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The Role of Abscisic Acid and Farnesol in the Alleviation of Water Stress

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Phil. Trans. R. Soc. Lond. B 1978 **284**, 471-482
doi: 10.1098/rstb.1978.0083

Phil. Trans. R. Soc. Lond. B. **284**, 471–482 (1978)
Printed in Great Britain

The rôle of abscisic acid and farnesol in the alleviation of water stress

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[Plate 1]

The complex responses of stomata which provide protection for land plants against excessive water loss are best understood if we consider them as occupying two lines of defence. The first line of defence consists of immediate responses to factors of the aerial environment, especially carbon dioxide concentration and water vapour pressure deficit, which ensure that the rate of transpiration is regulated to a level which can be supported by water uptake through the roots in moist soil. When the soil becomes dry, further controls become necessary, and the second line of defence comes into operation. A ceiling is imposed on the extent to which stomata can open, and an increase in the efficiency of water use is achieved, though at the expense of some reduction in the rate of photosynthesis.

A sesquiterpenoid, abscisic acid (ABA) plays a major part in the second line of defence. It is contained in the mesophyll chloroplasts in leaves of well watered plants and is released when the water potential falls; the synthesis of new ABA is also induced by water stress. Movement of ABA from the mesophyll to the guard cells is assumed to take place, because the chloroplasts of guard cells appear to be unable to form ABA in response to water stress. We suggest that farnesol, another sesquiterpenoid hitherto considered to have a separate rôle as a regulator of transpiration, is the agent responsible for altering the permeability of chloroplast envelope membranes, allowing the release of ABA into the cytoplasm.

The closure of stomata induced by ABA appears to be part of a series of integrated responses throughout the plant which helps to maintain turgor and growth when water is in short supply.

'*Can we look at guard cells as sense-organs which, when the leaf is threatened by want of water, perceive the coming danger before the rest of the leaf? This idea is not wholly fanciful.*'
Francis Darwin 1898 *Phil. Trans. R. Soc. Lond.* B **190**, 616.

Stomatal control of water use

Land plants require many defences to enable them to survive in a hostile environment. Their basic dilemma, that of acquiring CO_2 and light for photosynthesis while retaining an adequate internal water content, has been outlined in a previous Royal Society Discussion Meeting (Raschke 1976). Evolution has offered a way out of the dilemma by providing a nearly impermeable epidermis perforated with stomatal pores. This arrangement does, however, require a high rate of water use by the plant whenever photosynthesis is consuming CO_2 rapidly from the external air. The occasions are rare when plants can afford to lose water by transpiration at the high rate that occurs through fully open stomata, for the size and distribution of these pores is such that they can sustain a rate of evaporation nearly as great as that from a free water surface of the same area as the leaf (Meidner & Mansfield 1968). It is essential, therefore, that the plant has the capacity to adjust stomatal apertures to bring water consumption under

control. This control has to be linked closely to internal water status if the damaging consequences of water stress are to be avoided.

Because the total water content of most mesophytes is low compared with the amount they transpire each day, an optimum stomatal aperture has to be determined with precision to enable adequate gas exchange to take place, and also to ensure that the demand for water does not exceed the supply available through the roots. The way that this is achieved is complex, and it appears to involve several mechanisms which it is convenient to look upon as different 'lines of defence'. In order to show where the topic of this paper fits into a pattern of defensive strategies against water stress, we shall categorize these as 'first' and 'second' lines of defence.

The first lines of defence are the direct responses of the stomata to factors of the aerial environment. In particular there are the reactions to the atmospheric water vapour pressure deficit (v.p.d.) and to carbon dioxide concentration. The closure that occurs when v.p.d. is high can be rapid (Lange, Lösch, Schulze & Kappen 1971) and it appears to be of importance in bringing about adjustments of stomatal aperture according to the evaporative power of the atmosphere. A small increase in CO_2 concentration causes stomata to close, and this reaction will occur when wind breaks down the boundary layer on the surface of leaves and increases the CO_2 level around the guard cells. This does, therefore, provide a way of reducing stomatal opening in wind.

These stomatal reactions to external factors are probably of most importance in regulating transpiration when the water supply in the soil is not severely limiting. The first lines of defence do not achieve the major drop in transpiration that is required if there is a serious shortage of water.

The second lines of defence come into operation as the bulk water potential of the leaves falls when water uptake of the roots still lags behind transpiration in spite of the stomatal responses described above. A small decline in water potential usually occurs during the day and this may be tolerated by the plant, though the drop in cell turgor can be sufficient to reduce or prevent growth (Hsiao, Acevedo, Fereres & Henderson 1976). If, however, the decrease in water potential is sufficient to impair cell metabolism, then stomatal closure must take place. Protection is obviously most needed by those cellular components which are most sensitive to water stress. There is some reason to believe that one of the highly sensitive organelles is the chloroplast, for photosynthesis is severely inhibited in water-stressed leaves (Boyer 1976). Electron transport and photophosphorylation are found to be reduced in chloroplasts isolated from leaves having low water potentials (Keck & Boyer 1974), and there is a lower carboxylation activity (Jones 1973). The chloroplast can, therefore, only carry on its normal activities if it is protected from water stress.

