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PHYSIOLOGICAL EFFECTS OF A FILM-FORMING ANTITRANSPIRANT ON PLANTS

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PHYSIOLOGICAL EFFECTS OF A FILM-FORMING ANTITRANSPIRANT ON PLANTS

by

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LIST OF ABBREVIATIONS

A	-	Photosynthetic rate
A_i	-	Photosynthetic rate at atmospheric CO_2 concentrations
A_o	-	Potential photosynthetic rate
ABA	-	Abscisic acid
ANOVA	-	Analysis of variance
Ca	-	Calcium
CO_2	-	Carbon dioxide
C_i	-	Intercellular CO_2
C_o	-	Ambient CO_2
cv	-	cultivar
C.V.	-	Coefficient of Variance
CWF	-	Cool white fluorescent
E	-	Transpiration flux
Eq.	-	Equation
Fe-EDTA	-	Iron Ethylene diaminetetra-acetic acid
g_s	-	Stomatal conductance
g_w	-	Conductance to water vapour
IRGA	-	Infra-red gas analyser
h_r	-	Relative humidity
H_2O	-	Water
/	-	Stomatal limitation
LSD	-	Least Significant Difference
LWC	-	Leaf water content
m	-	Molecular mass
PAR	-	Photosynthetically active radiation
PEG	-	Polyethylene glycol
PMA	-	Phenylmercuric acetate
PPF	-	Photosynthetic photon flux
r_a	-	Boundary layer resistance to water vapour transfer
r'_a	-	Boundary layer resistance to CO_2 transfer
r_c	-	Cuticular resistance to water vapour transfer
r_{ep}	-	Epidermal resistance to water vapour transfer

r'_{ep}	-	Epidermal resistance to CO_2 transfer
r_f	-	Resistance of antitranspirant film to water vapour transfer
r'_f	-	Resistance of antitranspirant film to CO2 transfer
r'_i	-	Intercellular resistance to CO_2 transfer
r_s	-	Stomatal resistance to water vapour transfer
R	-	Gas constant (8.314 $J.mol^{-1}.K^{-1}$)
RSME	-	Root Square Mean Error
SEM	-	Standard error of the mean
SS	-	Sum of Squares
T	-	Absolute temperature
w_a	-	Water vapour density of the air
w_i	-	Water vapour density in the leaf
Ψ	-	Water potential
WUE	-	Water use efficiency

DECLARATION

I declare that this dissertation
for the degree M.Sc(Agric) at the University of Pretoria, has not been
submitted by me for a degree at any other university.

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Pretoria
July 1997

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by

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Degree	:	**M.Sc(Agric) Agronomy**

ABSTRACT

Antitranspirants could make an important contribution to crop production when optimum yields are required in areas of water scarcity. However, a decrease in photosynthetic rate is an important problem that has been experienced with the use of antitranspirants. Vapor Gard, a film-forming antitranspirant, is generally used in South Africa to alleviate transplant shock of vegetable seedlings. Experiments were performed in growth chambers and a glasshouse on butter lettuce, crisphead lettuce and bean plants, to quantify the effect of Vapor Gard on physiological processes. Vapor Gard did not decrease transpiration, except when measurements of bean plants were taken in full sunlight. Photosynthetic rates were not negatively affected. Micrographs showed that the antitranspirant film cracked over stomatal pores soon after application. Photosynthesis: Internal CO_2 curves showed that the film did not affect stomatal resistance. Differences between leaf temperatures of control and treated plants were not greater than 4°C.

INTRODUCTION

The use of antitranspirants to limit transpiration and alleviate the adverse effects of water deficits on plant growth, dates back to 300 B.C. (Gale & Hagan, 1966). Typically, 1% of the water that moves through the plant, from the soil to the atmosphere, becomes part of the biomass. Due to the scarcity of water in many parts of the world, the achievement of maximum crop yields with a minimum of water use, presents a major challenge to agricultural producers.

In the past metabolic inhibitors, which chemically restrict stomatal opening, were used as antitranspirants. It was found that most of these materials caused side effects such as reduction in photosynthesis. Antitranspirants that form a film over the stomata were found to be more inert and tended to cause fewer side effects than the metabolic inhibitors. Film-forming antitranspirants have been used to limit water loss in different crops for over thirty years. The alleviation of transplant shock of seedlings, such as lettuce, is an important application of antitranspirants. A film-forming β-pinene polymer (trade name Vapor Gard) is frequently used in South Africa (as in many other countries) for this purpose. There is evidence of the efficacy of antitranspirants, but no published local experimental results are available.

An antitranspirant that can suppress transpiration without inducing serious injury to treated plants, and without a proportional reduction in photosynthesis, can make an important contribution to effective water use in various crops. This is especially important in countries where scarcity of water has created pressure for the effective use of this resource.

When evaluating antitranspirants for their use in the field, their effects on plant processes should be quantified. Consequently, the main objectives of this study were to quantify the effects of Vapor Gard on the rates of transpiration and photosynthesis of treated lettuce seedlings under a variety of controlled and well-defined environmental conditions. Secondary objectives were to determine the effects of Vapor Gard on the water relations of the plants, leaf temperature and the effect of the film on the stomata.

CHAPTER 1

LITERATURE REVIEW

The possibility of reducing plant transpiration, thus saving water and alleviating the adverse effects of water stress on plant growth when transpiration exceeds the rate of water uptake, presents a tremendous challenge.

Loss of water through transpiration is a passive process in which water moves from the leaves to the air, mainly by vapour diffusion and partly by turbulent transfer. The driving force is the vapour density gradient between the air of the stomatal cavities and the air around the leaves. The following equation describes transpiration:

$$E = (w_i - w_a)g_w \qquad (1)$$

where

$$g_w = \frac{1}{r_a + r_{ep}} \quad \text{and} \quad \frac{1}{r_{ep}} = \frac{1}{r_s} + \frac{1}{r_c}$$

and E - the transpiration flux,

r_a - boundary layer resistance to water vapour transfer,

r_{ep} - epidermal resistance to water vapour transfer,

r_s - stomatal resistance to water vapour transfer,

r_c - cuticular resistance to water vapour transfer,

w_i and w_a - water vapour densities in the leaf and the air, respectively (Solárová, Pospíšilová & Slavík, 1981).

It is clear from Eq. (1) that transpiration may be reduced in four ways:

(i) by reducing the net energy input and (w_i),

(ii) by increasing the humidity of the air near the leaf (w_a),

(iii) by increasing the resistance to water loss (r_{ep}),

(iv) by increasing the resistance of the air near the leaf to vapour transport (r_a) (Poljakoff-Mayber & Gale, 1972; Solárová *et al.*, 1981).

An important requirement of any method for reducing transpiration, is that it should not interfere with plant growth. The reduction of transpiration in itself is not very difficult to obtain, but the main problem is to do so without having an adverse effect on photosynthesis and growth.

1.1 APPROACHES TO REDUCE TRANSPIRATION

Various methods exist that can be regarded as possibilities to reduce transpiration. The four most important are 1) increasing leaf reflectance with chemical sprays, which reduces the infrared radiation absorption; 2) windbreaks, which increase the air resistance to water vapour transfer; 3) enclosures in which the humidity builds up, thus decreasing the vapour density gradient from the leaf to the air; 4) applying chemicals which tend to close the stomata or form a film over the leaf surface, resulting in a physical barrier to diffusion and thus increasing leaf resistance to water vapour loss (Poljakoff-Mayber & Gale, 1972).

1.1.1 MODIFICATION OF LEAF REFLECTANCE

Trunks of fruit trees are often whitewashed after pruning has exposed them to the direct rays of the sun. This is done to increase reflectance and to prevent overheating (Poljakoff-Mayber & Gale, 1972). Abou-Khaled, Hagan & Davenport (1970) covered the upper leaf surfaces of various plant species with kaolinite (about 225mg.dm^{-2}) under laboratory conditions and measured leaf temperatures, photosynthesis and transpiration. A reduction in leaf temperature (3 to 4°C) and a decrease in transpiration (22 to 28%) under conditions of high light intensity, warm air, low relative humidity and low wind speed were found. This was ascribed to increased leaf reflectivity mainly in the visible spectrum. Photosynthesis was reduced only at low light intensities since the white coating raises both the light compensation and light saturation points of single leaves. Maximum benefit from kaolinite coatings was obtained under high incident energy and for species which were light saturated at relatively low light intensities or showed no effective self-regulating mechanism for water expenditure.

The reflective materials applied must stick and spread evenly on the plant surfaces, be non toxic, and must be sufficiently permeable to gases, so as not to interfere with respiration and photosynthesis (Abou-Khaled *et al.*, 1970). If a material reflecting above 700nm and transmitting below this wavelength could be developed, it would be a promising research direction (Poljakoff-Mayber & Gale, 1972)

1.1.2 WINDBREAKS

In advective situations where a significant amount of the energy taken up by the leaf originates from the air, windbreaks may be effective in decreasing the transpiration/photosynthesis ratio. Lowering of the wind speed decreases the amount of advective heat brought to the leaves and increases the water vapour content of the air near the leaves. A large quantity of water vapour is given off in transpiration, as compared to a relatively small quantity of carbon dioxide taken up in photosynthesis. Consequently, the percentage change in the air to leaf CO_2 gradient and the CO_2 concentration of the air behind a windbreak is small compared to the change in the leaf to air vapour gradient and the water vapour concentration. As a result, photosynthesis benefits from the reduced stomatal resistance that is often found in sheltered plants. Windbreaks may increase the photosynthesis/transpiration ratio both by decreasing transpiration and increasing photosynthesis (Poljakoff-Mayber & Gale, 1972).

1.1.3 ENCLOSURES

Covered enclosures such as plastic greenhouses present two advantageous possibilities for plant growth.

1. Transpiration is reduced and the development of water stress is retarded by the higher humidity that is maintained in the greenhouse.
2. It is commonly observed in greenhouses that the CO_2 concentration drops below the ambient level. This so-called CO_2 depletion (Heij & De Lint, 1984) is caused by CO_2 uptake by the crop and insufficient CO_2 influx. The addition of CO_2 to the air will raise the rate of photosynthesis (Mortensen, 1987). The stomatal closure response to high CO_2

concentrations tends to increase the ratio of photosynthesis to transpiration, thus increasing water use efficiency (WUE) on a unit leaf area basis (Gijzen, 1995). This is because the flux of water vapour from the leaf is relatively more sensitive than the flux of CO_2 to changes in the stomatal resistance (Poljakoff-Mayber & Gale, 1972; Wolfe, 1995).

Carbon dioxide enrichment seems like the ideal antitranspirant, as it operates favourably on both sides of the transpiration/photosynthesis ratio, but its practical application is limited to greenhouses.

1.1.4 INCREASE OF LEAF RESISTANCES

The surface of the leaf is the most logical place for increasing resistance to water movement and loss, since diffusion takes place mainly through the stomata (Poljakoff-Mayber & Gale, 1972, Salisbury & Ross, 1992, Nederhoff, 1995.) The coating of leaves with an inert material is probably the oldest method for reducing transpiration. References regarding the use of antitranspirants to ameliorate the detrimental effects of water deficits on plant growth date back to Theophrastus in 300 B.C. (Gale & Hagan, 1966).

