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STOMATAL BEHAVIOUR

Chemical control of stomatal movements

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Artificially induced partial closure of stomata can improve the efficiency with which plants use available water; in other words, there is a fall in the transpiration: photosynthesis ratio. The careful use of some metabolic inhibitors can achieve the required inhibition of stomatal opening, but properly controlled application under field conditions is difficult and metabolic inhibition of photosynthesis in the mesophyll can occur. In the search for a more specific inhibitor of the activities of stomatal guard cells, most attention has been focused on abscisic acid, a hormone which appears to function as an endogenous regulator of stomatal opening. External application of abscisic acid achieves a useful reduction in the transpiration: photosynthesis ratio. Studies are being made of the effects of chemical analogues of abscisic acid in the search for more active compounds. Another naturally occurring substance which induces stomata to close, all-*trans*-farnesol, has been found in water-stressed sorghum plants. Sorghum is better able to tolerate water shortage than most crops, and further studies of its physiological mechanisms should lead to a better understanding of how we can provide protection for more susceptible crops.

Introduction

It is widely believed that it would be useful if we could impose artificial controls on stomata of crop plants to improve the efficiency with which they use water, especially at critical stages of growth. Closure of stomata must inevitably increase the diffusion resistance to carbon dioxide entering the leaf, and may thus reduce the rate of photosynthesis. There are, however, theoretical grounds for believing that a given degree of stomatal closure would exert a larger proportional effect on water loss from the leaf than on CO_2 entering, because of differences in the lengths of their diffusion pathways (Meidner & Mansfield 1968). Evidence confirming the theoretical predictions has come from experiments in which stomatal opening was inhibited by chemical means. The findings of several authors are summarized in table 1.

Carbon dioxide appears to play a central role in the stomatal mechanism. An increase in atmospheric CO_2 concentration to two or three times the normal level is sufficient to induce partial closure of stomata in the majority of species that have been studied. There has been no better experimental evidence to illustrate the effect of CO_2 than that which came from the factorial experiments of Heath and his colleagues over 20 years ago, notably the one on 'Charter' wheat (Heath & Russell 1954).

There was great excitement in the early 1960s over several chemicals which could induce stomatal closure when applied to leaves as highly dilute solutions. Effects of CO_2 on stomata were sometimes forgotten in the scramble to study the possible practical application of these compounds, but it later emerged that many of their effects could be explained in terms of raised CO_2 levels in the treated leaves (Meidner & Mansfield 1966; Mansfield 1967). Some of the compounds in question (e.g. the α-hydroxysulphonates) achieved this by inhibiting photosynthesis, others (e.g. the synthetic auxins) probably did so by stimulating respiration.

The lessons learned from these studies of a decade ago are important. When a compound applied to the leaf to reduce transpiration (i.e. an 'antitranspirant') causes stomatal closure by first increasing the internal CO_2 concentration, it not only *increases the resistance* to CO_2 molecules diffusing across the epidermal barrier, but it also *reduces the concentration gradient* down which the diffusion occurs. Considerations of the relative effects of a given degree of stomatal closure on transpiration and photosynthesis (Meidner & Mansfield 1968) assume that the magnitude of the concentration gradient for CO_2 diffusion is not reduced. When it is substantially reduced CO_2 intake will be inhibited proportionally more than water vapour loss, and hence the efficiency with which water is used by the plant will be reduced.

