LABORATORY STUDIES OF THE EFFECT OF POTASSIUM SALTS ON LUCERNE DRYING

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ABSTRACT

The application of a simple solution of potassium carbonate to lucerne at the time of cutting speeds the drying of the crop to the safe storage level. In dipping grapes to accelerate drying, emulsifier and oil accompanies the potash and it is widely accepted that these act by causing air-filled spaces between wax platelets to be filled with water which then evaporates directly to the atmosphere. Our results indicate that the "K-hay" mechanism is probably different.

In laboratory tests, we have shown that alkalinity is not involved, that potassium formate and acetate are as effective as carbonate, while sodium carbonate or the deliquescent acetate have much less effect. These results indicate cell-level action of potassium ions which are unlikely to affect superficial waxes but are known to be important in the function of stomata.

In potometer experiments, surface treatment with potassium carbonate did not affect water uptake into cut stems during the day but treated shoots took up more water than untreated during the night, which suggests that stomatal closure is being affected by the treatment. Scanning electron micrographs of treated leaflets also indicated some interference with normal stomatal closure. This effect could be due to the uptake of potassium ions by guard cells and is a possible cause of the faster drying of treated lucerne recorded in field an in laboratory experiments.

INTRODUCTION

Tullberg and Angus (1972, 1978) showed that surface treatment of cut lucerne with potassium carbonate solution greatly decreased the time necessary for the crop to dry to the baleable stage (25% moisture relative to dry matter).

The idea came from the ancient, and still widely used, practice of dipping grapes in potash-oil emulsions to accelerate drying to raisins (Martin and Stott, 1957). Possingham *et al.* (1967) considered that the emulsion fills the interstices between the microscopic wax platelets which in the natural state are responsible for water-repellency of the cuticle, so enabling vapour diffusion to be replaced by liquid flow. Possingham (1972) found evidence for this reversible behaviour using scanning electron microscopy. This mechanism has been widely accepted in the case of grape drying and assumed to operate also in the "K-hay" process.

Tullberg and Angus (1978) consider the possibility of stomata being involved but dismiss this because gas flow under low pressure across the leaf was found to be decreased by potash treatment. They showed, however, that potassium carbonate alone is effective in lucerne drying. Since grape drying needs an oil (e.g. ethyloleate) and a powerful surfactant (e.g. sulphated butyloleate) in addition to potash in comparable amount, we think it probable that different mechanisms are involved. Wieghart *et al.* (1980) used the raisin formula on lucerne, gaining useful acceleration of drying and found that the potash made no useful contribution. Their dosages, however, were very heavy and uneconomic (1.8% of both oil and surfactant and 1.3% of potash on dry weight).

The object of the work reported here was to throw further light on the mechanism in the hope of improving drying rate still further or more reliably.

METHODS

Healthy shoots of lucerne (cv. Wairau), carrying 4 to 8 expanded leaves were cut from plants in greenhouse or paddock. For drying measurements, except those following potometry, stems were gripped, singly or in groups of 2 or 3, in the smallest type of office "bulldog" clip with jaws covered by rubber tube. These were suspended at 24 numbered stations on the periphery of a circular plate rotating slowly about its vertical axle so as to give all shoots the same exposure inside a ventilated box heated and illuminated by incandescent lamps. The lamps were turned off while the rotation was stopped for periodic removal of shoots for weighing. Temperature was between 28° and 33°C and relative humidity between 45 and 60%. Closer environmental control was not attemped for these preliminary, but necessarily lengthy, experiments but an untreated and potassium carbonate treated group were run in each test along with one or two other treatments for comparison, 7 replicates of 3 categories or 5 of 4 being accommodated.

When water loss from turgid shoots was to be examined, they were initially cut under water if possible, at once re-cut a few cm above, again under water, before transfer to small test tubes or to volumetric potometers. The latter comprised 3 mm i.d. transparent plastic tube, mounted horizontally on wood. Part of the tube was bent through 270° to terminate upwards about 5 mm above the

horizontal portion in a short rubber tube sealing the stem sufficiently well to stop sucking back. The position of the free meniscus was measured at intervals and a 1 mm diam. polythene tube served to refill the horizontal measuring tube as necessary. These units were very convenient for measurements on shoots exposed to full sunlight.

