STUDIES ON THE USE OF CETYL ALCOHOL AS A TRANSPIRATION SUPPRESSOR

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Summary

The incorporation of cetyl alcohol into the rooting medium (vermiculite) of wheat plants at a concentration of 5% (w/w) caused a significant reduction in transpiration (expressed on a leaf area basis) in three out of four experiments. However, this effect was accompanied by a significant reduction in photosynthesis (per unit leaf area) and also in plant dry weight. The concentration of phosphorus in the tissue of treated plants was not reduced. The presence of 1% cetyl alcohol caused no significant transpiration reduction, but did cause a significant depression in photosynthesis and dry weight. The dry weight suppression with 1% cetyl alcohol was more severe if soil was substituted for vermiculite. The application of cetyl alcohol as a foliar spray reduced transpiration rates but also caused the ultimate death of the treated tissue; the solvent alone did not affect the plants. It is suggested that cetyl alcohol reduces transpiration rates by increasing the resistance to the entry of water into the root system, but that the growth-inhibitory effects of cetyl alcohol preclude its use as a transpiration suppressor.

I. Introduction

The capacity of cetyl alcohol (hexadecanol; $CH_3(CH_2)_{14}CH_2OH$) to reduce evaporation from a free water surface was reported 36 years ago by Langmuir (1917), and this substance has had some successful practical application in Australia (Mansfield 1955). The monolayer formed by cetyl alcohol, while reducing evaporation, does not prevent the exchange of carbon dioxide and oxygen. If such a monolayer could be induced to form at the liquid–vapour interface in the leaf mesophyll perhaps transpiration would be reduced without affecting photosynthesis and respiration. By 1961 reports had appeared from three separate workers (Roberts 1961; Sale 1961; Woolley 1961) on the potential use of cetyl alcohol in reducing plant transpiration.

Roberts' (1961) experiments with cetyl alcohol seemed quite promising. Corn (*Zea mays*) was grown in either sterilized soil or vermiculite; transpiration rates were reduced if an octadecanol–hexadecanol mixture had been added to the rooting medium. Water loss was measured with a potometer, and the reduction was of the order of 40% compared with control plants. However, when we analysed these particular data statistically, the reduction was not significant. Assuming that the octadecanol–hexadecanol was producing an effect, Roberts then attempted to find out why. Carbon-labelled cetyl alcohol fed to the roots of corn seedlings produced very faint radioactivity in their tops; but the root system showed very strong activity. Roberts concluded that perhaps cetyl alcohol was affecting the rate at which the roots took up water, and compared the resistances to water movement through the root systems of treated and untreated plants. Under negative pressure between 12 and 22% more water could be drawn from the root systems of control plants

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than from plants grown in soil enriched with cetyl alcohol. However, no account was taken of the effect of treatment on the size of the root system; and results presented in this present paper indicate that cetyl alcohol can reduce root growth.

Contrary to Roberts' (1961) findings, Woolley (1961) and Sale (1961) have reported the absence of an effect of cetyl alcohol on transpiration rates, either when mixed with the soil or when applied as a foliage spray. Both of the latter investigators also report a depression of growth resulting from the mixing of cetyl alcohol into the plant rooting medium. Roberts states that cetyl alcohol had no effect on growth, but records no relevant growth data.

The experiments reported here were an attempt to confirm the results published by Roberts (1961), which were obviously important if reproducible and of wide application. The effects of cetyl alcohol were studied in relation to growth, transpiration, photosynthetic rate, and phosphorus uptake.

II. Materials and Methods

Cetyl alcohol was kindly supplied by Dr. W. W. Mansfield (Chemical Research Laboratories, C.S.I.R.O., Melbourne) and some was also purchased from British Drug Houses Pty. Ltd. The two samples had identical X-ray diffraction patterns, and also had the same physiological effects on plants. A small quantity of 1-^{14}C-labelled cetyl alcohol came from the Amersham Radiochemical Centre, England.

Experimental plants included wheat (cv. Sherpa), Algerian oats, and sugar-beet (cv. Braun "E"). Seedlings were grown in 600-ml glass beakers equipped with drainage arms. Each container housed four plants, and was filled with 75 g of vermiculite. The plants were watered weekly with 100 ml of half-strength Hoagland solution, and were grown under both glasshouse and growth-room conditions. The growth room had a 16-hr photoperiod and was illuminated from a combination of warm white fluorescent tubes and incandescent strip lamps, and gave an intensity of 1200 f.c. at the base of the plants. Air temperature was 21°C.