Effective protection depends upon the stomata, and it could be suggested that the best way of ensuring that they close at the right time would be for a 'distress signal' to pass from the affected chloroplasts to the guard cells. There is now some evidence that this is indeed the way in which the second line of defence is achieved. The first clues pointing to the nature of the mechanism that has since been elucidated in more detail came from observations that the growth regulator, abscisic acid (ABA), can cause stomata to close when applied exogenously to leaves (Mittelheuser & Van Steveninck 1969; Jones & Mansfield 1970), and that ABA is formed in large quantities when leaves wilt (Wright 1969). It was also observed that mutants which lack the ability to synthesize ABA wilt very easily because they cannot close their stomata to avoid water stress (Tal & Imber 1970; Tal & Nevo 1973). Some aspects of the control of

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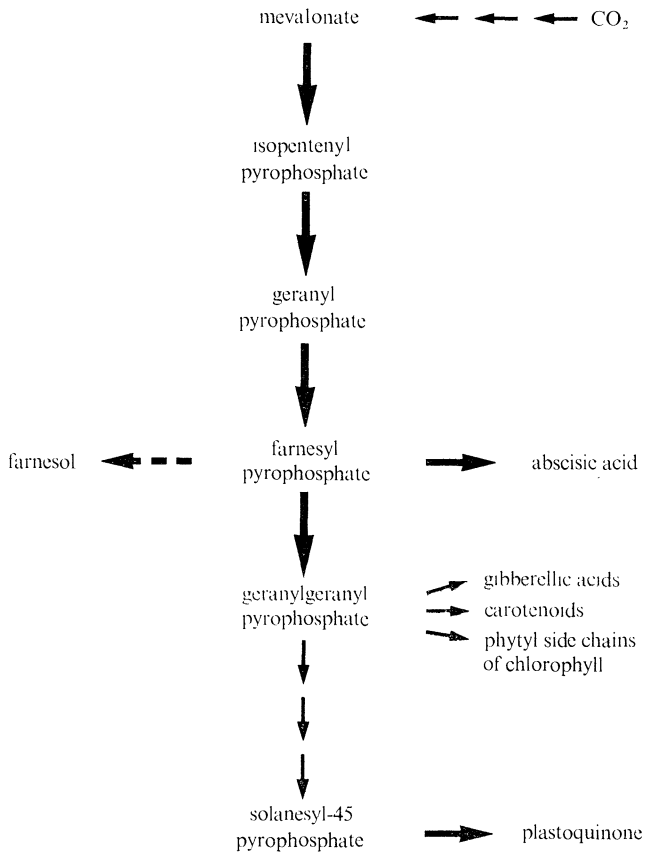
stomata by ABA have been covered in a previous review (Mansfield 1976), and the main object of this paper will be to examine in more detail the way in which ABA acts as a regulator of water use and turgor in land plants.

The regulation of stomatal apertures in plants under water stress

1. *Control by abscisic acid*

A knowledge of the mechanism by which ABA is formed, and the way its distribution is controlled, is necessary for a full understanding of its rôle as a stress hormone. The biosynthesis of ABA is known to involve the conversion of mevalonic acid (MVA) to isopentenyl pyrophosphate (IPP) which is incorporated into a C-15 precursor, all-*trans* farnesyl pyrophosphate (Milborrow 1971). This biosynthetic pathway, depicted in table 1, is involved in synthesis of all isoprenoids. The work of Goodwin and coworkers (see Goodwin 1965) established that the biosynthesis of the terpenoids involved in photosynthesis and formed when etiolated cells are illuminated is essentially confined to the chloroplasts. The envelopes of mature plastids are relatively impermeable to cytoplasmic or exogenous MVA or IPP. It is also accepted that there are enzymes located only in chloroplasts which lead to the formation of photosynthetically

Table 1. Chloroplastidic pathways of terpenoid biosynthesis to show the point of origin of farnesol and abscisic acid



involved terpenoids. There is evidence that the chloroplasts are the sites of ABA formation in aerial photosynthetic organs, and that they have a special ability to synthesize increased quantities of ABA in cells suffering water stress.

Considerations of the kinetics of ABA formation from labelled MVA in water-stressed leaves of avocado, and the low rate of penetration of externally applied MVA to the sites of ABA synthesis led Milborrow & Robinson (1973) to the view that these sites might be chloroplastic. Later, Milborrow (1974) showed for avocado that only the chloroplasts incorporated labelled MVA into ABA, and that there was less incorporation into intact than into broken chloroplasts. Thus the chloroplasts can be suggested as the main, if not the only, sites of ABA formation in green tissues. Loveys (1977) showed that nearly all of the ABA in the leaves of well watered plants of spinach was contained in the chloroplasts, but after 4 h of water stress the total ABA level rose 11-fold while the amount in the chloroplasts only doubled. These findings suggest that new ABA synthesized in the chloroplasts is rapidly released into the cytoplasm.