TABLE 1. EFFECTS OF CHEMICALLY INDUCED CLOSURE OF STOMATA ON TRANSPIRATION AND GROWTH

(The data for phenylmercuric acetate (PMA) are taken from studies in which no foliar injury was reported.)

author(s)/plant species/ treatments	observations
Zelitch & Waggoner (1962) *Nicotiana tabacum* PMA: 3.3×10^{-4} M, 1×10^{-4} M and 3.3×10^{-5} M	$(P_t/P_0)/(T_t/T_0)$ ranged from 1.40 to 0.96 and was significantly above unity; ($P < 0.05$); P_t, P_0 = photosynthesis in treated and control plants; T_t, T_0 = ditto, for transpiration
Shimshi (1963*b*) *Nicotiana tabacum* PMA: 9×10^{-5} M	ratio of growth over transpiration increased from 0.78 to 1.00 in well-watered plants, and from 0.83 to 0.93 in water-stressed plants; growth was indicated by leaf area
Slatyer & Bierhuizen (1964) *Gossypium* sp. PMA: 10^{-4} M	g transpiration/g dry tissue produced was 245 for controls and 180 for treated plants
Davenport (1967) *Festuca rubra* PMA: $10^{-3.5}$ M	g transpiration/g dry tissue produced was 2052 for controls and 1734 for treated plants
Jones & Mansfield (1972) *Hordeum vulgare* ABA and its methyl and phenyl esters, all at 10^{-4} M	g transpiration/g dry tissue produced was 560 in controls, 410, 390 and 390 respectively, after treatment with ABA, methyl-ABA and phenyl-ABA
Mizrahi *et al.* (1974) *Hordeum vulgare* ABA: 3.8×10^{-4} M	cumulative transpiration over 20 days was approximately 13 cm³ in controls and 5 cm³ in treated seedlings; total dry mass was 24 % greater in treated plants (over unspecified period); these figures applied to non-watered plants
Raschke (1974) *Xanthium strumarium* ABA: 10^{-5} M	g transpiration/g dry tissue was reduced by one half, while net photosynthesis was reduced only by one seventh

An antitranspirant of practical interest should, therefore, be capable of inducing stomatal closure without interfering with the ability of the underlying mesophyll to continue photosynthesizing. The partially closed stomata would offer protection from excessive water loss, and the only inhibition of photosynthesis would be due to the reduced availability of CO_2 resulting from the lower conductivity of the epidermis. Perhaps the ideal antitranspirant is CO_2 itself, for when an increase in CO_2 concentration closes stomata and reduces transpiration, the rate of photosynthesis actually increases because of the steeper concentration gradient for CO_2 diffusion. Unfortunately, problems of applying CO_2 in the field make its use impracticable. It is used in greenhouses to enhance the rate of photosynthesis, and as a result the stomata stay partially closed (Jones & Mansfield 1970*b*) but the saving of transpired water in this situation is of little practical consequence.

Can we find other chemicals with the necessary ability of inhibiting only the activities of the stomatal guard cells, and not exerting undesirable effects on other cells of the leaf? A chemical applied to the leaf surface might achieve this objective in two ways. It could have limited penetrability, entering only the guard cells and inhibiting their activity, but not gaining access to the other leaf cells. Alternatively it might be able to attack some specialized and essential process in the guard cells, but have no effect on other cells. An attempt to utilize the first of these modes of operation has been made with the inhibitor of photophosphorylation, phenylmercuric acetate (PMA). Under ideal conditions this compound does not penetrate leaves very readily and causes stomatal closure without destroying the photosynthetic apparatus in the mesophyll. It then achieves a useful reduction in transpired water with a proportionally smaller drop in photosynthesis (Shimshi 1963*a*, *b*). It is, however, difficult to predict the circumstances when PMA does not penetrate to the mesophyll, and indeed there is reason to believe that it easily does so (Squire & Jones 1971). Many field trials of this compound have been disappointing, there having been considerable toxicity to the crop under treatment. Nevertheless Waggoner & Turner (1971) after very extensive trials on forest trees, concluded that though PMA may not itself be of any practical appeal, they had at least demonstrated that the use of an antitranspirant can save a vast amount of transpired water.