Measurements of transpiration were made gravimetrically. The tops of the containers were sealed with paraffin wax, polythene sheeting, or sometimes with a fixed depth (4 cm) of polythene beads. After leaf area determination, all plant material was dried for 3 days at 80°C in an oven with forced draught. Transpiration rates have invariably been expressed on a leaf area basis.

Net photosynthesis was determined at a CO_2 concentration of 300 p.p.m. by measuring, with an infrared gas analyser, the CO_2 consumed by the plant from a gas stream recirculating at 13 l/min. The light flux was $13{\cdot}6\times10^4$ ergs/sec/cm^2 (from high-pressure mercury vapour lamps). Leaf temperature was 25°C. For chlorophyll analysis plant material was macerated in 80% acetone and the optical density of a clear extract measured at 6630 and 6450 Å (in a Unicam spectrophotometer) after the method of MacKinney (1941).

Phosphorus analysis followed a modification of the Berenblum and Chain (1938) phosphomolybdate method.

III. Results

(a) *Effect of Cetyl Alcohol on Transpiration, Photosynthesis, Dry Weight, and Phosphorus Uptake in Wheat*

(i) *Experiment 1.*—An initial unreplicated experiment was performed using wheat seedlings grown for 21 days in a glasshouse. Cetyl alcohol (C.S.I.R.O.) was added to the root medium on sowing at three concentrations—0, 10, and 40% of the dry weight of vermiculite in the pot. Each pot contained four seedlings. Treatment with 10 and 40% cetyl alcohol reduced transpiration, compared with the control, by 8 and by 40% respectively. However, whole plant dry weight was reduced by 40 and by 54% respectively.

(ii) *Experiments 2 and 3.*—In these experiments, wheat seedlings were grown for 21 days in the growth room. Cetyl alcohol (B.D.H.) was supplied at two levels, viz. 1 and 5% of the dry weight of vermiculite in each pot. There were 12 plants for each treatment plus eight controls. In experiment 2, measurements were made of

Table 1

EFFECT OF CETYL ALCOHOL ON CERTAIN PHYSIOLOGICAL CHARACTERS IN 21-DAY-OLD WHEAT SEEDLINGS

Experiments 2 and 3

Concn. of Cetyl Alcohol in Root Medium	Transpiration ($g/dm^2/hr$)	Dry Weight of Whole Plant (g)	Net Photosynthesis ($mg\ CO_2/hr/dm^2$)	Moisture Content of Tops (% fresh wt.)
Control	1·27	0·179	9·67	84·29
1% (w/w)	1·16	0·132**	6·29**	82·92
5% (w/w)	0·84**	0·122**	4·66***	78·51***

** Differs significantly from control, $P < 0·01$.
*** Differs significantly from control, $P < 0·001$.

transpiration and photosynthetic rate and of dry weight. Some of these results have been published previously (Neales and Kriedeman 1962) and are therefore only briefly summarized in Table 1. In experiment 3, photosynthesis was not measured, but the effects of cetyl alcohol on transpiration and dry weight observed previously in experiment 2 were confirmed. The moisture content data for the tops of control and treated plants presented in Table 1 were derived from experiment 3.

The most important feature of these results is that even though 1% cetyl alcohol caused a significant reduction in both dry weight and photosynthesis, there was no significant reduction in transpiration rate. While the addition of 5% cetyl alcohol reduced transpiration, it also reduced dry weight and photosynthesis significantly.

The reduction in growth with 1 and 5% cetyl alcohol may be partially attributed to the reduced rate of photosynthesis in treated plants. These plants showed severe chlorosis, and when some of the net photosynthesis data were expressed on a chlorophyll basis, rather than in terms of leaf area, treatment with cetyl alcohol showed no effect.

(iii) *Experiment 4.*—Phosphorus absorption was studied on seedlings grown for 40 days in the growth room, with 5% cetyl alcohol added to the rooting medium. There were 12 control and 12 treated plants. The results for dry weight, transpiration, and phosphorus content are given in Table 2.