Reduction of transpiration by coating leaves with chemicals can be accomplished, either by initiating the closure of the stomata, or by forming a film over the leaf area.

1.1.4.1 Closure of stomata

Theoretical analysis and experimental data indicate that stomata-closing antitranspirants should cause reduced transpiration rates, provided the applied chemicals do not damage the plant's internal photosynthetic mechanism (Solárová *et al.*, 1981).

Adjustments of stomatal aperture provide the primary short-term control of plant water status, and there is strong evidence for endogenous abscisic acid (ABA) playing a major role in stress-induced stomatal closure. A wide range of plant metabolites has been reported to cause some degree of stomatal closure. Many of these metabolites are related to ABA, such as ABA-esters (Jones & Mansfield, 1972), vomifoliol (Stuart & Coke, 1975) and xanthoxin (Raschke, Firn & Pierce, 1975). The concentrations of several short straight-chain fatty acids rise in stressed leaves, and when applied exogenously, can cause stomatal closure (Willmer, Don & Parker, 1978).

There has been a widespread interest in developing synthetic antitranspirants because of the perceived need to limit transpiration in dry environments. Some of these compounds, such as phenylmercuric acetate (PMA), may act by increasing chloroplast permeability and consequently ABA release (Milborrow, 1979), while others such as photosynthesis inhibitors and acetylsalicylic acid, may act via an increase of internal CO_2 concentrations. Compounds that raise the internal CO_2 concentrations are unlikely to be agronomically useful, for they inhibit the net photosynthesis (Jones, 1980). Compounds artificially decreasing stomatal aperture (by inducing the closure of stomata or arresting their opening), change in contrast to film-forming compounds, neither r_c nor r_a, but only r_s.

It is important to use the correct concentration of these materials to avoid phytotoxicity effects. Phenylmercuric acetate (PMA), in particular, must be used with care, since it is a mercury containing metabolic inhibitor (Davenport, Hagan & Martin, 1969)

1.1.4.2 Film-forming antitranspirants

According to Kreith, Taori & Anderson (1975), transpiration rate in a leaf treated with a film-forming antitranspirant can be described as

$$E = \frac{w_i - w_a}{r_a + (r_{ep} + \triangle r_{ep}) + r_f} \qquad (2)$$

Comparison to Eq.(1) shows that due to the application of a film-forming material leaf resistance increases with the additional resistance of the film (r_f) and a simultaneous change of r_{ep} to ($r_{ep} + \triangle r_{ep}$).

A corresponding equation (3) describes the net photosynthetic rate (A) of leaves treated with film-forming antitranspirants

$$A = \frac{c_a - c_i}{r'_a + (r'_{ep} + \triangle r'_{ep}) + r'_i + r'_f} \qquad (3)$$

where

c_a and c_i - CO_2 concentrations outside and inside the leaf respectively,

r'_a - boundary layer resistance to CO_2 transfer,

r'_{ep} - epidermal resistance to CO_2 transfer,

r'_i - intracellular resistance to CO_2 transfer and

r'_f - resistance of antitranspirant film to CO_2 transfer (Kreith, Taori &

Anderson, 1975)

An increase in the ratio of photosynthesis to transpiration can only be achieved if the film is more permeable to carbon dioxide than to water vapour. Otherwise a reduction in transpiration will cause a concomitant reduction in photosynthesis (Kreith *et al.* ,1975). The permeability of films is however lower for CO_2 than for H_2O and thus r'_f is higher than r_f (Solárová *et al.*, 1981, Woolley, 1967). The resistances of 1μ thick films of several polymers are represented in Table 1.1. The resistance to carbon dioxide relative to water ranges from about equality in polyethylene to an unfavourable difference in cellulose acetate. The obvious choice would be a material like polyethylene (Waggoner, 1981). However, if the objective is primarily to reduce water loss, film-forming antitranspirants of any kind can be used (Kreith *et al.*, 1975).

As permeability of films is relatively low for CO_2 and for H_2O, gas exchange is mostly taking place in the uncovered leaf areas. A decrease in transpiration (E) and photosynthesis (A) is also affected by the ratio of covered and uncovered leaf surfaces (Waggoner, 1981).

According to the properties of a film-forming compound or solvent the created films are of different thicknesses (from monomolecular to relatively thick layers) and the thickness is usually not uniform over the whole leaf surface (Waggoner, 1981). Properties of a compound and the method of application affect the ratio of covered and uncovered areas (Olofinboba, Kozlowski & Marshall, 1974).

Table 1.1 Permeabilities and resistances of 1μ thick films to gas and vapour exchange (After Waggoner, 1981)

	PERMEABILITIES (10^{-8} cm^2 sec^{-1} per atm)		RESISTANCES (sec cm^{-1})	
MATERIAL	CO_2	WATER	CO_2	WATER
Natural rubber	100	1100	56 - 100	9
Polyethylene	3 - 20, 9 - 14, 13, 19, 50	16 - 50, 44 - 88, 44 - 190, 130	200 - 3300	52 - 620
Ethyl cellulose	23, 33, 36	3900	280 - 430	3
Polystyrene	6 - 281, 7, 30	460, 550 - 770, 700 - 1000	330 - 1700	10 - 22
Cellulose acetate	4, 4 - 14, 7	1500 - 9700	720 - 2500	1 - 7
Pliofilm	0.1 - 14, 0.4 -2	10 - 250, 99	5000 - 10000	40 - 1000
Polyvinyl chloride	0.4, 0.81	280, 390 - 2200	12000 - 25000	4 - 36
Polyethylene glycol	0.007, 0.09	200	10^5 - $1.4*10^6$	50
Regenerated cellulose	0.001	36, 38	10^7	260 - 280

Successful compounds must be non toxic, not only to plants, but also to animals and man (Waggoner, 1981). The cost of the material and its application must be less than the value of the water saved and/or the increased yield of a crop for an antitranspirant to be economical (Kreith *et al.*, 1975).

Many materials were tested in the past. Information on some materials available as commercial products, like Wilt Pruf, Mobileaf, Clear Spray, Vapor Gard, Folicote etc., are presented in Table 1.2.

Table 1.2 Film-forming antitranspirants: trade names, chemical composition and distributors (Adapted from Solàrovà *et al.*,1981)

TRADE NAME	CHEMICAL COMPOSITION	MANUFACTURERS
A-C-3	Polyethylene emulsion	Allied Chemical Corporation
Adol 52	95% cetyl alcohol plus about 1.5% C_{14}-and 1.5% C_{18} alcohols	Archer Daniels, Inc., USA
Clear Spray	Latex	W.A. Cleary Corp., New Brunswick, N.J., USA
CS-6432	Wax-latex emulsion	Chevron Chemical Company, Richmond, Ca., USA
Dow silicone (emulsion XF-43531)	Dimethylpolysiloxane chains	Dow Corning Corp., Midland, Michigan, USA
Folicote	Paraffin Wax emulsion	Aquatrols Corp. Pennsauken, N.J.
Improved Wiltpruf	Polyvinylchloride	Nursery Specialty Products Inc., CT, USA
Keykote	Plastic wax emulsion	Key Chemicals, Inc., Ca., USA
Mobileaf	Wax emulsion in water	Mobil Chemical Company, Richmond, USA
OED Green	?	Nikken Chemicals Ltd., Japan
S-789	Copolymer dispersion of vinyl acetate-acrylate esters	Serafon Co., Rehovot, Israel
Vapor Gard	poly-1-*p*- menthen-8-9-diyl	Miller Chemical and Fertilizer Co., Hanover, USA
Wiltpruf NCF	Polyvinyl chloride emulsion in water	Nursery Specialty Products Inc., Greenwich, USA

1.2 POSSIBLE EFFECTS OF REDUCED TRANSPIRATION ON PLANTS

Apart from the obvious interests in transpiration reduction, antitranspirants challenge plant physiologists with such questions as: What will the effect of a reduction of the transpiration stream on the plant be? What physiological side effects will result from different types and concentrations of antitranspirants applied to different plants? (Gale & Hagan, 1966). Opinions on the significance of transpiration have varied in the past, from the belief that transpiration is entirely unnecessary and merely an inevitable consequence of plant structure, to considering it vital for processes such as leaf cooling and mineral uptake and transport (Gale & Hagan, 1966, Salisbury & Ross, 1992).

1.2.1 TRANSPIRATION AND LEAF TEMPERATURE

Leaf temperatures depend on a balance between net incoming radiation and factors of dissipation: mainly sensible heat exchange, long wave radiation and the latent heat of evaporation expended in transpiration. The factors of dissipation are interdependent. When transpiration is reduced by a resistance in the transpiration pathway, leaf temperatures will rise. This in turn will lead to a raise in transpiration rate (Gates, 1968).

In a study by Gates (1968) it was found that leaf temperatures were very similar to air temperatures. The data show that a relatively large reduction in transpiration (almost 40%) did not result in more than a 3°C rise in leaf temperature. This agrees with field measurements of several plants made by Gale, Poljakoff-Mayber, Nir & Kahane (1964).

Thames (1961) observed an interesting side effect. He found in a laboratory experiment at low wind velocity, that a wax antitranspirant had little effect on transpiration rates, but raised pine leaf temperatures about 2.4°C, an effect that he ascribed to a greenhouse effect trapping radiation within the film. It appears that leaf temperatures would only be significantly increased by a reduction of transpiration under extreme conditions of high incident radiation (Gale & Hagan, 1966) and very low wind velocity (Gale & Hagan, 1966; Salisbury & Ross, 1992). As pointed out by Gates (1968), and measured in some plants by Lange & Lange (1963), a complete termination of transpiration may under extreme conditions produce a 10% increase in leaf temperature. Such a rise may result in a detrimental increase in the respiration/photosynthesis ratio and may even be lethal.

While antitranspirants of the reflecting type cause a reduction in leaf temperature, the stomata-closing and film-forming types tend to increase leaf temperature by curtailing transpiration rates and thus reducing evaporative cooling. The increase in leaf temperature under normal conditions is relatively small, since thermal emission, rather than evaporative cooling, is the most important means of heat dissipation (Davenport *et al.*, 1969).

1.2.2 TRANSPIRATION AND ION UPTAKE AND TRANSPORT

There is no doubt that transpiration expedites ion transport within the plant. There appears to be some effect of transpiration rate on ion uptake, but this varies according to the type of plant and the specific ion involved. However, the use of antitranspirants, on a short term basis, and the concomitant decrease in transpiration, will not decrease the mineral

nutrient supply to such an extent that it will retard growth (Gale & Hagan, 1966; Davenport *et al.*, 1969; Win, Berkowitz & Henninger, 1991).