The idea of attacking some aspect of the mechanism within the guard cells necessary for their turgor changes is probably more likely to be successful. Whether this method is feasible must obviously depend on whether there are essential processes that are peculiar to the guard cells and that are susceptible to chemical inhibitors. Encouragement comes from several unusual features of guard cells that distinguish them from other leaf cells. There is, for example, the loss of starch from their chloroplasts during the day and its accumulation at night, the exact reverse of what happens in the mesophyll. When stomata open, the guard cells are rich in potassium (Penny & Bowling 1974) and malate (Allaway 1973). They also apparently contain large amounts of the enzyme phosphoenolpyruvate carboxylase (Willmer, Kanai, Pallas & Black 1973). Unfortunately, we do not understand the reasons for all the specialized features we observe, simply because we have such a poor understanding of the mechanism behind stomatal movements. After years of effort since the beginning of this century we remain in ignorance of the full significance of the changes in starch content of the guard cell chloroplasts, and even of why chloroplasts are present at all. However, potassium accumulation in guard cells does now seem to have been established as a major, though perhaps not the sole, factor behind their turgor changes and it may be suggested that any means of controlling potassium transfer between the guard cells and their neighbours should be an effective means of imposing controls on stomatal movements.

NATURALLY OCCURRING ANTITRANSPIRANTS

It has long been known that the stomata of mesophytes do not open to their full apertures when the plants are under water stress. There appear to be defence mechanisms which have evolved to give some protection to the plants under such conditions. It will be of value to look at these mechanisms for two reasons. First, we must consider whether the naturally occurring mechanism for closing stomata is sufficient to give adequate protection; in other words, is there any need to apply antitranspirants exogenously? Secondly, by studying endogenous mechanisms we may learn something of the nature of chemical compounds that can cause stomata to close.

Stomatal behaviour in plants under water stress

There have been many studies of the way stomata react when the plant is short of water, and two distinct aspects of their response have been identified: an early reaction leading to closure long before visible wilting is evident, and a prolonged after-effect the duration of which depends on the severity of the water stress. The initial reaction was studied by Heath & Mansfield (1962) and it was found for *Xanthium strumarium* that there was a considerable increase in the sensitivity of the stomata to CO_2 in the early stages of the development of water stress. A stream of CO_2-free air passing through the intercellular spaces largely prevented the closing reaction. The work of Heath & Meidner (1961) had shown that the stomatal closing response to water stress could not be explained in terms of increases in the intercellular concentration of CO_2, and hence the closure is presumably induced by the normal CO_2 content of the atmosphere. The supply of CO_2 to the leaf will be greater in wind which reduces the boundary layer resistance, and it is under such conditions that transpiration is greatest and stomatal closure most effective in reducing it (Bange 1953). Thus the increased stomatal sensitivity to CO_2 in the early stages of water stress provides an efficient means of regulating transpiration, coming into operation increasingly in wind when it is most needed.

The prolonged after-effect of wilting on stomata has been studied in many species, and most recently by Fischer (1970) and Allaway & Mansfield (1970). A typical pattern of behaviour, which has been observed for species such as *Taraxacum officinale* (Heath & Mansfield 1962) and *Rumex sanguineus* (Allaway & Mansfield 1970) is shown diagrammatically in figure 1. The stomata recover the ability to open to their full apertures slowly over a period of several days. During this time the leaves are fully turgid, and show no visible signs of having suffered from water stress. The stomata are not induced to open if the leaves are flushed with CO_2-free air, and hence accumulation of CO_2 or a change in the sensitivity to it cannot be held responsible for the continued closure.