TABLE 2

EFFECT OF CETYL ALCOHOL ON CERTAIN PHYSIOLOGICAL CHARACTERS IN 40-DAY-OLD WHEAT SEEDLINGS

Experiment 4

Concn. of Cetyl Alcohol in Root Medium	Transpiration ($g/dm^2/hr$)	Dry Weight of Whole Plant (g)	Phosphorus Concentration (mg P/g dry wt.)		Phosphorus Content (mg P)	
			Tops	Roots	Tops	Roots
Control	1·22	0·525	1·59	0·743	0·502	0·192
5% (w/w)	1·43	0·143**	1·97**	1·140**	0·254	0·110

** Differs significantly from control, $P < 0·01$.

It is apparent that phosphorus uptake, as assessed by the phosphorus concentration of tops and roots, is unhindered by the presence of cetyl alcohol in the rooting medium. The lower phosphorus content of treated plants is simply a reflection of their reduced dry weight. Thus it seems unlikely that impedance of nutrient uptake is the cause of the growth inhibition induced by cetyl alcohol.

From earlier experience, the significant dry weight suppression was anticipated, but the slight (though non-significant) increase in transpiration was not expected. The reason for this increase is not clear from the available data. Considering earlier growth-room experiments, the plants in experiment 4 were grown under identical conditions but were nearly twice the age of plants in experiments 2 and 3. This can only imply that the expression of an effect of cetyl alcohol on transpiration is somehow influenced by the state of vegetative development of the plants.

(*b*) *Comparison between Soil and Vermiculite as a Suitable Rooting Medium for Cetyl Alcohol Enrichment*

Cetyl alcohol is a long-chain aliphatic alcohol and it is therefore liable to be broken down by the soil microorganisms. It is also possible that the breakdown products are inhibitory to plant growth, although Roberts (1961) stated that cetyl

alcohol caused no effects on the growth of corn in either soil, sterilized soil, or vermiculite. We therefore compared the growth of plants in the presence of cetyl alcohol in both soil and vermiculite.

(i) *Experiment 5.*—Oats were raised in polythene pots in a glasshouse for 40 days using both soil (unsterilized) and vermiculite as rooting media. In treated pots, cetyl alcohol (C.S.I.R.O.) was added at a concentration of 1·0% of the air-dry weight. In the vermiculite series there were five control and five treated plants, and in the soil series there were 10 replicates.

The results in Table 3 indicate that the presence of cetyl alcohol in the rooting medium causes a significant reduction in the dry weight of both tops and roots. This decrease is clearly more severe for the soil-grown oats than in the vermiculite culture, but the reason for this greater severity is not apparent. Perhaps the plant absorbs some toxic product from the breakdown of cetyl alcohol by the soil microflora—a situation which would not exist in vermiculite. The difference between soil and vermiculite control dry weight may be attributed to differing nutritional conditions.

TABLE 3

EFFECT OF CETYL ALCOHOL ON THE DRY WEIGHT (G) OF OATS GROWN FOR 40 DAYS IN VERMICULITE AND IN SOIL

Experiment 5

Concn. of Cetyl Alcohol in Root Medium	Vermiculite			Soil		
	Tops	Roots	Whole Plant	Tops	Roots	Whole Plant
Control	0·248	0·135	0·383	0·383	0·252	0·635
1% (w/w)	0·106**	0·079**	0·185**	0·023***	0·052***	0·075***

** Differs significantly from control, $P < 0·01$.
*** Differs significantly from control, $P < 0·001$.

(c) *Movement of Cetyl Alcohol within the Plant*

In the work already described, the presence of cetyl alcohol in the rooting medium reduced transpiration rates in three out of four experiments. It was therefore of interest to investigate the extent to which this compound was translocated in the plant and to see if there was any evidence that cetyl alcohol did indeed form a monolayer at the evaporative surface of the leaf mesophyll cells.

(i) *Experiment 6.*—Six wheat seedlings aged 28 days and grown originally in soil were transferred to aerated half-strength Hoagland solution for the duration of the experiment. The six plants were maintained in a growth room at a light intensity of 700 f.c. and temperature of 19°C. Four days after transfer, four plants were each given 100 mg of unlabelled cetyl alcohol (to simulate conditions where plants are grown in the presence of this substance) and 4 hr later their root systems were

supplied with 1-^{14}C-labelled cetyl alcohol. This was administered as 7·2 μc in 2 ml 95% ethanol. At this point the two control cultures were given 2 ml 95% ethanol. Three days later all seedlings were harvested. One of the controls and two of the treated plants were dried between blotters in a mechanical press and then left in direct contact with X-ray paper for 10 days. The photograph of one such radioautograph is given in Plate 1, Figure 1.