Calcium movement within a plant depends mainly on the transpiration stream in the xylem (Hanger, 1979). Therefore, plant foliage and fruit compete for Ca as it moves with the transpiration stream from root to shoot. Since leaves transpire at a much higher rate than fruit, deficiencies of Ca often occur in fruits and tubers, e.g. blossom-end rot in peppers and tomatoes (Schon, 1993), Ca-deficiency related tuber necrosis in potatoes (Win *et al.*, 1991) and tipburn in lettuce and cabbage (Palzkill, Tibbits & Williams, 1976; Barta & Tibbits, 1986). Altering the leaf:tuber or leaf:fruit water potential gradients within a plant by the application of an antitranspirant on the leaves, may allow greater Ca accumulation in the tubers and fruit (Win *et al.*, 1991).

1.2.3 TRANSPIRATION AND WATER RELATIONS

Antitranspirants may offer some protection against water stress by slowing moisture loss from plants (Hummel, 1990). The use of antitranspirants consequently may also have positive effects on crop quality, maturity date and yield (Davies & Kozlowski, 1974; Davenport, Uriu & Hagan, 1975; Andersen, Buchanan & Albrigo, 1979; Steinberg, McFarland & Worthington, 1990). Research done on the effects of antitranspirants on water relations are summarised in Table 1.3.

It is clear from the data in Table 1.3 that the results are inconsistent, although the majority of results seem to be positive. These studies emphasize that the differences in species influence the efficiency and usefulness of antitranspirants.

Table 1.3 Effects of antitranspirants on water relations

AUTHOR(S) AND YEAR OF PUBLICATION	MATERIAL(S)	PLANT SPECIES	EFFECTS	COMMENT
Slatyer & Bierhuizen, 1964	Adol 52, OED Green, S-600, PMA	*Gossypium hirsutum*	Improved water use efficiency	PMA most promising
Davenport, 1967	PMA, DSA (metabolic)	*Festuca rubra*	Reduction in transpiration	Phytotoxicity
Davies & Kozlowski, 1974	Dow Silicone, CS-6432, Keykote, Folicote, Improved Wiltpruf, Clear Spray, Vapor Gard	*Fraxinus americana, Pinus resinosa*	Reduced water loss	Differences in species and in product efficacy
Davenport *et al.*, 1975	CS-6432 (wax-latex)	*Olea europaea*	Improved water balance	Possible increase in fruit growth
Martin & Link, 1978	Folicote, Clear Spray, Wiltpruf NCF	*Chrysanthemum morifolium*	Reduced water loss	Folicote depressed flower size and delayed flowering
Weller & Ferree, 1978	Vapor Gard	Golden Delicious Apples	Improved water balance	Decreased photosynthesis
Andersen *et al.*, 1979	Vapor Gard	Rabbiteye blueberry fruit	No significant changes	Increased leaf resistance and leaf temperature
Srinivaso Rao, 1985, 1986	PMA, 8-HQ, Kaolinite	*Lycopersicon esculentum*	Improved plant water status High values of relative water content	PMA and Kaolinite improved yield, Stomatal diffusive resistance increased

Table 1.3 Effects of antitranspirants on water relations (Continued)

AUTHOR(S) AND YEAR OF PUBLICATION	MATERIAL(S)	PLANT SPECIES	EFFECTS	COMMENTS
McDaniel, 1985	Folicote, All-Safe, Vapor Gard, Cloud Cover, Elvanol	*Hydrangea macrophylla*	Reduction in transpiration	Better results together with chlormequat
Berkowitz & Rabin, 1988	ABA	Bell peppers	Increased water potential	Increased leaf resistance
Hummel, 1990	Vapor Gard, Envy, Wiltpruf, Folicote, UC86177	Chinese elm, Crabapple, *Viburnum,, Lycopersicon esculentum, Petunia,*	Differences in species and in product efficacy	—
Steinberg *et al.*, 1990	Improved Wiltpruf NFC	*Prunus persica*	Reduction in water use	More research is needed
Nitzsche, Berkowitz & Rabin, 1991	Folicote	Bell peppers	Increased water potential	Increase plant growth
Shekour, McDavid & Brathwaite, 1991	Vapor Gard	Sweet corn	Improved plant water status	Increased growth
Win *et al.*,1991	Folicote	*Solanum tuberosum*	Increased leaf water potential	Reduction in tuber necrosis

These factors must carefully evaluated prior to recommending use of film-forming antitranspirants (Davies & Kozlowski, 1974).

An increase in water potential may increase other aspects of growth (Waggoner, 1981). Considering that fruit growth depends not only on the accumulation of photosynthates and minerals, but also on high cell turgidity, larger fruits (Davenport *et al.*, 1975, Andersen *et al.*, 1979) may result from antitranspirant applications.

Reduction of transpiration, by applying an antitranspirant to the foliage before transplanting could: 1) increase plant survival and 2) improve crop stand uniformity (Mungse & Bhapkar, 1983).

The alleviation of plant water stress experienced after transplant shock (a situation where transpiration may exceed water absorption, resulting in substantial plant water deficits) may increase transplanting success and ultimately lead to increased yield. Berkowitz & Rabin (1988) characterized transplant shock in pepper seedlings as a decline in water potential (by as little as 0.6 MPa) within hours after transplanting and lasting for as short as 24 hours. In their study, when transplants were irrigated within a day after planting out, the transitory stress led to yield reductions. Berkowitz & Rabin (1988) applied ABA and Nitzsche *et al.* (1991) applied Folicote (Table 1.3) and in both instances significant increases in plant water status and subsequent fruit yield of Bell peppers were demonstrated. It has been shown that applications of exogenous ABA increase leaf resistance (r_L) and water potential (Ψ) for several days (McKee, 1978). Enhancement of seedling water status for several days after transplanting by Folicote application resulted in less stress-induced leaf abscission and enhanced plant growth throughout the growing season (Nitzsche *et al.*, 1991).

1.2.4 PHOTOSYNTHESIS

Since plant growth depends on the accumulation of raw materials, particularly through photosynthesis (Davenport, Uriu & Hagan, 1974), the effects of antitranspirants on photosynthesis should be considered. From a superficial examination, it seems probable that an increase in the resistance across the leaf-air interface, would affect both processes to a similar degree since both transpiration and photosynthesis involve gaseous diffusion across this zone. More detailed examinations have however, revealed an important difference in the two diffusion pathways (Slatyer & Bierhuizen, 1964).

Transpiration involves the evaporation of water from sites primarily located in the walls of internal mesophyll cells, and its diffusion through two resistances, connected in series, to the bulk air outside the leaf [$r_a + r_{ep}$ in Eq.(1)]. Photosynthesis also involves these internal and external resistances since CO_2, in entering the plant, must pass through the same pathway in the reverse direction. However, the CO_2 must diffuse through an additional resistance [r'_i in Eq. (3)] in the liquid of the mesophyll cells. The total resistance to diffusion is thus smaller for transpiration than for photosynthesis and increasing the resistance of the leaf with an antitranspirant should, theoretically, have less effect on photosynthesis than on transpiration (Slatyer & Bierhuizen, 1964, Gale & Hagan, 1966, Kramer & Boyer, 1995).

Stomata may be more open beneath an antitranspirant film, because of an improved leaf water potential. The wider stomata under the film could result in increased photosynthesis if the permeability of the films to CO_2 could be improved without impairing their resistance to water vapour loss

(Davenport *et al.*, 1974).

It is not only the percentage coverage of leaves and/or fruit that influences photosynthesis, but also environmental conditions. Photosynthesis of *Phaseolus vulgaris* plants treated with a copolymer dispersion of vinyl acetate-acrylate (S-789) was increased under hot, dry growing conditions but decreased under cool, humid conditions (Gale, 1961). Transpiration demand (Gale, 1961), soil moisture (Gale *et al.*, 1964) and degree of coverage seem to influence the effects of coatings on photosynthesis (and transpiration).

1.3 MISCELLANEOUS APPLICATIONS

Except for the more obvious uses (such as the reduction of transpiration with the possibility of an increase in plant water status and improved photosynthesis) film-forming antitranspirants may have other practical applications.

1.3.1 PRESERVATION AFTER HARVEST

Post harvest application of Vapor Gard and gibberellic acid to improve the shelve life of mangoes was investigated by Khader (1992). A Vapor Gard treatment led to a decrease in weight loss of fruit, and a pronounced retardation of ripening was observed when fruits were treated with Vapor Gard and gibberellic acid (Khader, 1992).

Application of Plantgard, Wiltpruf NCF, Mobileaf, Nu-Film 17, and Vapor Gard were compared on mature *Citrus sinensis* cv. Valencia trees for reducing fruit weight loss after harvest. Sprays of 2% Plantgard deposited

little material, Wiltpruf NCF and Nu-Film 17 were intermediate and Mobileaf and Vapor Gard added substantial material to the natural epicuticular leaf coating two weeks after application. Reduction of fruit weight loss two weeks after spraying was similar in magnitude to the additional coating measured on the leaves. Vapor Gard at 4% was still effective five months after application (Albrigo, 1977).

Leatherleaf fern fronds desiccate rapidly after harvest, with frond water potential decreasing from -0.45 MPa at harvest to -1.75 MPa within 30 minutes (Nell, Barrett & Stamps, 1983). Pre-harvest applications of 5% Cloud Cover, 2.5% Vapor Gard and 10% Wiltpruf had no positive effect on water loss or longevity of fronds. Post-harvest dipping of fronds in the antitranspirants was not effective in reducing frond curl or in preventing rapid frond desiccation that occurs after harvesting. It did however, nearly double the vase life compared to fronds that received a water dip (Nell, Conover, Barrett & Poole, 1985).

1.3.2 CONTROL OF FOLIAR DISEASES

Powdery mildew (*Erysiphe* spp.) is an important disease in many plants and its control is not easy (Kamp, 1985). Intensive use of several fungicides, mainly systemic, has resulted in the development of fungicide-resistant strains of the targeted pathogen. Vapor Gard and Wiltpruf effectively controlled powdery mildew on *Hydrangea macrophylla*, *Lagerstroemia indica* (Ziv & Hagiladi, 1984) and on *Zinnia elegans* (Kamp, 1985).

Antitranspirants controlled the powdery mildew on Zinnia plants more effectively than a registered fungicide. It is possible that the additional

epidermal coating interrupted fungal pathogen development on the leaf surface. The additional coating repels the film of free water on the leaf surface, and can cause enough change on the surface to disorient pathogen germ tubes (Kamp, 1985).

1.3.3 PROPAGATION

Plants grown *in vitro* usually do not possess protective mechanisms against desiccation. Reduced epicuticular waxes (Sutter, 1988) and impaired stomatal function (Marin, Gella & Herrero, 1988) have been noted in these plants. The possible use of an antitranspirant for acclimatizing micropropagated walnut plantlets was investigated by Voyiatzis & McGranahan (1994). Plantlets dipped in a latex polymer, Anti Stress 550, generally matched the standard method of acclimatization. The survival rate of treated plants was higher than that of the controls and they accumulated significantly more dry matter, apparently because their new leaves could photosynthesize longer than the controls. *Ex vitro* plantlets are also subjected to desiccation due to rapid water loss when they are transferred to the greenhouse or field.