The role of abscisic acid

Wright (1969) and Wright & Hiron (1969) observed that wheat leaves which had been allowed to wilt contained greatly increased amounts of abscisic acid (ABA-see figure 2). They suggested that after-effects of wilting on physiological processes might be explained by the effects of this hormone. Little & Eidt (1968) had already observed that exogenous applications of ABA could reduce transpiration. Jones & Mansfield (1970*a*, 1972) found that surface applications of ABA and its derivatives to leaves were followed by behaviour identical to that in figure 1, i.e. a slow recovery taking several days after a single application to the leaves at the rate of approximately 0.02 μg cm^{-2}. Tal *et al.* (1970, 1971) showed that the absence of stomatal control over transpiration in the *flacca* mutant of tomato could be restored if it was supplied with ABA. Thus a picture has emerged of the role of ABA in the stomatal responses that take place after wilting. It may also play a part in the early responses to water shortage, before wilting becomes apparent. It has been shown that stomata react very quickly to ABA (Cummins *et al.* 1971) and further that ABA may enhance their response to CO_2 (Raschke 1974). This may be the reason for their greater sensitivity to CO_2 during the development of water stress. It is still not clear, however, why there is a difference in the CO_2-sensitivity of stomata between the early stages of water stress, and the later stages after rewatering when they do not open fully. If we accept that ABA is responsible for the increased sensitivity to CO_2, we cannot postulate the involvement of the hormone in the after-effects of stress without assuming that its mode of action has by then become different. This is a question worthy of further study.

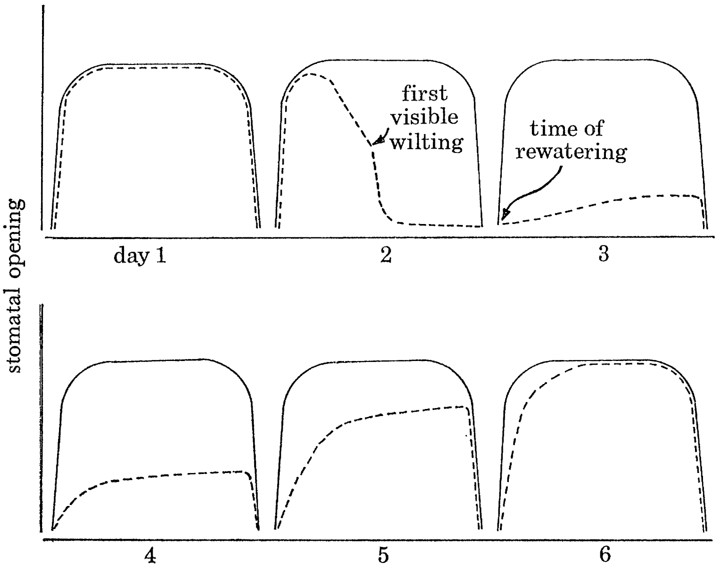

Figure 1. Stomatal behaviour during wilting, and the after-effect following re-watering. Diagrammatic, but based on data for *Taraxacum officinale* (Heath & Mansfield 1962) and *Rumex sanguineus* (Allaway & Mansfield 1970).

Exogenous applications of ABA to close stomata

A single surface application of a 10^{-4} M solution of ABA to leaves of *Xanthium strumarium* resulted in a suppression of stomatal opening for up to 9 days (Jones & Mansfield 1970*a*). Subsequent tests of its value as an antitranspirant were made on barley seedlings, and a single treatment reduced transpiration by about 50% averaged over a 9 day period. In barley as in *Xanthium* the effects of treatment had practically worn off after 9 days. Mizrahi *et al.* (1974) applied solutions of ABA at 3 day intervals to barley seedlings, some of which received an ample water supply, and some of which were deprived of water. In water-deprived seedlings, ABA reduced the rate of water consumption and caused an increase in the accumulation of dry matter. In other words there was an increase in the efficiency with which the available water was utilized.

These data seem to provide the answer to a question posed above: is there any advantage in the exogenous application of a compound that can be formed endogenously in response to water stress? That there is some advantage probably indicates that the time of the appearance of endogenous ABA was not ideal under the experimental conditions employed, i.e. it was too late to be of any benefit. If, under field conditions, we can anticipate the arrival of water deficiency and apply ABA to conserve the remaining water that is available, we might be able to give a crop better protection than it obtains from its own endogenous defence mechanism, which only comes into operation when the water shortage is already severe.