Wellburn & Hampp (1976) have shown that the envelope membranes of etioplasts are modestly penetrable with respect to ABA, but that there is a marked increase in penetrability after an hour of greening which rises to a peak after 2 h, and persists for at least 8 h after illumination. This demonstrates the existence, in plastid envelopes, of a capacity to change with respect to passage of the hormone, which could be utilized in a regulatory manner. The occurrence of ABA in the xylem and phloem transport pathways (Lenton, Bowen & Saunders 1968; Hoad 1973) indicates that it can move from its sites of synthesis to other parts of the plant.

The following is suggested as a working hypothesis which could usefully be tested in future work.

(*a*) ABA is present in the mesophyll chloroplasts of well watered plants. It might be retained there because of a barrier imposed by the plastid envelopes, or because it is in a 'bound' form. This could be in the form of a glucose ester (abscisyl-β-D-glucopyranoside) which Milborrow (1971) has shown to be produced when labelled ABA is supplied exogenously to tomato plants.

(*b*) Conversion of any 'bound' ABA to the 'free' form, and/or changes in nature of the plastid envelopes, occurs when the plant suffers sufficient water shortage to cause a fall in the water potential of the photosynthetic tissues.

(*c*) This ABA released into the cytoplasm moves from cell to cell through plasmodesmatal connections to the epidermis, and then to the guard cells, where it induces stomatal closure. The likely pathway is indicated in figure 1, plate 1.

(*d*) The loss of stored ABA induces fresh biosynthesis of more ABA which continues to be released from the chloroplasts as long as the water potential remains low.

(*e*) The release of ABA stops when the water potential is restored to a favourable level, and synthesis of new ABA by the chloroplasts ceases.

(*f*) The ABA released by the chloroplasts during the period of stress remains active in the guard cells for a period of several days, and full stomatal opening is therefore not restored immediately.

The several stages of this supposed sequence of events receive varying degrees of support from the literature. While there is substantial evidence that the chloroplasts are the centres of synthesis of ABA, there has been little exploration of the extent to which they might store ABA in a 'bound' form, or of the way in which the envelopes of mature plastids might control its release. Indirect evidence that there is compartmentalized ABA which is released in the early

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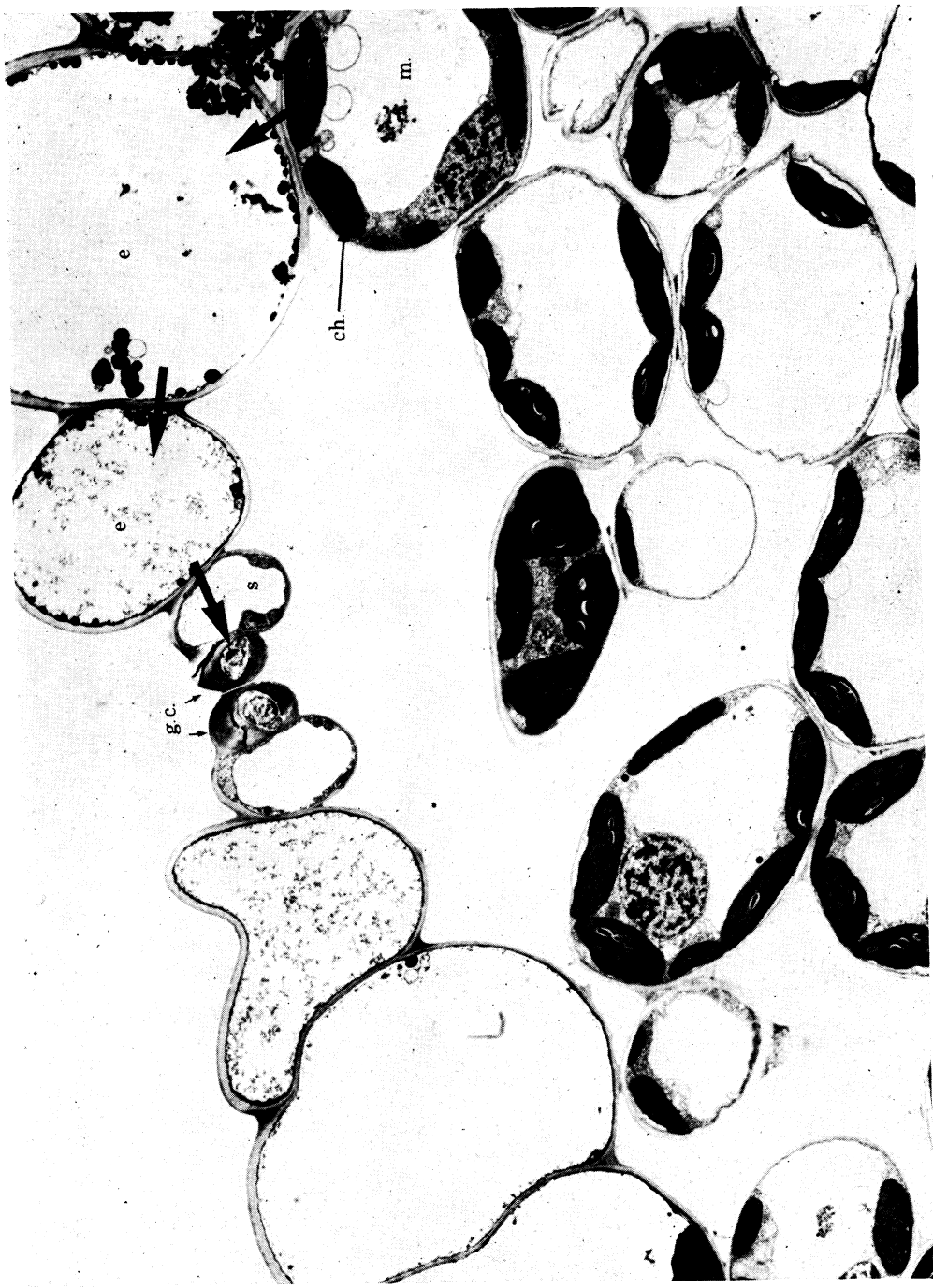