1.4 PHYTOTOXICITY

Because phytotoxicity and impaired growth caused by antitranspirants may be encountered, the effectiveness of such chemicals in acclimatization depends on the species, and must therefore, be used with caution (Kamp, 1985). The potential for the use of polymer based antitranspirants is high, because most of them are biodegradable, readily available and stable over a range of temperatures and humidity (Kamp, 1985).

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CHAPTER 2

GENERAL PROCEDURES

2.1 PROPERTIES AND INSTRUCTIONS OF THE ANTITRANSPIRANT: VAPOR GARD

Vapor Gard is a β-pinene polymer product containing di-1-p-Menthene as the active ingredient (Table 1.2) and is manufactured by Miller Chemical & Fertilizer Corporation, Penn., USA. The product is a natural film-forming compound, derived directly from pine resin, with 4 % of the formulation as an emulsifier. The amount of active ingredient in the formulation does not determine the activity. Activity is governed by the film which is formed and this is determined by refinement. The product is also non-ionic. In contrast with many other antitranspirants, no additional spreader is needed with Vapor Gard. Only sunlight is needed for the protective film to set (Vapor Gard Label Specifications, 1983[1]).

According to the specimen lable, vegetable seedlings such as tomato, celery, cabbage and lettuce should be sprayed with, or dipped in Vapor Gard at the rate of 2% before transplanting, to reduce transplant shock. Vapor Gard, mixed with water at the rate of 5 and 10% was sprayed on to the plants with a pressurized hand spray, commonly used to apply herbicides or fungicides. The plants were sprayed to runoff and no irrigation was applied for at least an hour. Gale (1961) found that applying

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[1] Miller Chemical & Fertilizer Corp., Hanover, Penn., 17331, U.S.A.

a film-forming antitranspirant (S-789) during hot dry weather was superior to spraying during cool, humid conditions. In the latter case the spray may penetrate the leaf and increase the risk of phytotoxicity. Application of Vapor Gard in the following experiments was thus limited to hot and sunny days.

No information on the permeability of Vapor Gard for carbon dioxide or water vapour is available.

An attempt was made to quantify the effect of the antitranspirant film on the transmittance of photosynthetically active radiation (PAR). A 5% Vapor Gard concentration was sprayed on a glass cuvette and placed in a spectrophotometer[2] together with a control. An insignificant amount (0.01 absorbance units) of light was absorbed at 630nm.

The film-forming antitranspirant will be referred to as Vapor Gard (the trade name) throughout the study.

2.2 PLANT MATERIAL

Seedlings of *Lactuca sativa* [cultivars Buttercrunch (butter lettuce) and Empire 2000 (crisphead lettuce)] and *Phaseolus vulgaris* [Teebus] were used in the experiments. Seeds were sown in seed trays with one part sand and one part peat moss. Lettuce seedlings were transplanted when the second pair of leaves were approximately 30mm long. Bean seedlings were transplanted when the second pair of leaves had opened. A nutrient solution (Nitsch, 1972) was supplied to the plants twice a week in the

[2]In cooperation with Mr. S. de Meillon, Department of Plant Science, University of Pretoria

growth chambers and every day when they were grown in the glasshouse. Insecticides (Metasystox and Red Spider Cide) were used to control red spider mite and aphids.

2.3 GAS EXCHANGE SYSTEM

A CIRAS-1 (PP Systems, Herts, U.K.) differential CO_2/H_2O non-dispersive infrared gas analyzer (IRGA) was used for all photosynthesis, transpiration and stomatal conductance measurements. CIRAS uses four independent infrared gas analyzers, two each for CO_2 and H_2O. One pair of CO_2 and H_2O analyzers have a common inlet/outlet and are defined as the reference, and similarly for the other pair of analyzers, defined as the analysis. Measurements are expressed as absolute concentrations for the reference, and as the difference between the reference and the analysis concentration. Ciras-1 is an open (or differential) system where there is a net flow of air through the system. The inlet air is dried before entering the IRGA by passing the air through columns of anhydrous calcium sulphate (drierite) and a molecular sieve to eliminate the effects of water vapour on the measurements of the CO_2 flux. A constant inlet CO_2 concentration is achieved by inserting a small disposable CO_2 cartridge with a running time in excess of 8 hours. Soda lime is used to absorb CO_2 to maintain the set CO_2 concentration (CIRAS-1 Combined infrared gas analysis system Operator's Manual, 1994).

The flow rate of air through the cuvette was set at 200 ml min^{-1} and leaf temperature was determined by an energy balance calculation. The reference CO_2 concentration was set at 345 μmol mol^{-1}.

A broad leaf cuvette with a total leaf area of 2.5 cm^2 was used for all the measurements. A fan in the cuvette provides air movement over the leaf to minimize the thickness of the boundary layer. Cuvette temperature is measured with precision thermistors. The photosynthetically active radiation (PAR) sensor is a filtered Silicon cell (CIRAS-1 Parkinson leaf cuvette Operator's Manual, 1994).

2.4 DATA ANALYSES

Analysis of variance (ANOVA) was performed using the General Linear Model Procedure of SAS (1989). The ANOVA Tables are given in Appendix A and tables are referred to in the text with the prefix A. Effects were considered to be significant at $P<0.10$ unless otherwise stated. When significant, means were separated using Fisher's Least Significant Difference (LSD).

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CHAPTER 3

MICROSCOPIC APPEARANCE OF VAPOR GARD ON THE LEAF SURFACE

3.1 INTRODUCTION

Leaf stomata provide the main passage for water vapour for transpiration and for carbon dioxide for assimilation. Because film-forming anti-transpirants increase leaf resistance to carbon dioxide and water vapour diffusion (by increasing stomatal and cuticular resistances), photosynthesis and transpiration can be reduced. However, changes in stomatal aperture (Penman & Schofield, 1951) and modifications of epidermal conductance (Solárová, Pospíšilová & Slavík, 1981) do not necessarily affect transpiration and assimilation to the same extent. Theoretically, antitranspirant application should reduce transpiration more than photosynthesis because, in addition to the stomatal and leaf boundary layer resistances to diffusion, carbon dioxide encounters resistance between the leaf's sub-stomatal cavities and the chloroplasts. If this mesophyll resistance is of similar magnitude to stomatal and boundary layer resistances, increased stomatal resistance would cause a relatively greater reduction in transpiration than in photosynthesis (Gale & Hagan, 1966; Cowan & Troughton, 1971; Anderson & Kreith, 1978).

When stomata are open, their resistance is considerably lower than that of the cuticle. Therefore most water vapour escapes through the stomata, and transpiration rates are largely controlled by stomatal aperture. (Gates,

1968, Kramer & Boyer, 1995).

Increases in the photosynthesis/transpiration ratio have been shown to occur with application of metabolic inhibitors (Slatyer & Bierhuizen, 1964), but film-forming antitranspirants have generally resulted in transpiration and photosynthesis reductions of similar magnitude (Slatyer & Bierhuizen, 1964; Parkinson, 1970). This has been attributed to partial coverage of the leaf by the antitranspirant film. Where the film is present, diffusion of both carbon dioxide and water vapour is apparently negligible and antitranspirant effectivity is a function of percentage coverage of the leaf (Anderson & Kreith, 1978, Waggoner, 1981)

At the outset of this investigation the physical appearance of the antitranspirant film on the leaf surface, leaf morphology and the effect on the stomata was studied with the aid of a scanning electron microscope.

3.2 MATERIALS AND METHODS

Two week old butter lettuce plants were grown in a glasshouse at 24 ± 2°C day/12 ± 2°C night temperatures. The plants were watered to approximately field capacity, whereafter leaves of the same physiological age were sprayed with Vapor Gard (5%). Three hours were allowed for the film to dry before leaf segments were sampled for the microscopic study.

Leaf segments (7mm by 4mm) of control and treated plants were excised from the centre of the leaf and immediately plunge frozen in liquid propane at -175 °C. The frozen leaf tissue was transferred to recesses in a copper block (60 x 60 x 60mm) precooled with liquid nitrogen. The block was fitted with a heater and thermocouple. The block containing the leaf

segments was placed in a Fisons high vacuum unit and when a vacuum of approximately 5 $x10^{-4}$ Torr was reached, the temperature of the block was still below -120°C. Pumping and freeze drying were done overnight and the next morning the block was heated to 25°C before removing it from the vacuum unit. The material was mounted on stubs and sputter coated with gold (Polaron E 5200 sputter coater) whereafter it was examined in a Jeol JSM 840 scanning electron microscope (Van der Merwe - Personal communication)[3].

The whole procedure of sampling and preparation was repeated two weeks after application to get an indication of the persistence of the polymer film on the leaf surface.

3.3 RESULTS AND DISCUSSION

Comparative micrographs of the control and treated plants are illustrated in Figure 3.1. Micrographs taken after the first application illustrates the appearance of the freshly applied film on the leaf surfaces. The natural appearance of the epidermis was illustrated by the control sample (Figure 3.1a). Epidermis cells were clearly visible and most of the stomatal pores were open (Figure 3.1b).

By comparing Figures 3.1a and 3.1c, the film coating obtained with a 5% Vapor Gard application is clearly evident. Most of the stomata were completely covered by the Vapor Gard film as illustrated in Figure 3.1c and 3.1d.

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[3]C. van der Merwe - Electron Microscopy Unit, University of Pretoria, Pretoria, 0002.