There was no detectable radioactivity in the tops of either treated or control plants but the roots of treated plants were active. From the original radioautographs it was quite apparent that most roots of the treated plants showed a narrow region of higher activity on each side. This was taken to represent an original pellicle of active material which had been squashed to either side of the root during the period for which they were pressed between the blotting paper.

(ii) *Experiment 7.*—Plant material similar to that used in experiment 6 was maintained in non-aerated half-strength Hoagland solution in the glasshouse for the duration of the experiment. Five pots, containing a total of 15 plants, were treated with 7·2 μc of 1-^{14}C-labelled cetyl alcohol in 2 ml 95% ethanol. One control pot (containing three plants) was given only 2 ml 95% ethanol.

Seven days later all seedlings were harvested. The plants were pressed as in experiment 6 but were left in direct contact with X-ray paper for about 10 weeks. The radioautographs were similar to the one in Plate 1, Figure 2.

In this case the tops of the treated plants showed very faint activity. But as in Roberts' tracer experiment this merely indicates that some labelled substance has entered the tissue. There is no evidence that this substance is the original 1-^{14}C-labelled cetyl alcohol. Because of the long exposure time of this radioautograph, it is not possible to localize the site of the activity within the root system.

(*d*) *Foliar Application of Cetyl Alcohol*

It was apparent that little, if any, cetyl alcohol was reaching the leaves of plants whose roots were in contact with this compound. Therefore the following experiment (No. 8) was performed to test the effect that cetyl alcohol has on transpiration when applied directly as a foliar spray.

Sixteen plants of wheat and of sugar-beet were grown in vermiculite under glasshouse conditions for 30 days. Eight of the plants of each species were treated, the other half were taken as controls. Treated plants were sprayed with an atomized solution of 10% cetyl alcohol (C.S.I.R.O.) in ether (as used by Sale 1961) which left an even white deposit on the surface of the leaves. Control plants were sprayed with the same volume of solvent only.

Transpiration was measured over 48 hr following spraying; treated sugar-beet showed a reduction of 59% in transpiration compared with the controls, and wheat showed a 54% reduction. Within 24 hr after completion of transpiration measurements, treated tissue became very flaccid, and was dead at the end of the fourth day. Control plants remained normal. This extreme toxicity of cetyl alcohol and also the suppression of transpiration is contrary to Sale's (1961) results, but it is very likely due to an excessively heavy application of cetyl alcohol.

IV. Discussion and Conclusions

The possibility of conferring upon plants a degree of drought resistance by chemical treatment is most attractive. This could be attained either by increasing drought tolerance, or by reducing transpiration. Cetyl alcohol has been used recently with the second aim in view (see Introduction). Halevy and Kessler (1963) indicate that treatment with growth-retarding substances possibly increases drought tolerance.

The results of the first five experiments reported in this paper are summarized in Table 4. These results demonstrate that the presence of cetyl alcohol did reduce foliar water loss in three out of the four experiments where transpiration was measured. However, in all of them there was also a reduction in growth; and this is most severe when cetyl alcohol is incorporated into soil (expt. 5). This substance is therefore toxic to growth. Such an observation needs emphasizing because, firstly, it is contrary to Roberts' (1961) claim and, secondly, from experiments 2, 3, and 4 there was a significant depression of growth without any attendant reduction of transpiration. Sale (1961) and Woolley (1961) both reported that cetyl alcohol depressed plant growth in a manner similar to our findings.

Table 4

Summary of results for plants grown in rooting media enriched with cetyl alcohol

Expt. No.	Plant Used	Age (days)	Rooting Medium	Concn. of Cetyl Alcohol (% w/w)	Transpiration Rate (as % of control)	Whole Plant Dry Weight (as % of control)
1	Wheat	21	Vermiculite	10	92·4	61·4
				40	73·1	46·4
2	Wheat	21	Vermiculite	1	91·3	73·7
				5	65·9	68·2
3	Wheat	21	Vermiculite	1	89·7	60·7
				5	75·0	52·3
4	Wheat	40	Vermiculite	5	117·5	41·1
5	Oats	40	Vermiculite	1	—	48·3
			Soil	1	—	11·8

The fact that cetyl alcohol significantly reduced growth *and* transpiration in some experiments also calls for comment. From Table 4 we see that a reduction in transpiration rate only occurs when growth is also reduced. The mechanism of this undoubted effect on transpiration is obscure but two alternatives at least appear possible.