Treatment was by dipping. Shoots were held upside down by the clip and at least 95% of the area immersed for approximately 10 seconds. After being shaken in a standard manner, the applied solution remaining amounted to between 8 and 12% of the fresh weight. In early experiments, wetting agent was added to each test solution but, unless used in amount thought likely to interfere with drying behaviour, this tended to leave trapped air bubbles. Later it was found that, after a dip in more powerful wetting solution, the shoots could be dipped successively in at least two lots of water and still remain wet overall. The preferred practice was to wet, pass through one lot of water and then into the test solution. In this way good cover could be obtained but with very little residual wetting agent on the surface-dried leaves, which recovered their unwettability. It seems necessary to have a volatile component in the wetting solution to secure rapid spontaneous spread on young lucerne leaves. We standardised on a 10% solution of Teric DN (dinonylphenol condensed with 15 ethylene oxides) containing also 1% n-octanol, this stock diluted 1 to 30 for use.

Tullberg and Angus dipped in potassium carbonate solutions up to a concentration of 10% with a maximal effect at about 2%. If retention is the same as in our work, the latter corresponds to about 1% on dry weight. A moderate crop yielding only 3 tonnes/ha would need 30 kg/ha, considerably more than the 4-10 kg/ha considered economic and giving a palatable product (Crocker and Lodge, 1981). Anticipating that, with the efficient wetting agent dip, a more uniform cover would be obtained than in field practice and considering it desirable to keep below the concentration for maximum effect to retain greater sensitivity to change, we used 0.25% in early potash dips but later used 0.75%.

To examine the appearance of the cuticle surface and stomata, leaflets from treated and untreated stems were sampled throughout the drying process and freeze-dried. They were coated with a thin (20 mm) conducting layer and examined in a Cambridge Stereoscan IIa scanning electron microscope. Some additional collapse of the leaf epidermal cells seems to have occurred during freeze-drying. In fully turgid leaves this effect exposed stomata to ready viewing but no evidence of its affecting guard cell structure was found.

RESULTS AND DISCUSSION

Rate of Drying

Tullberg and Angus (1978) state that potassium carbonate solution does not need wetting agent addition. However, we found that when fresh greenhouse grown lucerne shoots were dragged under the solution they retained a silvery appearance due to an air film and came out dry except for droplets trapped in axils and on tips. Older leaves weathered in the field were more easily wetted depending on history of exposure. Even the very water-reflective leaves of the pea crop became temporarily water-wettable after strong wind, (Dewey *et al.*, 1956). In field application it may be disadvantageous to use a spray of good wetting properties. Drops deflected from the first leaves hit will have more chance of deeper penetration of the swath and of being stroked by the conditioning bar on to the stems where potash is believed to have had its greatest effect. We wished to avoid these complications in the present tests but point out that they may be important and be a source of variability in field practice. The effect of wetters in the spray must be examined under field conditions.

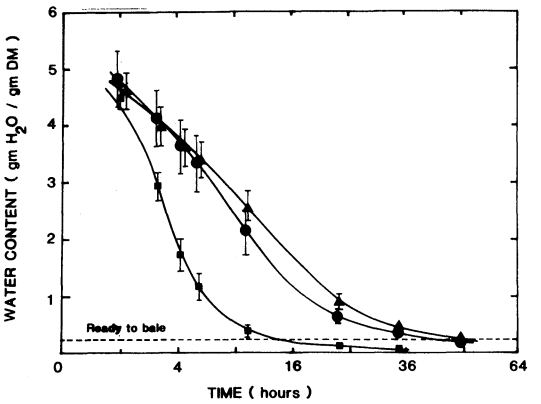

Figure 1: **3 groups of cut shoots; 7 reps. each, drying together.**
- ● **dipped in 0.5% wetter, left on**
- ▲ **dipped in 0.5% wetter, then water**
- ■ **dipped in 0.5% wetter then water, then 0.75% K_2CO_3**

Time on square root scale.