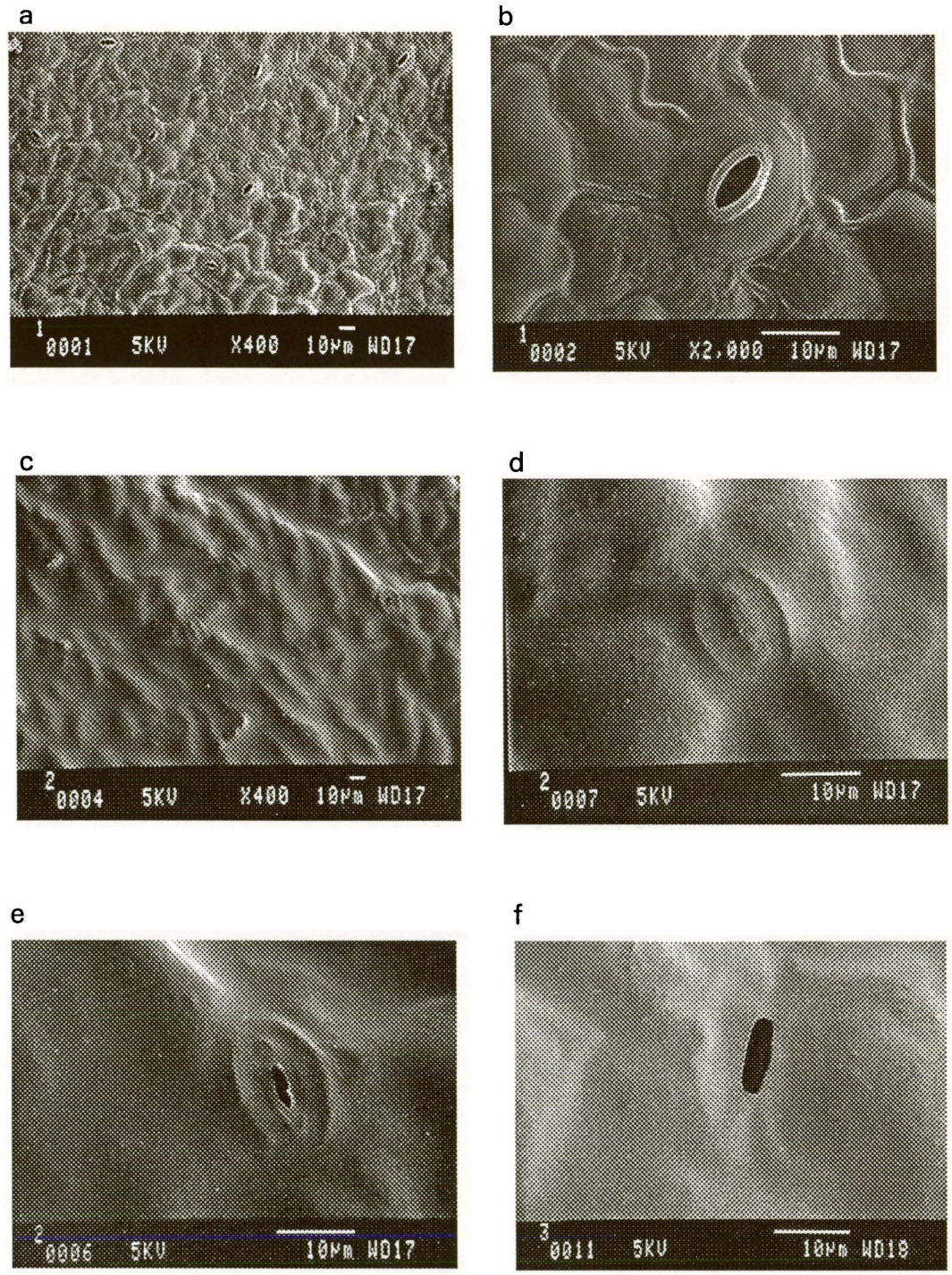

Figure 3.1 Physical appearance of Vapor Gard on leaf surfaces a few hours after application.
(a) Control leaf sample, (b) Open stomata of control, (c) Treated leaf sample, (d) Covered stomata of treated leaf sample, (e) Cracking over stomatal pore of treated leaf sample and (f) increased incidence of cracking.

It was impossible to see from the micrographs whether the stomatal pores were open or not. The micrographs suggested that the film cracked directly over most of the stomatal pores, possibly as a result of functioning of the guard cells (Figure 3.1e). Some of the cracks developed into larger cavities (Figure 3.1f).

These observations were similar to that of Davies & Kozlowski (1974) who treated *Fraxinus americana* (ash tree) with 20% Wilt Pruf. In these studies micrographs taken shortly after treatment also showed cracking of the film over stomatal pores.

Micrographs from samples taken two weeks after application, showed that the film coating was still evident over the leaf surface although it appeared wrinkled (Figure 3.2a). Cracking of the film did not only occur directly over the stomata, but also around them (Figure 3.2b).

The larger extent of the cracking after two weeks suggests disintegration of the film. This is in accordance with the findings of Anderson & Kreith (1978) who found that Mobileaf and Wiltpruf decayed slowly over 10 to 14 days.

A film that covers the entire leaf surface may minimise transpiration rates, but may also have negative effects on photosynthesis. Waggoner (1981) suggested that degree of coverage may be an important factor in the application of an antitranspirant film and that incomplete coverage may be necessary to avoid extreme decreases in photosynthetic rates.

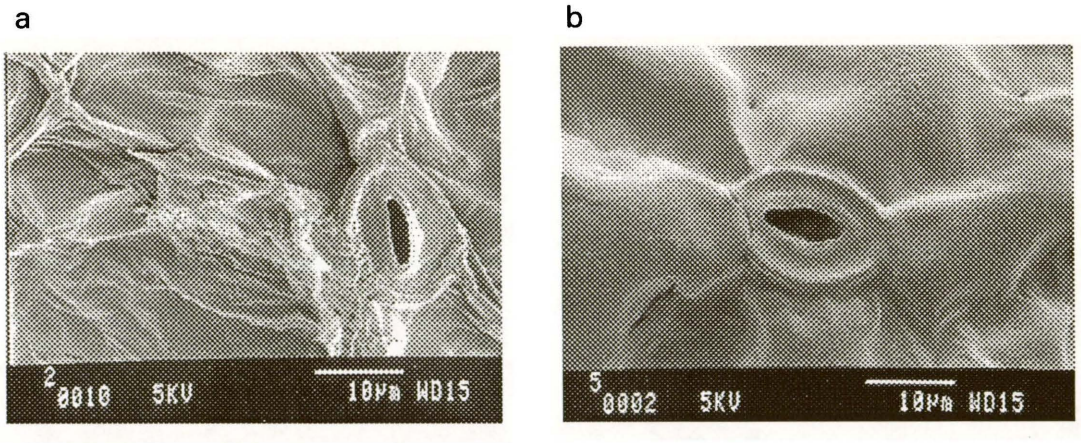

Figure 3.2 Treated leaf surface after two weeks indicating wrinkling of film and cracking over stomata (a) as well as cracking elsewhere (b).

The assembled data suggest that, even with reasonable careful application, the Vapor Gard film did not remain intact and that cracking of the film over the stomatal pores occurred soon after treatment. It therefore seems unnecessary to make a deliberate effort to ensure an imperfect coverage.

3.4 CONCLUSIONS

The efficient and even coating of the leaf surfaces by the Vapor Gard film suggests that it creates an additional resistance to transpiration and photosynthesis pathways. This implies that the application of a film-forming antitranspirant will affect the rates of these processes, at least for a few days after application.

In succeeding experiments attempts were made to quantify the effects of such a film on transpiration and photosynthetic rates.

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CHAPTER 4

EFFECTS OF VAPOR GARD ON TRANSPIRATION AND PHOTOSYNTHESIS

4.1 INTRODUCTION

Decreased evaporation from large bodies of water has been achieved by covering them with suitable materials. Similarly, application of film-forming antitranspirants which decrease transpiration, could save water and reduce the inhibitory effects of dehydration on leaf metabolism (Kramer & Boyer, 1995). However, the effects of the antitranspirant on other physiological and physical activities that affect plant performance must also be determined. The suppression of transpiration, if achieved without serious injury to treated plants, could be of considerable practical value. The benefit of this suppression would be greatly enhanced if it could be obtained without a proportional reduction in photosynthesis (Slatyer & Bierhuizen, 1964).

Stomata not only control the loss of water vapour but also the diffusion of CO_2 into the leaf. When water loss is decreased by the application of an antitranspirant, diffusion of CO_2 into leaves may also be reduced (Salisbury & Ross, 1992, Kramer & Boyer, 1995). Transpiration rates are mainly determined by the resistances to water vapour diffusion from the evaporating surface to the bulk air outside the leaf [r_a + r_{ep} in Eq.(1)], but CO_2 encounters an additional resistance [r'_i in Eq. (3)] to diffusion in the liquid of the mesophyll cells. The total resistance to diffusion is therefore

smaller for transpiration than for photosynthesis, and increasing the resistance of the leaf with an antitranspirant should, theoretically, have less effect on photosynthesis than on transpiration (Slatyer & Bierhuizen, 1964, Gale & Hagan, 1966, Anderson & Kreith, 1978, Kramer & Boyer, 1995).

Davies & Kozlowski (1974) treated *Fraxinus americana* (white ash) with Vapor Gard and measured the rates of transpiration and photosynthesis. Seedlings treated with Vapor Gard exhibited reduced transpiration for eight days after application. The photosynthetic rate was decreased during the entire experimental period of 32 days by a mean of 5mg $CO_2.dm^{-2}.hr^{-1}$. Similar results were obtained by Davenport, Uriu & Hagan (1974) who found that the film-forming antitranspirants, CS-6432 and Mobileaf, had not only decreased transpiration (by 66 and 43 percentage units, respectively) of treated sugar beet leaves after 24 hours, but also significantly reduced photosynthesis (by 73 and 53 percentage units, respectively) over a fourteen day period.

Three experiments were carried out under controlled environmental conditions, to determine the influence of Vapor Gard on transpiration and photosynthesis. The first experiment was conducted in growth chambers with lettuce and bean plants. In the second experiment, transpiration and photosynthetic rates of bean plants grown in a glasshouse were measured in full sunlight. In the third experiment, measurements of butter lettuce were taken under a high pressure sodium light source.

Transpiration and photosynthetic rates were measured to test the hypothesis that Vapor Gard limits transpiration without negatively affecting photosynthesis.

4.2 EXPERIMENT 1 - GROWTH CHAMBER TRIAL

4.2.1 MATERIALS AND METHODS

Butter and crisphead lettuce and bean seedlings that had been raised in seed trays in a glasshouse were transplanted into one part sand and one part peat moss (v/v) in 150mm diameter pots. Before the antitranspirant was applied, one day was allowed for the plants to acclimatize to the conditions in the Conviron PGW 36 growth chambers. The growth chambers were programmed for a 12-hour photoperiod, and a 22°C day/16°C night thermal regime. All the plants were watered similarly and adequately, regardless of any differential moisture extraction which might have resulted from the antitranspirant treatment.

According to Tibbits & Langhans (1993), cool season crops such as lettuce will develop effectively under cool white fluorescent (CWF) lamps, if the lamps are closely packed across the ceiling of the growth chamber. The quantity of lighting required by plants is a function of the level of irradiance from the lamps and the length of time during which irradiance is provided daily. Generally a level of 552 μmol.m^{-2}.s^{-1} provided for twelve hours daily is sufficient for cool-season crops (Tibbits & Langhans, 1993). In this growth chamber experiment light was supplied by CWF and 40 W incandescent lamps. Photosynthetic photon flux (PPF) at plant height was between 220 and 270 μmol.m^{-2}.s^{-1}.

Three treatments (control, 5% and 10% Vapor Gard) were randomly allocated to a total of 72 seedlings (three treatments x three test crops x eight replications). The seedlings were sprayed to runoff on both the adaxial and abaxial sides of the leaves with 5% and 10% Vapor Gard. The

control plants were sprayed with water. Figure 4.1 illustrates the general appearance of the plants in the growth chamber at the stage when transpiration and photosynthetic measurements commenced.

Figure 4.1 General appearance of the plants in the growth chamber at the stage when transpiration and photosynthetic measurements commenced.

The leaves were allowed to dry before the first measurements were made. Three sprayed leaves of similar age of each plant were randomly selected. Rates of net photosynthesis and transpiration were measured simultaneously with a Ciras-1 Portable Photosynthesis System three hours after the plants were sprayed and then on days 2, 6, 7, 9, 12, 15, 18 and 21. Measurements were made on the same leaves for the entire experimental period (Figure 4.2) .