FIGURE 1. Assumed route for the movement of abscisic acid from the sites of its biosynthesis in chloroplasts to its 'target' areas in the epidermis, the guard cells. The route could be symplastic up to the subsidiary cells, but as there are no plasmodesmatal connections between guard and subsidiary cells, it must be apoplastic over this short distance. g.c., guard cells; s, subsidiary cell; e, epidermal cell; m, mesophyll cell; ch, chloroplast. Electron micrograph of section through leaf of *Phleum bertolonii* kindly supplied by K. Oates and F. I. Woodward.

(*Facing p.* 474)

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stages of water stress comes from the work of Beardsell & Cohen (1975). They found for maize that stomatal closure began before the level of ABA had risen appreciably in the leaves. Unfortunately the possibility that other factors might control this early response of the stomata cannot be ruled out. The problem will only be solved when attention is paid to changes in the ABA content of chloroplasts, and the permeability of their envelopes to the hormone, during the early stages of water stress. The work of Itai *et al.* (1978) has shown that radioactively labelled ABA quickly accumulates in stomatal guard cells when presented exogenously to isolated epidermis. A rapid transport of an endogenous store of the hormone to the guard cells (the 'target' area) and its accumulation there could result in a substantial stomatal response to an amount of hormone which is small when measured on a whole leaf basis.

The guard cells of most plants contain chloroplasts, and they are often the only cells of the epidermis to do so. It might, therefore, be surmised that the ABA which controls stomatal aperture is formed within the guard cells themselves, and that transport from the mesophyll is unnecessary. The work of Loveys (1977) provides important evidence that in spite of the presence of chloroplasts, the epidermis of *Vicia faba* is unable to synthesize its own ABA. There was no detectable increase in the ABA content of epidermal tissue after exposure to an osmotic stress which was sufficient to induce ABA formation in the intact leaf. If epidermis was removed from leaves which had previously been stressed it was, however, found to contain large quantities of ABA. Lovey's data thus provide strong evidence that the ABA which reaches the guard cells has travelled from the mesophyll cells.

According to Milborrow & Robinson (1973) the only known latent source of ABA, the glucose ester, is not usually present in unstressed tissue in concentrations above one-third of the free ABA. This amount is far too low to account for the enormous rise in ABA concentration which occurs when plant tissue wilts. Wright (1969) and Wright & Hiron (1969) found that the ABA content of wheat leaves rose by about 40 times during the first half hour of wilting. Most of this ABA appears to arise by synthesis rather than by release from a 'bound' form (Milborrow & Robinson 1973). Thus stage (*d*) of our suggested sequence of events appears to be of major importance, coming into operation very quickly when wilting is severe. The formation of new ABA can, however, cease almost immediately when water stress is removed, and the level of 'free' ABA falls appreciably in a few hours (Hiron & Wright 1973).

As the level of ABA declines when turgor is regained after wilting, the amount of the glucose ester rises appreciably (Hiron & Wright 1973). It is possible that this represents a store of 'bound' ABA which could provide an equilibrium level of 'free' ABA to maintain a reduced opening of stomata during the recovery period which often lasts for several days (Mansfield 1976). This provides an alternative to suggestion (*f*). The actual reason for depressed stomatal opening during the recovery period will be difficult to assess until a method is available of determining the ABA level in the guard cells themselves.

There is thus a substantial amount of evidence supporting several aspects of the sequence we have outlined. The identification of the chloroplasts at the sites of ABA formation, and the need for transport into the epidermis and then to the stomata, enables us to fit ABA into the classical concept of hormonal action in which a regulatory substance is released in one location and transported some distance to a 'target' area. It is not altogether surprising that the chloroplasts in the guard cells do not manufacture their own ABA. There is evidence that they do not perform the normal range of chloroplast functions, for they appear to lack the ability to fix CO_2 via the Calvin cycle (Raschke & Dittrich 1977). One of their rôles may be to act as

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amyloplasts, with a store of starch which can be utilized in the production of organic acid anions which, with potassium ions, contribute a major source of the solutes required for the turgor changes (Allaway 1973). They do, however, probably maintain photosystem I activity, and manufacture ATP by cyclic photophosphorylation which contributes to the energy requirements of active ion movements (Willmer & Mansfield 1970; Humble & Hsiao 1970; Das & Raghavendra 1974). Their function in the guard cells may thus be primarily concerned with the turgor changes. It is worth noting that because of the large reversible changes in water potential which are essential for the production of these turgor changes, chloroplasts within the guard cells are not well placed to act as sensors of the bulk water potential of the leaf.