Fig. 1 shows the drying of shoots cut from the same source, 7 replicates each, under the same conditions, comparing three treatments just prior to drying (1) dip in standard wetter, followed by water; (2) dip in standard wetter, followed by water then 0.75% K_2CO_3; (3) dip in standard wetter, put to dry without wash. Bearing in mind that the two washed groups recover unwettability as soon as they dry and that the other group has a very high wetting residue it seems very unlikely that the much greater effect of potash is directly concerned with wetting of pores.

A probable primary effect of potassium carbonate on accessible cells would be an increase of pH. Although sodium carbonate has been reported as being ineffective, this could be due to its lower solubility, allowing it to crystallise out on the leaf surface whereas potassium carbonate remains liquid at normal field humidities. Two tests were made for the significance of alkalinity:

1. Cut lucerne was packed loosely into a 1 litre beaker and concentrated ammonia solution pipetted to the

bottom. Ammonia is known to be rapidly absorbed by leaves and will be retained by the slightly acid sap. 0.05% NH_3 on the fresh weight was used, equivalent to about 8 times the dose of potash left by dipping on similar stems set to dry at the same time. The ammonia had no effect on drying rate.

2. Shoots were dipped in potassium solution over-neutralised (ca. 10% excess) with acetic acid. Drying was not significantly slower than when carbonate was used. Formate also was nearly as effective as carbonate. Since the ineffectiveness of sodium carbonate could be due to its limited solubility and since alkalinity is apparently unnecessary, a run was carried out with sodium formate, a very deliquescent salt. There was a small decrease of drying time compared with controls but much less than with potassium formate or carbonate.

Several other potassium salts have been tested. Chloride is active, but definitely less so than carbonate. This could be due to limited solubility — a deposit can be seen to crystallise on the leaf surface. Laurate, at the equivalent concentration, with the addition of octanol, combines very rapid wetting with probably enhanced membrane permeability but its effect was about half of that of carbonate. This result is not consistent with Possingham's mechanism being applicable to lucerne. Perhaps laurate could cause potassium to be lost from the surface by too general a penetration of the leaf surface. To test the opposite, potassium alginate was used, in expectation that potassium would be held on the surface by the non-penetrating macroanionic lattice. It was totally ineffective.

It was thought that addition of non-crystallising sugars (Golden Syrup) to the carbonate might provide additional fluidity on the surface and it did slightly increase the drying effect but definitely so only when deliberately harsh drying conditions (35°, 20% r.h.), unlikely to be reached in a swath in the field, were used.

These variants were suggested by physico-chemical considerations. Another was tried for economic reasons. The main electrolyte constitutent of whey is potassium. Raw whey would contain too much sugar to be usable but much of this could be removed. Potassium lactate solution was tested. This salt, although also deliquescent, was much less effective in promoting drying than carbonate, acetate or formate. Since malate has been found to be a major companion of K^+ in guard cells (Allaway, 1973) potassium malate solution was tried. It was even less effective than lactate.

The results leading to these conclusions are shown in Figs. 2 and 3.

Cell-level chemistry rather than surface physics seemed to be implicated. This strongly suggests involvement of the guard cells, sensitive organs accessible to externally applied chemicals and known to be subject to change of potassium content during their function (see Raschke, 1975; Jarvis and Mansfield, 1981).

Effect on water loss from turgid shoots

One might expect an effect on stomata to make itself evident during the diurnal variation of water loss from turgid plants. The interpretation of the results is however ambiguous as is illustrated by the data summarized in Fig. 4.