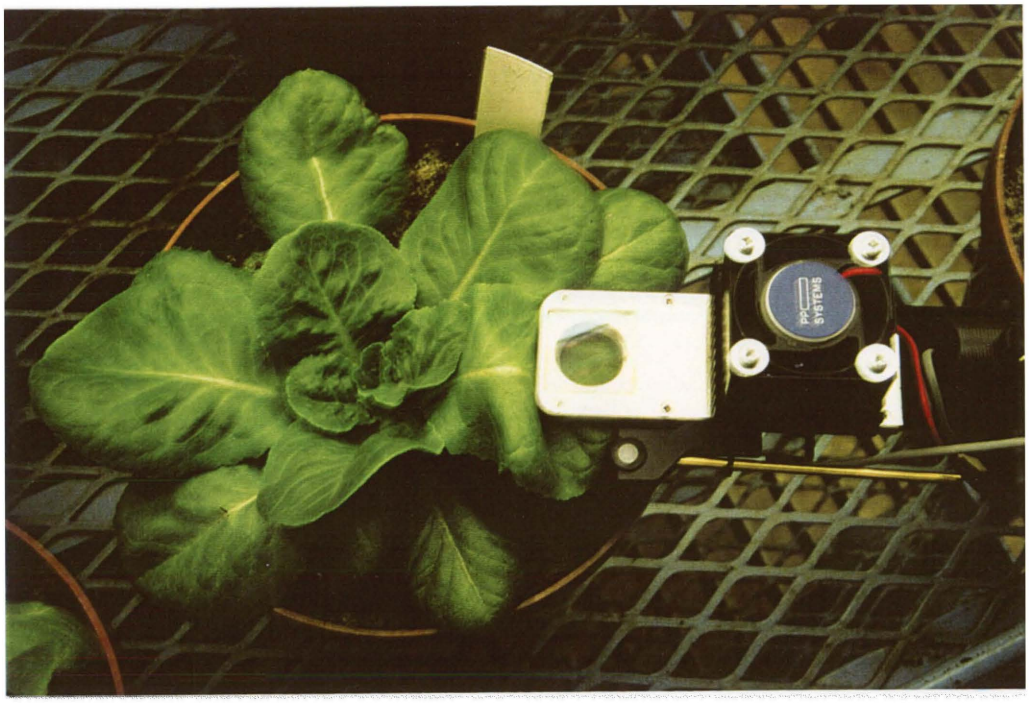

Figure 4.2 Ciras-1 cuvette clamped on butter lettuce leaf to measure transpiration and photosynthetic rates.

4.2.2 RESULTS AND DISCUSSION

4.2.2.1 Transpiration

The effect of antitranspirant treatments on the rates of transpiration of the three test crops is illustrated in Figure 4.3. In general the antitranspirant had no noticeable effect on the rates of transpiration of any of the three crops. Not even a consistent trend of slightly reduced transpiration rates occurred.

Relatively low rates of transpiration occurred under the growth chamber conditions. In both lettuce cultivars the transpiration rate declined over the experimental period. As measurements were performed on the same leaves for the duration of the experiment, the decline might have been due to ageing of the leaves. This phenomenon was less prominent in the bean plants.

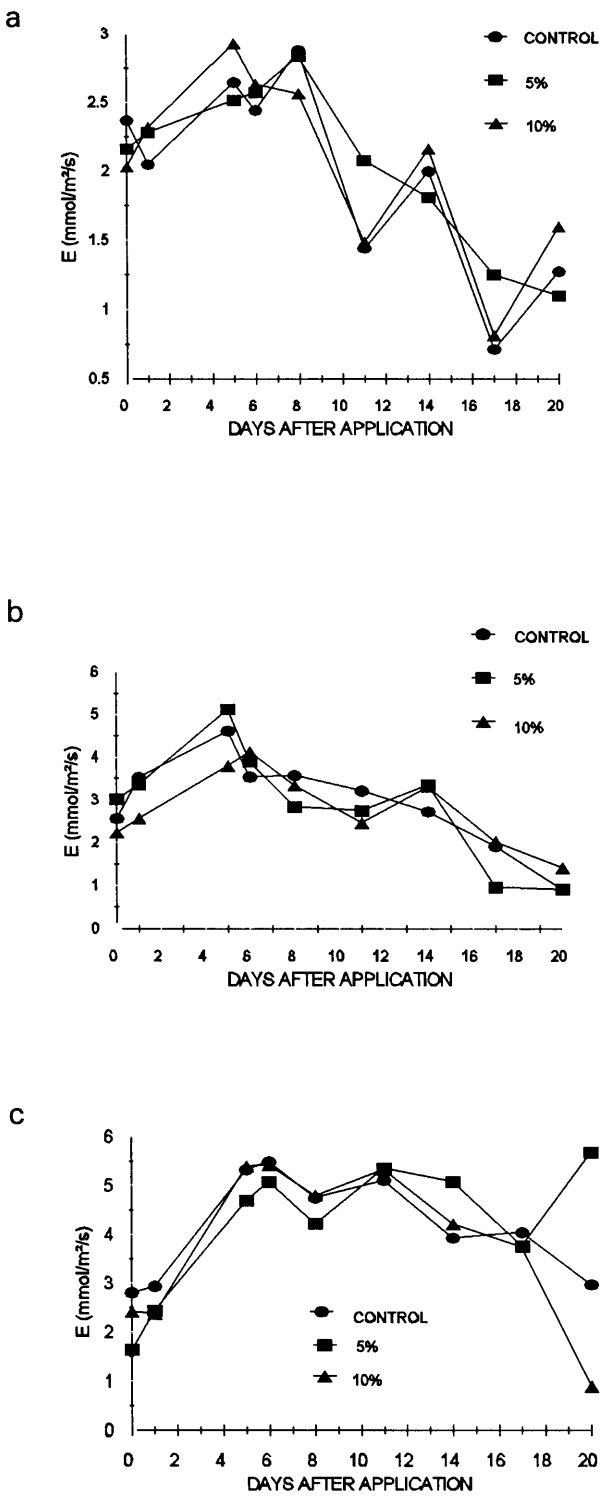

Figure 4.3 Effects of Vapor Gard on mean transpiration rates of a) Butter lettuce b) Crisphead lettuce and c) Beans. Day 0 represents the measurement taken three hours after the plants were treated (Appendix Tables A1, B1).

Considering the effective coating of the leaf surface observed in the micrographs (Figure 3.1), and considering the results of Davies & Kozlowski (1974) who found a decrease in transpiration rate after a Vapor Gard application, the results obtained in this experiment were totally unexpected.

4.2.2.2 Photosynthesis

The effect of the antitranspirant treatments on the photosynthetic rate of the three test crops is illustrated in Figure 4.4. Davies & Kozlowski (1974) and Anderson & Kreith (1978) reported that film-forming antitranspirants reduced photosynthesis of *F. americana* and *Elymus canadensis* by 50%, when grown in growth chambers. In contrast, no reduction in photosynthetic rate was observed in this experiment, although small but inconsistent effects were noticed.

Three hours after application it was observed that the antitranspirant treated leaves (both concentrations) tended to show slower photosynthetic rates, though this was not statistically significant ($P=0.1109$). These observations did not persist during subsequent measurements however. (Figure 4.4a). The photosynthetic rate of crisphead lettuce leaves was not affected by the presence of a Vapor Gard film (Figure 4.4b). Bean leaves treated with 10% Vapor Gard tended to show an increase in the photosynthetic rate eight and 11 days after application ($P=0.14$ and 0.18, respectively). The 5% concentration resulted in an increase ($P=0.05$) in photosynthetic rate on the 20th day after Vapor Gard application (Figure 4.4c).

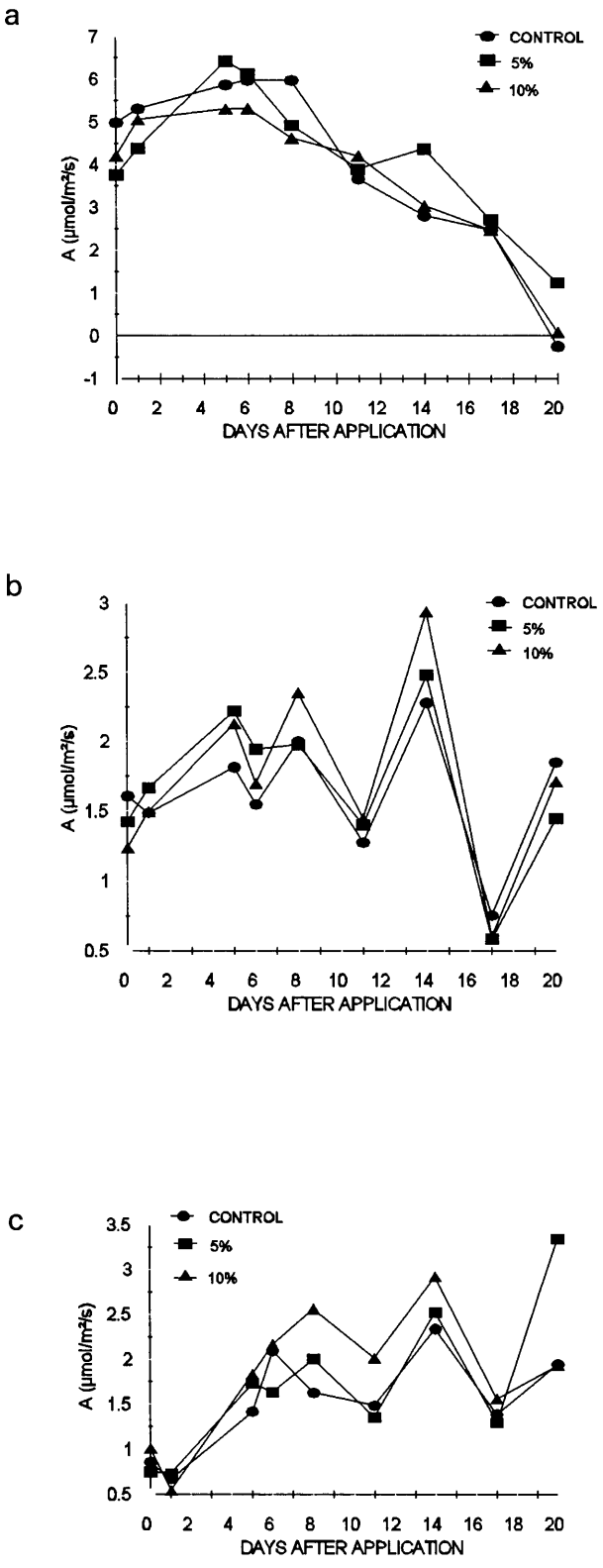

Figure 4.4 Effects of Vapor Gard on mean photosynthetic rates for a) Butter lettuce and b) Crisphead lettuce and c) Beans. Day 0 represents the measurement three hours after application of Vapor Gard (Appendix Tables A2 and B2).