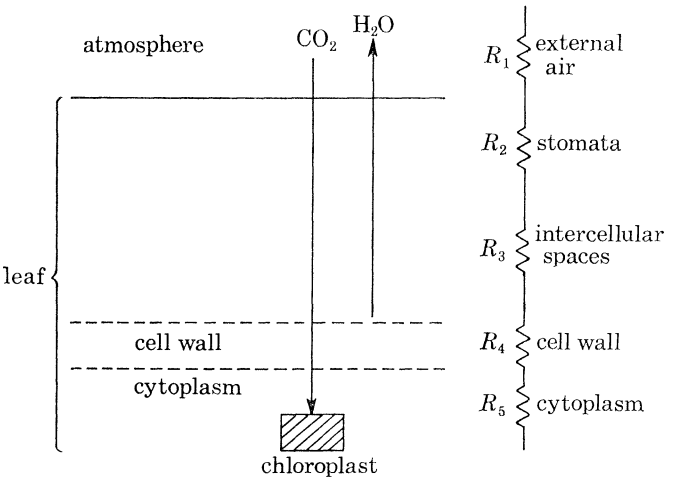

FIGURE 2. Diagram to show diffusion resistances in series encountered by molecules of H_2O leaving the leaf $(R_1+R_2+R_3)$ and CO_2 entering $(R_1+R_2+R_3+R_4+R_5)$.

2. *After-effects of water stress*

The failure of the stomata to recover their full opening potential for some time after rewatering has been looked upon as a 'safety mechanism' enabling the plants to regain full turgor more rapidly (Dörffling, Streich, Kruse & Maxfeldt 1977). In cases where the after-effect is not of long duration this is an acceptable explanation, but where recovery takes several days other reasons must be sought. In many mesophytes the stomata take 3 or 4 days to recover even after full turgor has been restored (Heath & Mansfield 1962; Allaway & Mansfield 1970; Fischer 1970). The explanation may be found if we consider the concept of 'water use efficiency'. There are theoretical grounds for believing that a given degree of stomatal closure would exert a larger proportional effect on transpiration than on CO_2 entering the leaf, because of differences in the lengths of the diffusion pathways for H_2O vapour and CO_2. The various resistances encountered are shown diagrammatically in figure 2. There have been many experimental indications that a reduction in stomatal aperture has the effect of reducing the transpiration : photosynthesis ratio or, in other words, of increasing the efficiency of water use. This improved efficiency can only be achieved at the expense of some reduction in photosynthesis, but large amounts of water can be saved with a relatively small drop in the rate of dry mass increase. For example, Raschke (1974) found for *Xanthium strumarium* that when transpiration was reduced by one-half after ABA treatment, photosynthesis was only reduced by one-seventh.

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With this information in mind we can assess the rôle of the after-effect of water stress on stomata as follows. Once water becomes scarce for a plant growing under temperate conditions it is unlikely that an abundant supply will become available as a result of just one period of rain. It is therefore desirable that the new supply of water should be used efficiently even though the rate of dry mass increase may have to be reduced. The tentative opening of stomata after the relief of water stress achieves the necessary increase in water use efficiency, avoiding a wasteful consumption that would be disastrous if the renewed water supply were only temporary.

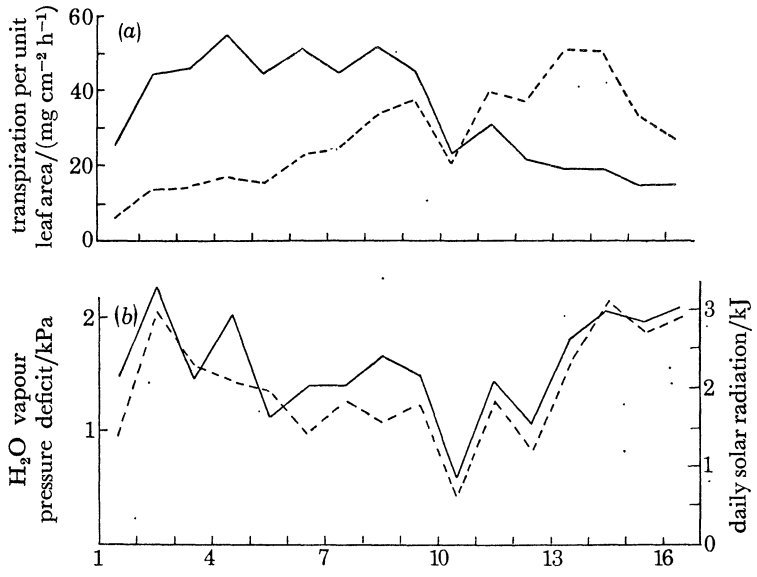

FIGURE 3. (*a*) Effects of the methyl ester of abscisic acid on the rate of transpiration of non-irrigated plants of coffee. Plants were sprayed with a 10^{-4} M solution on day 0. ——, Control; – – –, treated with ABA methyl ester. (*b*) Amounts of solar radiation (——) and water vapour pressure deficits (– – –) throughout the experiment, which was conducted out of doors in Angola.