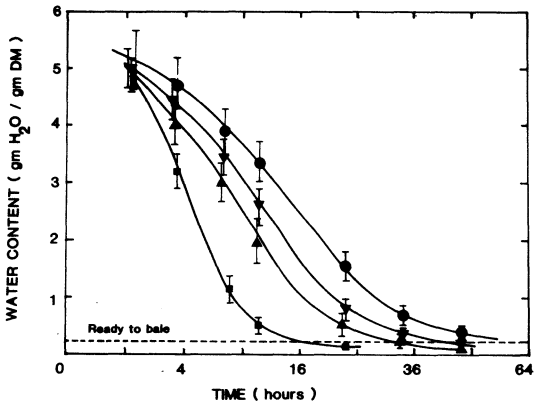

Figure 2: **4 groups of cut shoots, 5 reps. each drying together, all dipped in 0.5% wetter, then water (●); then water, then 0.75% K_2CO_3 (■); then water, then equiv. conc. K Lactate (▲); then water, then equiv. conc. N Formate (▼).**
Time on square root scale.

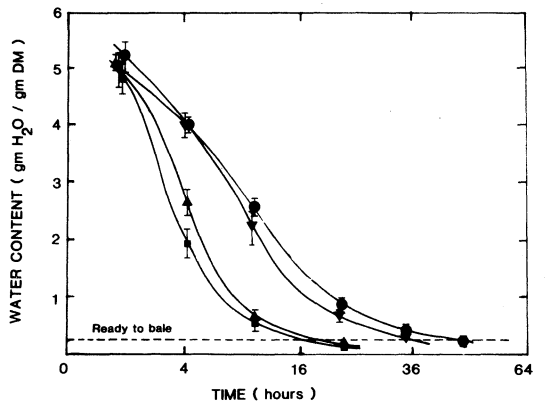

Figure 3: **4 groups of cut shoots; 5 reps. each drying together**
All dipped in 0.5% wetter
then water (●)
then water then 0.75% K_2CO_3 (■)
then water then eq. conc. K Formate (▲)
then water then eq. conc. K Malate (▼)
Time on square root scale.

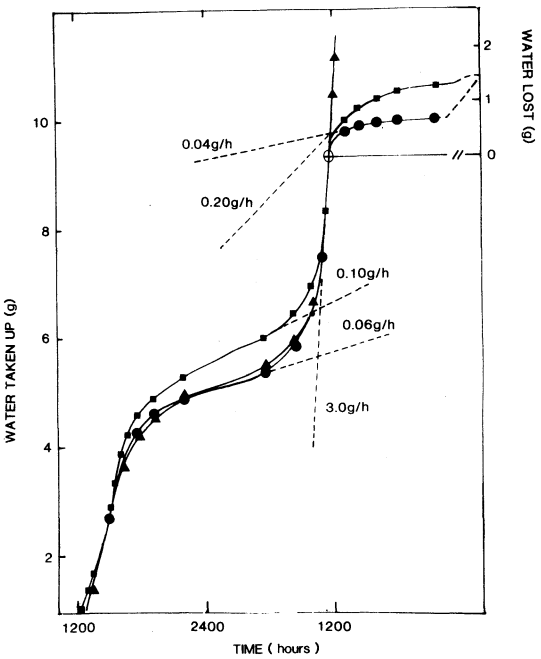

Figure 4: **3 well-matched shoots, cut ends in water. One untreated (●) and one K_2CO_3 -dipped (■) had water supply removed at this point. One untreated continued to take up water (▲). Figures by ——— tangents are rates of uptake or loss in g./h. Uptake scale adjusted to make equal at circled point.**

Examination of a large number of cut shoots reveals more variation in transpiration than in subsequent drying. In this experiment, 7 shoots were selected from 20 placed in potometers, for similarity of uptake. Three were treated. Four which showed later evidence of wilting or irregularity of uptake were discarded, leaving one treated and two controls. When the second day uptake had approached its peak rate, water was removed from the treated and one control stem and measurement continued gravimetrically though keeping the specimens in the same location alongside blotting paper evaporimeters. For better comparison, the water loss scale was adjusted to equate the values at the start of drying but no more than 15% correction was necessary.

