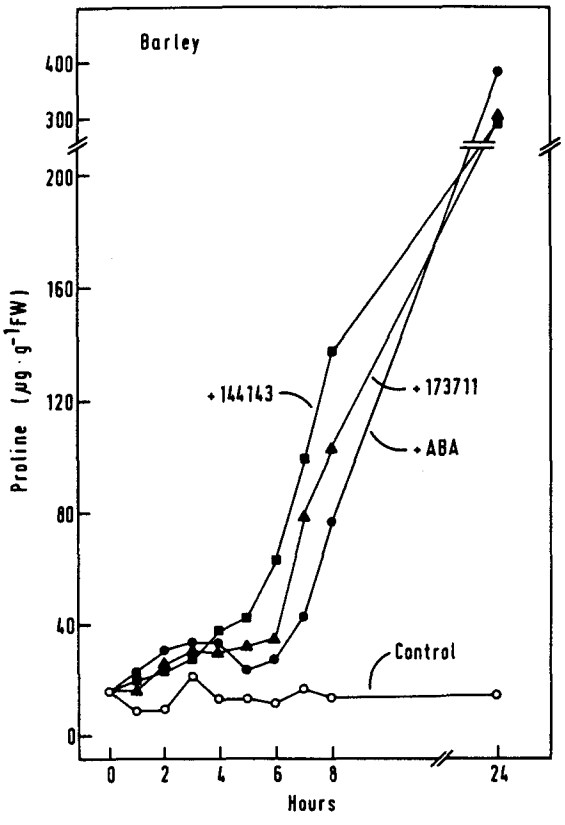

Fig. 8. Changes in proline accumulation of detached barley leaves with time, at 21°/16°C, in response to 10^{-4} mol L^{-1} ABA, LAB 144143, and LAB 173711.

rate of cell multiplication (*Lemna* fronds). Not only was cell elongation and multiplication affected but germination as well. The compounds delayed the time of initiation and completion of seed germination. To test for their effectiveness as abscission agents, both explants and intact plants were used. In both cases, abscission was enhanced for ABA- as well as analogue-treated plants compared to control plants. For intact plants, however, the effectiveness of ABA and the analogues as abscission accelerators was low. This low response may be a consequence of inadequate substance uptake through the cuticle and/or its rapid inactivation. Addicott (1982) suggested that higher concentrations of the substances and, we add, repeated treatments are needed to induce a response in intact plants, which is unlikely to be economical for large-scale agricultural use. Hence, this apparently limits the use of ABA and the analogues as chemical defoliants for practical application.

Foliar sprays of the compounds on intact barley leaves reduced transpiration and, vice versa, increased diffusion resistance. This agrees with the results obtained by Jung and Grossmann (1985). To further support these data, the effect of the analogues on stomatal behavior using epidermal strips was investigated. Both ABA and the analogues caused an apparent decrease in stomatal pore width, suggesting that the compounds induced stomatal closure directly by affecting stomatal turgor. In addition, the effect of ABA and the analogues on diffusion resistance persisted several days without markedly affecting CO_2 uptake. ABA and the analogues are effective in inducing stomatal closure, not only via foliar spray but via the root system as well (Rademacher et al. 1987). The latter effect can be used as an alternative application method in order to prevent high light-induced degradation of the compounds which may be encountered using spray applications under field conditions. Rademacher et al. (1987) showed that application of the analogue LAB 144143 at concentrations of 10^{-5} and 10^{-6} mol L^{-1} via the roots of wheat plants during anthesis improved the ratio of transpired water to yield. Application of 10^{-5} and 10^{-6} mol L^{-1} of LAB 14443 and LAB 173711 in barley and tomato plants, respectively, reduced plant water consumption by about 30%. It is likely that ABA, as well as the analogues, cause an increase in the hydraulic conductivity of the roots (Ludewig et al. 1988), in addition to the induction of stomatal closure, and thus minimize the water consumption of the plant. Two strategies are then suggested by which the analogues enable the plants to resist drought: (1) by stomatal closure to reduce the loss of water from the leaves; and (2) by improvement of water uptake by the root from the soil. This paves the way for a possible practical application of the analogues.

Not only was water balance affected by the analogues, but membrane stability as well. Chilling is known to cause an increased leakage of electrolytes from the membrane indicating an injury (Flores et al. 1988, Patterson et al. 1976, Wright and Simon 1973). Our results show that foliar application of the analogues prior to chilling reduced leakage of electrolytes. The mechanism of action of the analogues seems to differ from that of some other cryoprotectants (e.g., mefluidide and triadimefon). Mefluidide (Tseng et al. 1986, Zhang et al. 1986) and triadimefon (Asare-Boamah and Fletcher 1986) increase the level of ABA in corn and bean plants, which possibly then activates the protective system that enables the plant to minimize injury from chilling. In contrast, the ABA analogues simulate the action of ABA and act per se. An increase in the level of ABA after application of the ABA analogues has not been observed (Dörffling, unpublished results). This direct action of the analogues may be advantageous over the indirect action of the other cryoprotectants, particularly in regard to their practical use.

Another aspect affected by ABA and the ana-

logues is the leaf proline level. Incubation of detached barley leaves in ABA showed a continuous increase in the levels of proline as the incubation time was prolonged. This result agrees with that of Aspinall et al. (1973), Rajagopal and Andersen (1978), and Stewart (1980). Proline accumulation may result from the activation of the synthesis of proline from glutamic acid by ABA, which is similar to the effect of water stress (Stewart 1980). Similarly, an increase in the proline level with treatment of the analogues was observed. This accumulation of proline as induced by ABA and the analogues may also be a protective mechanism for plants to water and low-temperature stress conditions.

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