The first of these is that cetyl alcohol obstructs the entry of water into the plant's root system, and this is in some measure substantiated by experiments 6 and 7. Here 1-[^{14}C]cetyl alcohol when fed to the roots of wheat seedlings gave little indication that this substance is translocated in any quantity to the evaporative surfaces in the leaves. Further evidence for a reduction in the amount of water

entering the plants comes from experiment 3 (Table 1). Here the moisture content of the tops of treated plants is significantly lower than controls; and since the loss of water from the tops of treated plants is also less than controls, the actual amount of water entering the plant must have been reduced.

The second alternative is that the reduction in transpiration is a consequence of the growth inhibition induced by the cetyl alcohol treatments. Perhaps leaf morphology is altered in such a way that water loss is reduced.

On the available evidence then, it appears probable that cetyl alcohol does not reach the leaves in sufficient quantity to affect transpiration rates if administered to the rooting medium, although if sprayed onto leaves it can reduce transpiration but it also kills the treated tissue.

We conclude that cetyl alcohol is unlikely to be of use as a transpiration suppressor until it can be shown to depress transpiration rate with little or no concomitant depression of growth.

V. Acknowledgments

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VI. References

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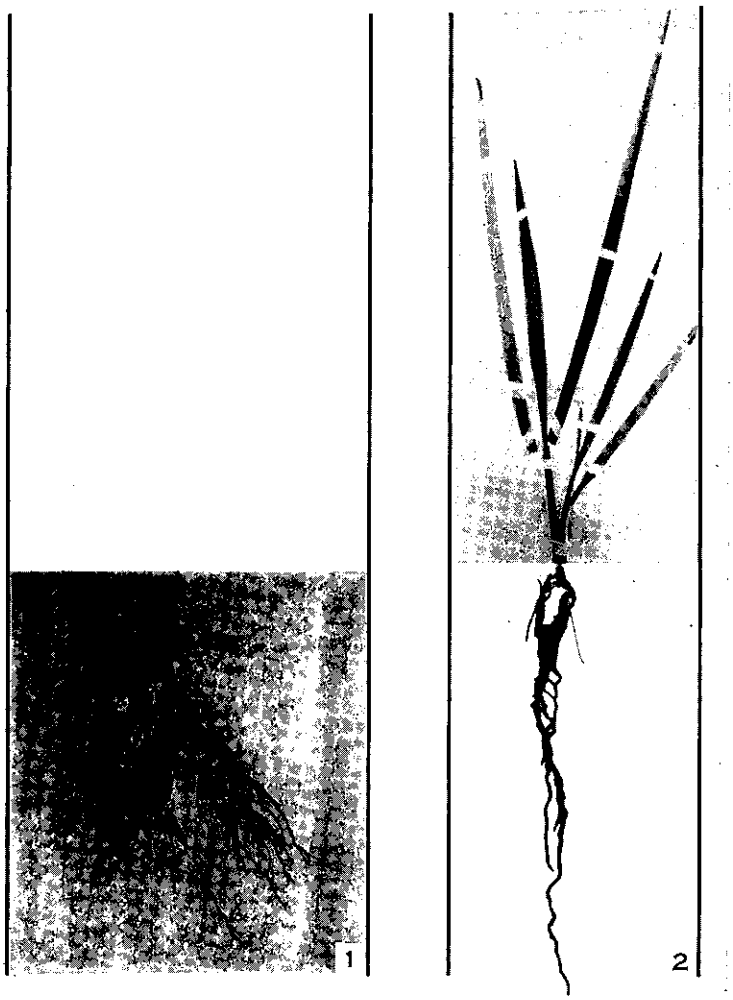

Figs. 1 and 2.—Radioautographs of wheat seedlings whose roots were fed 1-[^{14}C]cetyl alcohol. Fig. 1: plant material left in contact with the X-ray plate for 10 days (expt. 6). Fig. 2: plant material left in contact with the X-ray plate for about 10 weeks (expt. 7).