The maximal rate of transpiration during the day was about 3.0 g/h and persisted in the turgid control during the first 1.5 hours of drying of the other shoots. The minimum rate of transpiration at night ranged from 0.06 g/h to 1.0 g/h with no significant difference. The ratio of day to night transpiration rates was 40:1 in this case but was only 6.5:1 for blotting paper. This gives the permeance of the leaf at night as about 0.16 of that in full light but this will be a minimum figure since the illuminated wet blotting paper would have reached a lower temperature than the dry leaf surface.

The difference when wilting is advanced is beyond doubt and much greater. There is now no atmospheric difference to allow for. After 1.5 h of wilting the rates of loss are 0.04 g/h for control and 0.2 for treated shoots giving permeance values of 0.013 and 0.07.

The residual permeance at night is usually considered to measure that of the coherent areas of cuticle between stomata (Gates, 1968). In the present case it is about 0.2 of the full day value. After 1.5 h of wilting the value in the control has fallen to about 0.013 although the water content is still 70% of the initial amount. At this level it can be assumed that the vapour pressure gradient is not much affected. Either the stomata are much more efficiently closed by wilting than by darkness or the inter-stomatal permeance is much reduced by wilting, or wilting creates new resistance in deeper tissues or some equivalent combination of these mechanisms. On the evidence of loss rate only it is impossible to distinguish between effects on stomata and effects on inter-stomatal cuticle. However, it is clear that, to produce the effect observed in the wilted state, where the plant has greatly increased its resistance to further drying, a potash effect on stomata need only be equivalent to very partial opening.

The effect on drying is seen in this case to operate after delay of 24 h in the turgid state although about 7 times the resident water content has passed through the stomata. Advantage was taken of this to apply potash internally. One tenth the concentration applied externally in one tenth of the volume of resident water was used. There was no effect on water uptake and no effect on subsequent drying, although 7 times the dose producing a major effect by external application was taken up and would be expected to be swept through into the peripheral tissues. The result is surprising in view of the known mobility of potassium in crops, much more than the resident amount being returned during a season in rain wash (Tukey, 1970). Presumably it must be excreted locally, e.g. by the hydatodes. To be effective in assisting drying, potash must be applied externally and it remains active on the leaf surface for more than 24 h.

Electron microscopy

Scanning electron microscopy was employed in an attempt to determine whether the leaf stomata or cuticle were being modified by the potash treatment. The cuticle on both surfaces of lucerne leaflets is covered with wax platelets approximately 1 μm square by 0.1 - 0.2 μm thick. They seem randomly oriented, though standing perpendicular to the cuticle surface. Dipping in the potassium carbonate/wetting agent solution did not modify this layer.

Stomata, about 130/mm² and with an aperture about 9 μm long, are present on both surfaces on the leaflets. On the stem, stomata are less frequent (ca. 60/mm²) but the aperture is longer (ca. 15 μ m). In micrographs the aperture is defined by prominent epidermal ledges through which the guard cell surfaces may be observed. These form the