A protective mechanism of this kind is of obvious value for plants growing in a natural situation, but is not always ideal for plants under cultivation. If water is not available to provide irrigation, a greater efficiency of water use may be required of a crop *before* the effects of water stress become sufficient to induce the formation of ABA. Forecasting of the weather, and a knowledge of soil water content, can indicate when water conservation is desirable. A means of providing artificial controls over the movements of stomata would therefore be of use to agriculturists, and it has been proposed that chemicals might be applied for this purpose to the leaf surfaces within crops. Many different compounds have now been tested, of which the most successful have been abscisic acid or its analogues or derivatives (Jones & Mansfield 1972; Orton & Mansfield 1974; Mansfield 1976). External application of small doses of ABA (in the region of 0.02 μg cm^{-2} sprayed onto the leaf surface) can mimic the effects of endogenously produced ABA, causing partial closure of stomata which persists for a week or more. The potential value of this for a non-irrigated crop is illustrated by the data for coffee in figure 3. Plants sprayed with the methyl ester of ABA at the start of the experiment used water more economically than the controls for 10 days, by which time the stomata of the untreated plants were closing owing to water stress. At the end of the experiment the treated plants still had enough water available to permit a high rate of transpiration, this being made possible by

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the reduced consumption of water up to day 10. The need to repeat the application of ABA (or a derivative) at intervals of around one week constitutes the major drawback to the practical use of this technique. There is still a need for exploration of the activities of analogues of ABA, in the hope of finding one capable of exerting a more persistent effect (see Mansfield 1976).

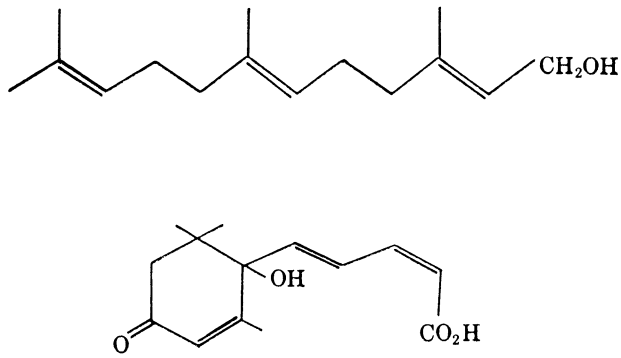

FIGURE 4. All-*trans* farnesol (top) and abscisic acid (bottom).

3. *The rôle of farnesol*

Sorghum is a tropical grain crop renowned for its ability to grow in dry climates. Its superiority in this respect over maize has attracted much attention, and studies under field conditions led Glover (1959) to the view that a major difference between the two species is the duration of the after-effect of water stress on stomata. The stomata of sorghum recover much more quickly than those of maize, enabling it to return to an unimpeded level of photosynthesis. Its deeper rooting habit may enable it to adopt this different strategy of water use.

Our own recent research has been much concerned with the mechanism of the stomatal response to drought in sorghum. Extracts from water-stressed plants revealed the presence of another sesquiterpenoid in addition to ABA; this was all-*trans* farnesol, the structure of which is shown in figure 4 (Ogunkanmi, Wellburn & Mansfield 1974; Wellburn, Ogunkanmi, Fenton & Mansfield 1974). It is possible that this may have arisen from farnesyl pyrophosphate by hydrolysis during the extraction procedure, but subsequent studies have revealed that an exogenous supply of all-*trans* farnesol is able to induce stomatal closure in sorghum, implying that this compound may itself have a physiological rôle. The stomatal closure is reversible, for there is a marked recovery of transpiration after 2 days (figure 5).

In previous publications we have pursued the idea that farnesol has a separate rôle as an antitranspirant in sorghum, in addition to any participation of ABA (Fenton, Davies & Mansfield 1977). We now direct attention to another possibility, namely that the function of farnesol lies in its ability to alter the permeability of chloroplast membranes, and that its action on stomata is the result of its allowing a release of ABA from the chloroplasts in the mesophyll. As we have already noted above, the work of Loveys (1977) has shown that most of the ABA in the leaves of well watered plants is contained in the chloroplasts. We now suggest the following sequence of events. When the water potential of the chloroplast falls, there is a block in the conversion of farnesyl pyrophosphate to geranylgeranyl pyrophosphate, and some production of farnesol then occurs (see table 1). This alters the permeability of the chloroplast envelope membrane, and there is a release of ABA into the cytoplasm. More ABA formation then occurs from the pool of farnesyl pyrophosphate. Support for this idea comes from the

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observed effects of all-*trans* farnesol on isolated chloroplasts (Fenton, Mansfield & Wellburn 1976). It inhibits the O_2 evolution stimulated by bicarbonate and by phosphoglyceric acid through its disruptive effects on membranes. This property is possessed only by farnesol and its tertiary alcohol isomer, nerolidol (Wellburn *et al.* 1974). Other homologous isoprenoid alcohols are either inactive (e.g. geraniol) or show only small activity (geranylgeraniol). Although farnesol will cause structural damage when applied to isolated guard cells, its ability to exert a reversible effect not involving damage has not been demonstrable. Foliar sprays of fine emulsions of farnesol have no effect on the stomata of sorghum. Only if a leaf is immersed in the emulsion (concentration equivalent to 2×10^{-4} M) for 30–60 min is a reversible effect on the stomata observed (Fenton *et al.* 1977). This is consistent with the view that the farnesol acts in a physiological manner only after it has penetrated to mesophyll cells in whose chloroplasts the endogenous ABA is situated.