The mechanism of action of abscisic acid

Mansfield & Jones (1971) found that ABA caused a reduction in the solute concentration in the guard cells, judging from determinations of solute potential. The surrounding subsidiary cells were not, however, affected. Thus ABA appeared to inhibit the accumulation of solutes, and therefore the turgor increase, in the guard cells required for stomatal opening. It was also shown that ABA prevented the accumulation of potassium in guard cells, and increased the starch content of their chloroplasts. It has now been established that there is active transport of potassium into and out of guard cells as stomata open and close (Penny & Bowling 1974). It would appear, therefore, that ABA is an inhibitor of such transport, but the precise mechanism of its action remains unknown. Mansfield & Jones (1971) were unable to find any effect of ABA on uptake of ions into leaf tissues as a whole, and concluded that the hormone has a specific effect on ion transport to and from guard cells. Thus experience with ABA encourages the view that it is possible to attack selectively the essential processes in the guard cells which are responsible for stomatal movements.

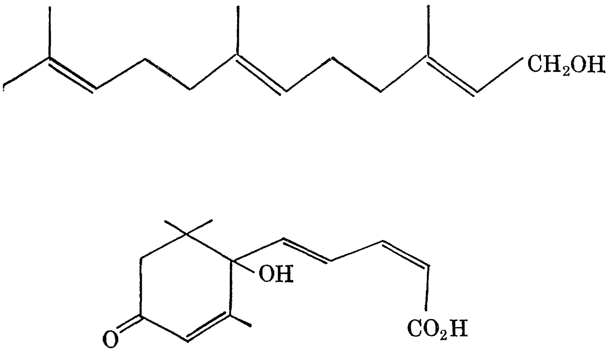

Figure 2. Chemical structures of all-*trans*-farnesol (top) and abscisic acid (bottom).

ABA is a hormone of widespread occurrence, and many roles have been suggested for it. While by no means all these roles have been fully established, there is no doubt that application of ABA to plants is likely to exert other effects in addition to closing the stomata. These have not yet been considered fully, but it is encouraging that in the experiments of Jones & Mansfield (1972) and Mizrahi, Scherings, Malis Arad & Richmond (1974) shoot growth was not severely inhibited by ABA. The latter authors did find a reduction in the rate of growth of the shoot, but regarded this as an advantage under conditions of water shortage. Growth was slower, but it continued after control plants were dying as a result of drought, with the result that ABA-treated plants showed an increase in dry mass compared with the controls. ABA was found to reduce root growth, but the disadvantage of this was probably overcome by the decreased resistance to movement of water across the roots after ABA treatment (Tal *et al.* 1971; Glinka 1972). ABA does not induce stomatal closure by causing an increase in the intercellular CO_2 concentration. It has little effect on CO_2 compensation, and ABA-induced stomatal closure is not reversed if CO_2-free air is flushed through the leaf (Jones & Mansfield 1970*a*; Orton & Mansfield 1975).

In its mechanism of action, therefore, ABA approaches the properties of an ideal chemical antitranspirant. It has a direct effect on stomatal guard cells and does not reduce the concentration gradient for CO_2 diffusion into the leaf. On the other hand it does exert other physiological effects and on this account it may fall short of the ideal properties we are seeking in a compound suitable for use in the field.

Molecular requirements for ABA-like activity

Full exploitation of the effects of ABA for control of transpiration in the field may depend on a knowledge of the molecular requirements for its activity. In the case of other plant hormones, notably the auxins, molecules having the correct structure can mimic the effects of the natural compound. Synthetic compounds, because they are not broken down in the plant, can exert greater and more prolonged action.

Studies of structure–activity relationships among analogues of ABA have been made by using the lettuce germination and the *Avena* coleoptile assays (McWha, Philipson, Hillman & Wilkins 1973). The response to different analogues differed in the two assays, perhaps indicating different molecular requirements for different physiological processes. It is, therefore, possible to conceive of an analogue which might exert an effect on stomata resembling (or greater than) that of ABA but which has little effect elsewhere in the plant. With this possibility in mind Orton & Mansfield (1975) studied the responses of stomata to ten of the few available analogues of ABA. Three of them showed activity comparable to that of ABA and it was possible to make some preliminary suggestions of structure-activity relationships. It was disappointing that none of the analogues was more active or persistent than ABA, but the number of compounds tested was very small compared with the large number of possible structural variations. The manufacture and testing of larger numbers of analogues may be required before further progess can be made. An operation of this scale is not possible with the resources of the average university laboratory, but one may hope that it will eventually be undertaken by an industrial organization.