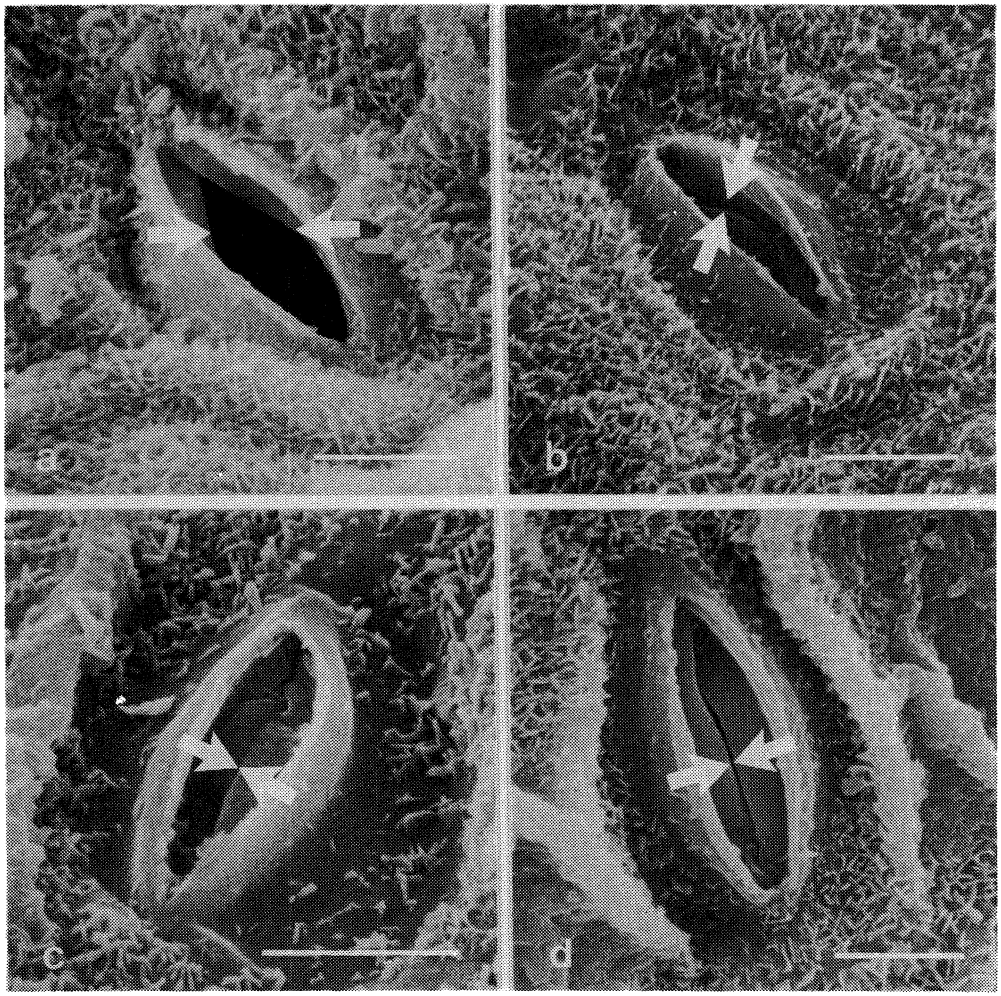

Figure 5: **Micrographs illustrating stomatal pore structures in different conditions. Beneath prominent epidermal ledges may be seen the surfaces of pairs of guard cells. The limits of the pore between are indicated by the white arrows. The permeance of the stoma to water vapour leaving the leaflet is proportional to the cross-sectional area of the pore.**

(a) after 4h in light — the leaflet was snap-frozen within 30s of the shoot being excised and dipped in potassium carbonate solution. The guard cells are fully turgid and the pore wide open.

(b) after 4h in darkness — this leaflet was fully turgid when taken from a plant supplied with water to excess each day. The guard cells have closed together but some pore is evident.

(c) during wilting — the shoot had been excised from the plant 4.5h earlier and had lost 0.45 of its initial water content. The guard cell surfaces are tightly appressed.

(d) during wilting following dipping in potassium carbonate solution — the shoot had been left to wilt for 35m and had lost 0.14 of its initial water content. A narrow pore is apparent between the guard cells. The bar in each micrograph represents 5 μm.

pore leading to the substomatal cavity and, lying far apart or closely apposed, regulate the egress of water vapour from the cavity. The response of the guard cells to light is illustrated in the micrographs, Figs. 5 a and 5 b. In the former, taken from a leaflet exposed to light of intensity about half full-sunlight for 4 hours before being excised, the fully turgid guard cells are forming a wide open pore of high permeance. In darkness, the guard cells lose turgour and close, as in Fig. 5 b taken from a leaflet sampled 4 hours after nightfall, lowering the permeance. Incomplete closure of the pore, as in the upper part of this stoma, was evident in 2/3rds of the stomata examined.