Ageing of the treated leaves, and to a lesser extent shading, might explain the observed decrease in the photosynthetic rate of the lettuce plants over the experimental period.

The low light intensities in the growth chambers (270 μmol.mol^{-1}), might have been insufficient for optimal photosynthesis according to Tibbits & Langhans (1993). A similar trial was performed in a glasshouse at the same time. Measurements were taken inside the glasshouse where the PPF was 560μmol.m^{-2}.s^{-1}. No differences between treatments were found in either the transpiration or photosynthetic rates. Consequently, a second trial was conducted in a glasshouse and measurements were made in full sunlight where the PPF was higher.

4.3 EXPERIMENT 2 - GLASSHOUSE TRIAL

To minimize the effect of possible variation among bean leaves, a different concentration of Vapor Gard was applied to each pinna of one compound leaf. The experiment was conducted in a glasshouse and measurements were made out of doors in full sunlight.

4.3.1 MATERIALS AND METHODS

Bean seedlings were grown in a glasshouse at 26°C day and 15°C night temperatures. Treatments were allocated randomly to the first compound leaf of ten plants when the second compound leaf had emerged. One of the pinna was used as a control and was sprayed with water and the other two pinnae were sprayed either with 5% or 10% Vapor Gard. Data were analysed as a complete randomized block design where plants were used as blocks.

Measurements of the transpiration and photosynthetic rates commenced three hours after the plants were treated. Subsequent measurements were made on days two, three and four. Although the plants were grown in a glasshouse, the measurements were made outside in full sunlight, where the PPF ranged from 1800 to 2000 μmol.m^{-2}.s^{-1}.

4.3.2 RESULTS AND DISCUSSION

4.3.2.1 Transpiration

The effect of the Vapor Gard treatments on the rate of transpiration of bean leaves is depicted in Figure 4.5.

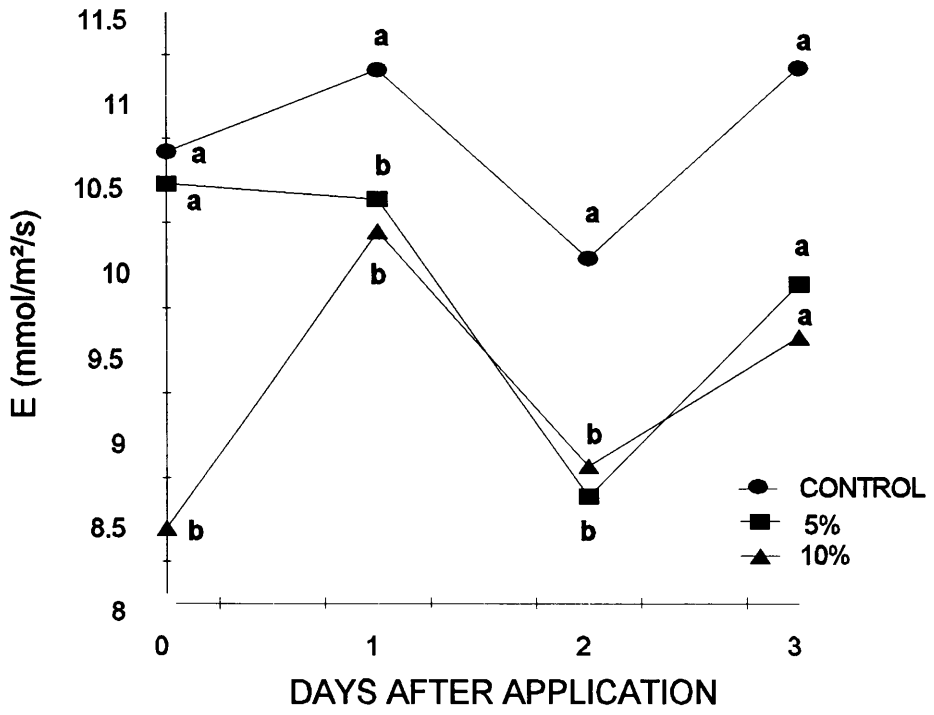

Figure 4.5 Effects of Vapor Gard on mean transpiration rates over a four-day period for bean leaves treated with 5% Vapor Gard. Each data point represents the mean of eight pinnae (Appendix Tables A3 and B3).
[a,b] Values on the same day with similar superscripts do not differ significantly (P<0.1).

The different reaction of the treatments between day 0 and day 1, can only be explained by slower cracking of the 10% concentration film over the stomata. Both the 5% and 10% treatments showed decreased transpiration rates compared to the control on day one (by 6.9 and 8.6%, respectively) and on day two (by 13.9 and 12.2%, respectively). The differences between the treated and control plants that were observed in this study are, however, much lower than the 57% decrease in transpiration that Davenport *et al*. (1974) recorded for sugar beet .

4.3.2.2 Photosynthesis

Figure 4.6 illustrates the effect of Vapor Gard treatments on the photosynthetic rate of bean leaves. Photosynthetic rates were not negatively affected by the Vapor Gard treatments on any day. On the contrary, a tendency of an increase in photosynthetic rate was observed for the 5% treatment on the first day, though this was not statistically significant ($P = 0.18$). Photosynthetic rates of the 10% treated plants tended to be lower than those of control plants immediately and one day after treatment, though these differences were not statistically significant ($P > 0.2$). It can be speculated that the 10% film did not crack over the stomatal pores as soon as the 5% film did. It seems likely that substantial cracking only occurred after one day allowing CO_2 to diffuse in for assimilation.

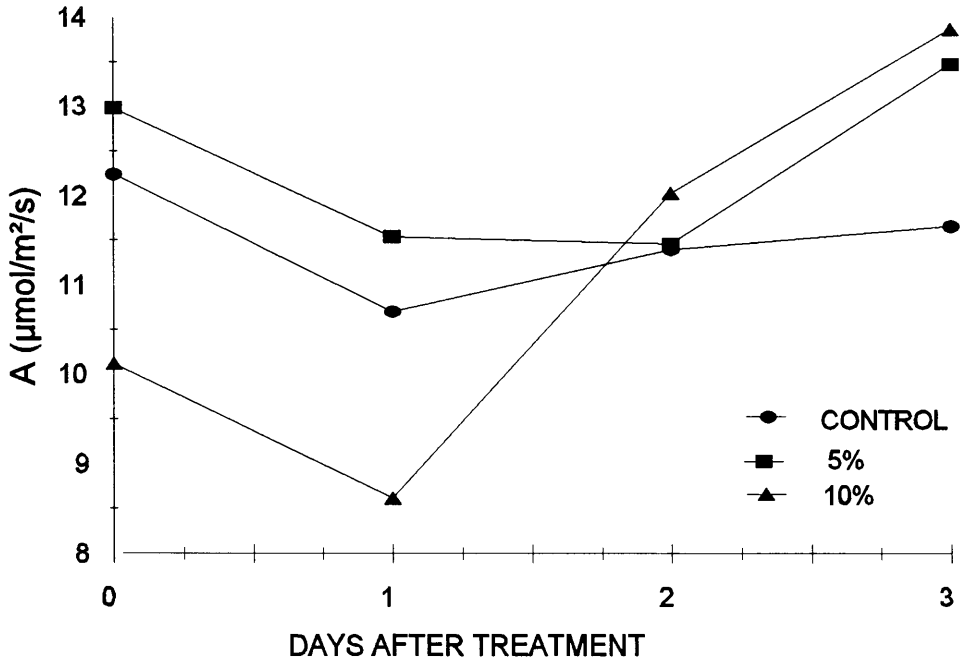

Figure 4.6 Effects of Vapor Gard on mean photosynthetic rates over a four-day period for bean leaves treated with 5% Vapor Gard. Each data point represents the mean of eight pinnae (Appendix Tables A4 and B4).

The transpiration/photosynthesis (E/A) ratio of the 5% treated plants showed a decrease compared to the ratio of the control plants (Figure 4.7). This decrease in E/A ratio is the result of the decrease in the transpiration rate without a change in the photosynthetic rate. This lower ratio indicates a higher water use efficiency by the 5% treated plants.

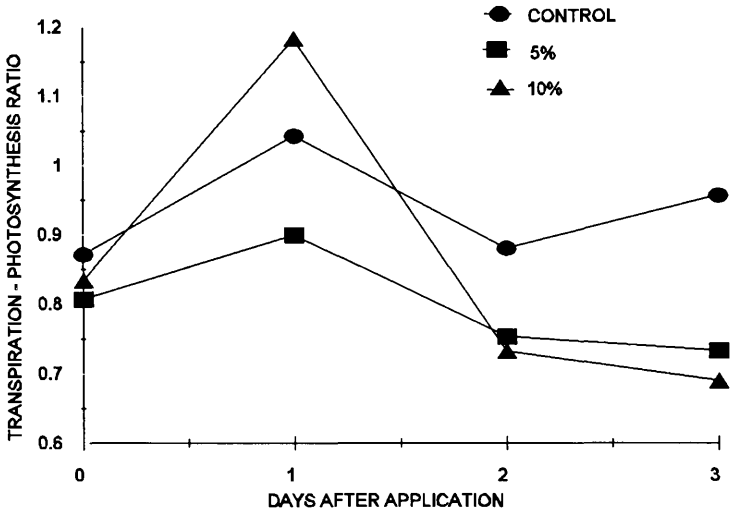

Figure 4.7 Effect of 5% Vapor Gard on the transpiration/photosynthesis ratio of bean seedlings (Appendix Table B5).

Since no significant differences in either the transpiration or photosynthetic rates between the treatments were detectable after two days, the experiment was terminated after the third day.

4.4 EXPERIMENT 3 - DIFFERENTIAL APPLICATION ON UPPER AND LOWER LEAF SURFACES

An experiment was conducted to determine whether Vapor Gard applied to one or both sides of the butter lettuce leaves would affect the transpiration/ photosynthesis ratio differently. In this experiment lettuce was grown in a glasshouse and the transpiration and photosynthetic rates were measured under the constant photon flux of a high pressure sodium lamp.

The interface between the leaf surface and the air mass has a complicated structure and leaf resistances to water loss are rarely the same on the top and bottom surfaces of the leaves. According to Gale & Poljakoff-Mayber (1968) the two surfaces are unequal conductors of water vapour and CO_2. Partial closure or coverage of the stomata on one side of a leaf may have a different effect on the transpiration/photosynthesis ratio than the same degree of coverage applied to the other side or both sides (Waggoner, 1965). Waggoner treated tobacco leaves with PMA on either the upper or lower epidermis. He found that a change in resistance of lower stomata substantially changed transpiration and photosynthesis, but that a change in resistance of upper stomata had little effect.

According to Bolhàr-Nordenkampf & Draxler (1993) sunflower has 27 to 326 stomata per mm^2 on the upper and 132 to 184 per mm^2 on the lower surface. No published data were available regarding stomatal frequency per square millimetre of lettuce leaf surfaces. When the number of stomata on the two sides of a butter lettuce leaf was counted, it was found that there are approximately 150 stomata per mm^2 on the adaxial side and 69 per mm^2 on the abaxial side.