STOMATAL BEHAVIOUR IN SORGHUM

In the above discussion we have considered the after-effect of drought on stomatal behaviour, and have ascribed to ABA the role of a stress hormone through the action of which the plant is protected. Studies of maize and sorghum under field conditions, however, led Glover (1959) to a different view of the after-effect, which he considered to be a disadvantage. Stomata of these two tropical grain crops behaved similarly up to and including the onset of drought, and in both species the stomata were less open as the drought became more severe. While experiencing the water stress, maize plants opened their stomata slightly during the morning, but they were closed for the rest of the day. Even during severe drought the stomata of sorghum appeared to be open to a small extent all day long. The main difference between the species was, however, in the rate of recovery after rewatering. Although both regained turgor and the leaves seemed normal (i.e. the wilting had not caused visible damage), the stomata of sorghum recovered quickly whereas those of maize remained closed for 10 days or more. Glover suggested that the superiority of sorghum over maize as a grain crop for use in dry regions lay in this ability to recover quickly to a normal pattern of stomatal behaviour.

Prolonged closure of stomata would inhibit photosynthesis and reduce the yield of the crop. Ogunkanmi (1973) performed experiments with maize and sorghum under laboratory conditions and confirmed that the stomata of sorghum are capable of a much more rapid recovery.

Our recent research in Lancaster has been much concerned with sorghum and its ability to tolerate drought. We have studied its pattern of stomatal behaviour and have attempted to determine the part played by ABA, and whether other factors are involved. Extracts from sorghum under increasing levels of water stress showed increasing amounts of endogenous ABA, but bioassays revealed the existence on the chromatograms of another factor which could induce stomatal closure. This substance, which was shown to be all-*trans*-farnesol, also increased in concentration in water-stressed plants (Ogunkanmi, Wellburn & Mansfield 1974; Wellburn, Ogunkanmi, Fenton & Mansfield 1974). The structure of this compound is shown alongside that of ABA in figure 2. Both ABA and farnesol are sesquiterpenoids, and so it might be possible for one to be converted to the other in order to exert an effect. However, a consideration of their speed of action and their effect within the guard cells led Wellburn *et al.* (1974) to the view that they act quite differently.

The role, if any, of farnesol as a factor in the drought tolerance of sorghum has not yet been fully established. This research has, however, shown the feasibility of preparing extracts from plants under water stress and testing the material eluted from segments of chromatograms in a bioassay in which the stomatal response is measured directly (Ogunkanmi, Tucker & Mansfield 1973). A systematic search for endogenous antitranspirants is now feasible and there is the possibility of our finding other compounds which can affect stomata directly.

FUTURE STUDIES

The fact that we have not completely elucidated the mechanism of drought resistance in sorghum is evidence of our general lack of understanding of the physiological mechanisms that give a plant protection. The unsuitability of maize for growing in dry regions indicates that the endogenous mechanism involving ABA is, of itself, insufficient to give the necessary protection. This and similar crops may benefit from externally applied chemicals which would induce a more expedient pattern of stomatal behaviour.

In relation to the main subject of this paper it is, however, pertinent to note that sorghum is able to tolerate drought without the need for externally applied chemicals. In other words, physiological mechanisms are available to enable plants to tolerate water stress, and the essential problem is that most of our crop plants do not possess them. If physiologists can elucidate the mechanisms, geneticists will be able to examine the possibility of producing new varieties with the required characters. Only if this is not possible will it be necessary to think of chemical treatment as a long-term solution to the problem.

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