Amongst shoots allowed to wilt there was considerable variation in stomatal pore appearance, perhaps because the potash treatment was not uniform. In 65% of 43 stomata examined on leaflets of untreated shoots that had been allowed to wilt for between 6 minutes and 35 h, the guard cell surfaces were tightly appressed, as shown in Fig. 5c. This example was observed on a leaflet 4.5 h after excision of the shoot when 0.45 of initial water content had been lost. But similar examples were found in 65% of stomata on leaflets allowed to wilt for 6 min, during which an estimated 0.03 of initial water content only had been lost. On leaflets of shoots that had been dipped in potassium carbonate solution the ratio of tightly closed to partially open stomata was almost reversed. Of the 42 observed in which the two guard cell surfaces could be seen, 64% were open as illustrated in Fig. 5d.

It is not possible to estimate the relative permeances of the stomatal structures displayed but the observations are in accord with the potometer measurements presented in Fig. 4. The response of the guard cells to loss of shoot water content is rapid and results in tight closure of the stomatal pore — more so than the onset of darkness. The action of potassium salts appears to limit the ability of guard cells to respond to wilting, leaving a stomatal pore of greater permeance for water vapour than untreated leaves.

CONCLUSION

The faster drying rate of lucerne treated with potassium carbonate was also achieved when potassium was combined with other anions such as acetate or formate. Chloride was less effective, and potassium malate caused only slightly faster drying than the control. Such chemical specificity and antagonism strongly suggest that the effect on drying occurs at the cellular level, most likely involving the guard cells.

Observations of lucerne leaflet surfaces with the scanning electron microscope supported this conclusion by showing that treatment with potassium carbonate interferes with the tight closing of stomata which is their normal reaction to wilting. Many stomata in treated leaflets remained slightly open even when 0.14 of their initial water content had been lost. A small interference with stomatal closure could explain the increased rate of drying in treated shoots.

The suggestion that stomatal closure and interference with the mechanism of closure affects the drying rate of lucerne encourages further study.

REFERENCES

Allaway, W.G. 1973. Accumulation of malate in guard cells of *Vicia faba* during stomatal opening. *Planta 110:* 63-70.

Crocker, G.J. 1981. Potassium carbonate speeds up lucerne haymaking. *Agricultural Gazette of N.S.W. 92 (3):* 33-36.

Dewey, O.R., Gregory, P., Pfeiffer, R.K. 1956. Factors affecting the susceptibility of peas to selective dinitroherbicides. *Proceedings 3rd British Weed Control Conference 1:* 313-326.

Gates, D.M. 1968. Transpiration and leaf temperature. *Annual Review of Plant Physiology 19:* 211-238.

Jarvis, P.G., Mansfield, T.A. (Eds.) 1981. "Stomatal Physiology." Society for Experimental Biology: Seminar Series 8. Cambridge University Press, esp. MacRobbie, E.A.C. Ionic relations of stomatal guard cells. pp. 51-70.

Martin, R.J.L., Stott, G.L. 1957. The physical factors involved in the drying of sultana grapes. *Australian Journal of Agricultural Research 8:* 444-459.

Possingham, J.V., Chambers, T.C., Radler, F., Grncarevic, M. 1967. Cuticular transpiration and wax structure and composition of leaves and fruit of *Vitis vinifera. Australian Journal of Biological Science 20:* 1149-1153.

Possingham, J.V. 1972. Surface wax structure in fresh and and dried sultana grapes. *Annals of Botany 36:* 993-996.

Raschke, K. 1975. Stomatal action. *Annual Review of Plant Physiology 26* 309-340.

Tukey, H.B. 1970. The leaching of substances from plants. *Annual Review of Plant Physiology 21:* 305-324.

Tullberg, J.N., Angus, D.E. 1978. The effect of potassium carbonate solution on the drying of lucerne 1. Laboratory Studies. *Journal of Agricultural Science, Cambridge 91:* 551-555.

Tullberg, J.N., Angus, D.E. 1972. Increasing the drying rate of lucerne by the use of chemicals. *Journal of the Australian Institute of Agricultural Science 38:* 214-215.

Wieghart, M., Thomas, J.W., Tesar, M.B. 1980. Hastening drying rate of cut alfalfa with chemical treatment. *Journal of Animal Science 51 (1):* 1-9.