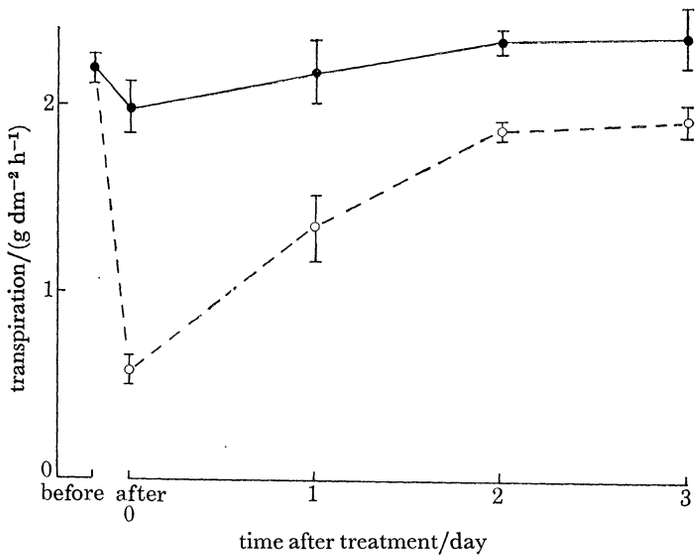

FIGURE 5. Transpiration of leaves of *Sorghum bicolor* before and after a 30 min treatment with farnesol, which was applied as a fine aqueous emulsion equivalent to 2×10^{-4} M. ●, Control leaves; ○, treated leaves. Standard errors of treatment means are shown. After Fenton, Davies & Mansfield (1977).

4. *Mechanism of action of ABA in guard cells*

During stomatal opening, guard cells take in potassium ions from the surrounding epidermis in exchange for hydrogen ions (Raschke & Humble 1973). Electroneutrality appears to be maintained by the generation in the guard cells of organic anions such as malate (Allaway 1973) though in some species chloride ions entering from outside can have a significant rôle (Raschke & Fellows 1971; Schnabl & Ziegler 1977). ABA has been shown to inhibit the uptake of K^+ ions and the disappearance of guard cell starch, both of which accompany stomatal opening (Mansfield & Jones 1971; Horton & Moran 1972). It has been suggested that ABA exerts its effect upon the H^+ expulsion mechanism within the plasmalemma of the guard cells (Raschke 1975). The rapid response to small amounts of ABA gives support to this view; for example, Cummins, Kende & Raschke (1971) found that a 10^{-7} M solution of ABA could initiate closure within 5 min of application. Rayle (1973) found that ABA inhibited the excretion of H^+ ions from coleoptiles, and Malek & Baker (1978) have shown that ABA

inhibits proton efflux into the perfused hollow petiole of *Ricinus*. The fact that inhibition of H^+ extrusion is common to the action of ABA in different locations in the plant thus lends support to the view that this is its primary effect upon the guard cells.

Stomatal responses to ABA seen as part of an integrated reaction of the plant to water stress

Two other effects of ABA have been identified which, acting in conjunction with the responses of stomata, could enable the plant to overcome some of the consequences of water stress.

Glinka & Reinhold (1971, 1972) found that ABA increased the permeability of carrot root tissue to water, and Collins & Kerrigan (1973) came to a similar conclusion from studies of exudation from isolated maize roots. Clarkson (1974) has pointed out that these results are likely to reflect, at least in part, an increase in active salt transport into the xylem. It is possible that ABA both increases the hydraulic conductivity of the root to water and raises the rate of ion flux into the xylem. A reduction in the resistance to radial water flow across the root could be important in making more soil water available to the shoot, for it can readily be shown that the resistance of the roots limits the flow of water into the shoot by the simple experiment of removing the root system and putting the base of the stem in water. The usefulness of the reaction of root cells to ABA, as a means of relieving water stress in the shoot, might depend on transport of ABA down to the root after its release from the chloroplasts. Hocking, Hillman & Wilkins (1972) showed that transport of ABA does take place from leaves to roots, and Hoad (1973) observed that some of the ABA produced by young leaves under water stress moves into the phloem for translocation to other plant parts. Tal & Nevo (1973) made an important study of three 'wilty' mutants of tomato, which have a low endogenous ABA content and have difficulty maintaining turgor because of abnormal stomatal behaviour. It was found that the resistance to water flow across the roots was greater in the mutants than in normal tomato plants, suggesting that this factor is closely linked to the effects of ABA in the leaf.

Malek & Baker (1978) have shown that ABA inhibits the active proton flux thought to be responsible for the co-transport of sucrose in phloem loading in *Ricinus*. A decrease in the rate of transport of sugars out of the leaf would enable the mesophyll cells to retain more solutes for the maintenance of turgor, which is necessary for a normal rate of growth to continue. The first effect of a fall in turgor may be on the expansion of cells, which is dependent on the pressure exerted by the protoplasts against the cell walls (Hsiao *et al.* 1976).

The extent to which these other effects of ABA operate with stomatal responses to relieve water stress is a matter for speculation, since the different processes have never been studied simultaneously in one plant species. More detailed studies are required of the distribution of ABA from leaf cells as their water potential falls, and of the integrated reactions in different parts of the plant.

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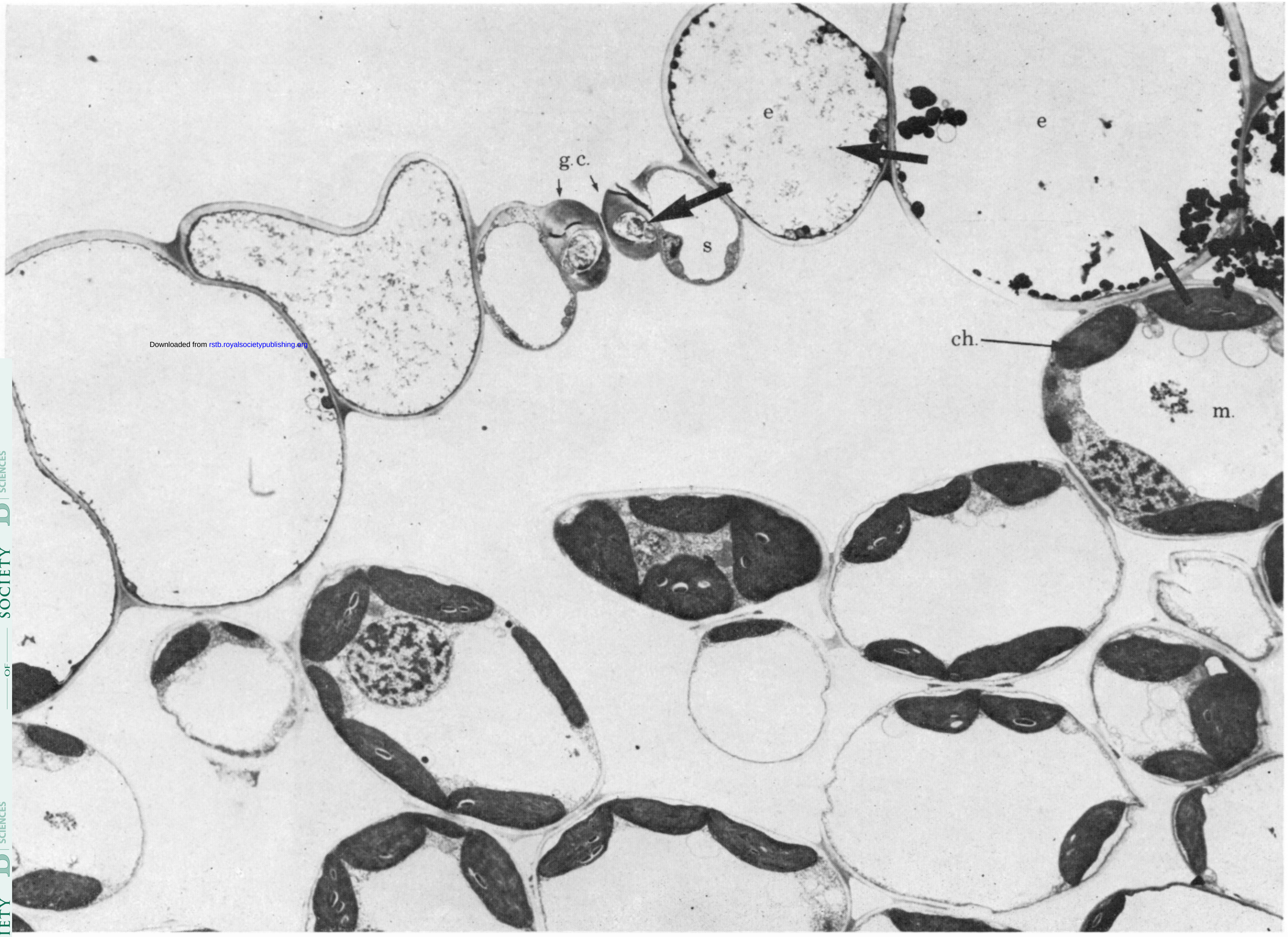

FIGURE 1. Assumed route for the movement of abscisic acid from the sites of its biosynthesis in chloroplasts to its 'target' areas in the epidermis, the guard cells. The route could be symplastic up to the subsidiary cells, but as there are no plasmodesmatal connections between guard and subsidiary cells, it must be apoplastic over this short distance. g.c., guard cells; s, subsidiary cell; e, epidermal cell; m, mesophyll cell; ch, chloroplast. Electron micrograph of section through leaf of *Phleum bertolonii* kindly supplied by K. Oates and F. I. Woodward.

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