4.4.1 MATERIALS AND METHODS

Butter lettuce seedlings were transplanted in 150 mm pots and grown in a glasshouse. Four treatments (control and plants sprayed with a 5% concentration of Vapor Gard on one or both sides) were randomly assigned to 24 seedlings. Net photosynthetic and transpiration rates were measured simultaneously under a high pressure sodium light lamp with a PPF output of 1200 $\mu mol.m^{-2}.s^{-1}$, at a distance of 200 mm from the light source (Figure 4.8). Sodium high pressure lamps have greater photosynthetic

radiation output and better longevity than CWF lamps. Their low level of 400-500nm (blue) wavelengths makes them of questionable use in enclosed environments (Tibbitts & Langhans, 1993). For this reason sunlight was allowed through the window of the laboratory and a fluorescent light was added.

Figure 4.8 Butter lettuce plant under high pressure sodium lamp.

Measurements were made three and eight days after the plants were treated. After two weeks Vapor Gard was reapplied to the same plants as the original treatments. Photosynthetic and transpiration rates were measured two days after the second application.

Only the 5% concentration was used since it had given the most positive results concerning a decrease in transpiration without a concomitant decrease in photosynthesis in the previous experiments.

4.4.2 RESULTS AND DISCUSSION

4.4.2.1 Transpiration

The effect of the different treatments on the transpiration rate of butter lettuce leaves is illustrated in Figure 4.9. None of the treatments caused a significant reduction in the transpiration rates, either after the first or second application.

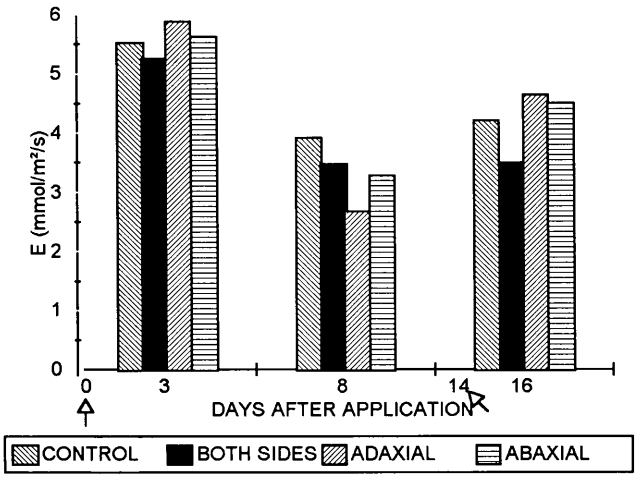

Figure 4.9 Mean transpiration rates of treated butter lettuce leaves on days three, eight and 16. Arrows indicate time of Vapor Gard application (Appendix Tables A6 and B6).

4.4.2.2 Photosynthesis

Figure 4.10 summarizes the effect of the different treatments on the photosynthetic rates of the butter lettuce leaves. No significant differences in the photosynthetic rates were observed, although the treatment on the adaxial side of the leaf surface tended to increase ($P=0.18$) the rate of photosynthesis on the third day after the first Vapor Gard application.

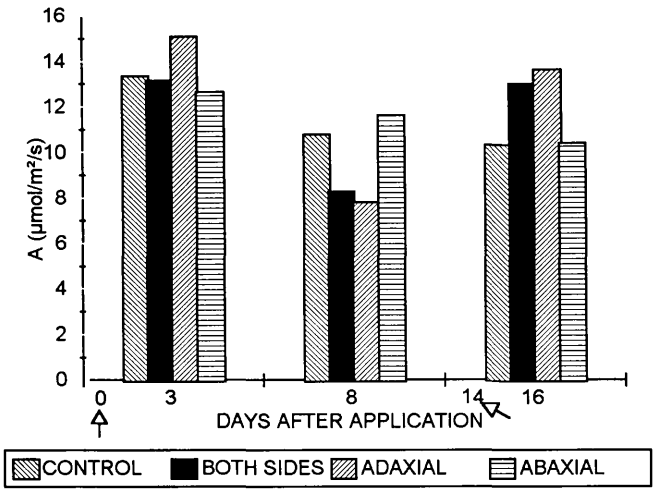

Figure 4.10 Mean photosynthetic rates of treated butter lettuce leaves on days three, eight and 16. Arrows indicate time of Vapor Gard application (Appendix Tables A7 and B7).

A field trial was done, but cool wet conditions dominated during the growing period and no significant differences in the transpiration or photosynthetic rates between the treated and control plants occurred.

4.5 CONCLUSION

In neither the growth chamber trial nor the differential application trial did Vapor Gard reduce transpiration or affect photosynthesis, though a decrease in transpiration rate of bean plants in full sun did occur. The photosynthetic rate was not reduced in any of the experiments and actually appeared to be increased by the 5% treatment on beans, when measurements were made in full sunlight. Based on these findings, the hypothesis that Vapor Gard decreases transpiration without negatively affecting net photosynthesis can not be accepted.

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CHAPTER 5

INFLUENCE OF VAPOR GARD ON WATER RELATIONS OF LETTUCE PLANTS

5.1 INTRODUCTION

Plant water status strongly influences plant growth and biomass production particularly through its effect on leaf and root extension, even though the amount of water used directly in the biochemical reactions of photosynthesis is small compared to that transpired or stored by plants at any time (Beadle, Ludlow & Honeysett, 1993). The photosynthetic rate of a crop under water stress will decline because of stomatal closure and the effects of water deficits on chloroplast processes (Salisbury & Ross, 1992). Drought is an important factor limiting the production of field crops. If an antitranspirant like Vapor Gard could decrease transpiration without affecting photosynthesis detrimentally, the severity of drought could be reduced by an improvement in the water-use efficiency.

Research on the effects of antitranspirants on the water relations in plants are summarised in Table 1.3 (p14,15). Although most of the results showed improved water balances in the treated plants, the antitranspirants had no effect in a few instances. Vapor Gard was one of the antitranspirants that showed inconsistent results. Davies & Kozlowski (1974) treated three-year-old seedlings of *Fraxinus americana* with 5% Vapor Gard. The plants were maintained in a growth chamber for the duration of the experiment. The transpiration/photosynthesis (E/A) ratio

was calculated for control and treated plants to show the effect of the antitranspirant on the amount of water lost per unit of CO_2 fixed. They found that Vapor Gard treatment resulted in higher E/A ratios after the eighth day compared to the control plants. This higher E/A ratio indicated lower water use efficiency of treated plants as photosynthesis was reduced more than transpiration. Hummel (1990) reported that a Vapor Gard treatment reduced water loss of tomato plants, petunias and impatiens.

Since no substantial reduction in transpiration rates occurred in the trials presented in Chapter 4, one pot experiment was conducted to determine the effects of Vapor Gard on water use of plants under water stress conditions. Water potential of the growing media was lowered and plant weight and water uptake over time recorded.

5.2 POLYETHYLENE GLYCOL TRIAL

5.2.1 MATERIALS AND METHODS

Individual crisphead lettuce seedlings were transplanted to 2L pots, each containing 1900g nutrient solution diluted to 50% of the standard concentration. Iron chelate (Fe-EDTA) at 1mL/L water was added during the second week. Each pot was covered with a polystyrene disk to prevent evaporation from the water surface. The plants were kept in a glasshouse at 24 ± 2°C day/ 12 ± 2°C night temperatures.

Twenty nine days after transplanting, twenty four healthy and well developed plants were selected for the experimental treatments. Twelve plants were randomly sprayed with Vapor Gard (5% concentration) to

obtain good coverage of all exposed leaf surfaces, while 12 control plants were untreated. The water potential of the nutrient solution was decreased to -1100kPa, by adding 250mg polyethylene glycol (PEG 6000) per kg diluted nutrient solution, for six of the Vapor Gard treated and six of the control plants. The water potential of the nutrient solution was confirmed with an AquaLab water activity meter (Model CX2, Decagon Devices, Inc., Pullman, Washington, U.S.A) and using the equation:

$$\Psi = \frac{R\,T}{m} \ln(h_r)$$

where Ψ = water potential ($J.kg^{-1}$)

R = gas constant (8.314 $J.mol^{-1}.K^{-1}$),

T = Kelvin temperature (K),

m = molecular mass of H_2O (0.018$kg.mol^{-1}$) and

h_r = relative humidity.

The loss of water and change in weight of plants were recorded every second day. This was achieved by weighing the intact plants plus containers, whereafter the plants were removed and the water allowed to drip from the roots for one minute before the weight of the nutrient solution remaining in the containers was recorded. Janes (1974) applied a similar procedure. The volume of the solution was restored to 1900g. The plants were weighed every second day for two weeks.

5.2.2 RESULTS AND DISCUSSION

Data on the amounts of water lost by transpiration are summarised in Table 5.1. Where the plants were grown in the nutrient solution without the osmoticum, the untreated control plants lost 1056 mL over the 14 day

observation period. Although the Vapor Gard treated plants used 15% more water than the control plants, this difference was not significant. These results support that of the growth chamber trial (Chapter 4) where it was shown that Vapor Gard treatment had no effect on transpiration rate.

Table 5.1 Mean[1] amount of water (g) added to the plants over a 14-day period

		DAYS							
		2	4	6	8	10	12	14	TOTAL
UNSTRESSED	CONTROL	187	133	151	192	148	123	122	1056
TREATMENT	VAPOR GARD	209	152	173	214	165	148	156	1215
	SEM[3]	29.4	26.1	31.9	43.1	31.3	29	38	223.0
PEG [2] -	CONTROL	35	16	18	20	10	9	8	123
STRESSED									
TREATMENT	VAPOR GARD	44	30	28	29	18	18	14	175
	SEM[3]	14.2	7.2	7.4	6.4	4.1	4.1	4.3	47.7

[1] Mean of six plants per treatment
[2] PEG = Polyethylene Glycol 6000
[3] SEM = Standard error of the mean (Appendix Table A8)

According to Davenport, Fisher & Hagan (1972) antitranspirant films curtail transpiration by offering a physical resistance to the water vapour passage. The resulting increased leaf water potential increases the turgidity of the guard cells and results in wider open stomatal apertures. Wider stomatal apertures decrease the stomatal resistance to the passage of water vapour out of the leaf. They found that the stomatal apertures of *Vicia faba* under a Mobileaf film were greater than those of control leaves. The greater water uptake by the Vapor Gard treated plants in this experiment may also be the result of wider open stomata.

Where the water potential of the nutrient solution was lowered with PEG, the plants used considerably less water than the unstressed plants and showed severe wilting symptoms after 7 days. There was, however, no differences in water uptake between the treated and untreated plants. Visually the Vapor Gard treated plants appeared healthier than the control plants, and the adverse effect of the osmoticum was obvious (Figure 5.1).

Figure 5.1 Plant appearance 10 days after commencement of treatments. The two plants on the left were in the nutrient solution and the two on the right in the PEG-stressed solution. The two centre plants were treated with Vapor Gard.

From Figure 5.2, depicting the change in weight of the plants, it can be seen that plant growth declined in all the treatments.

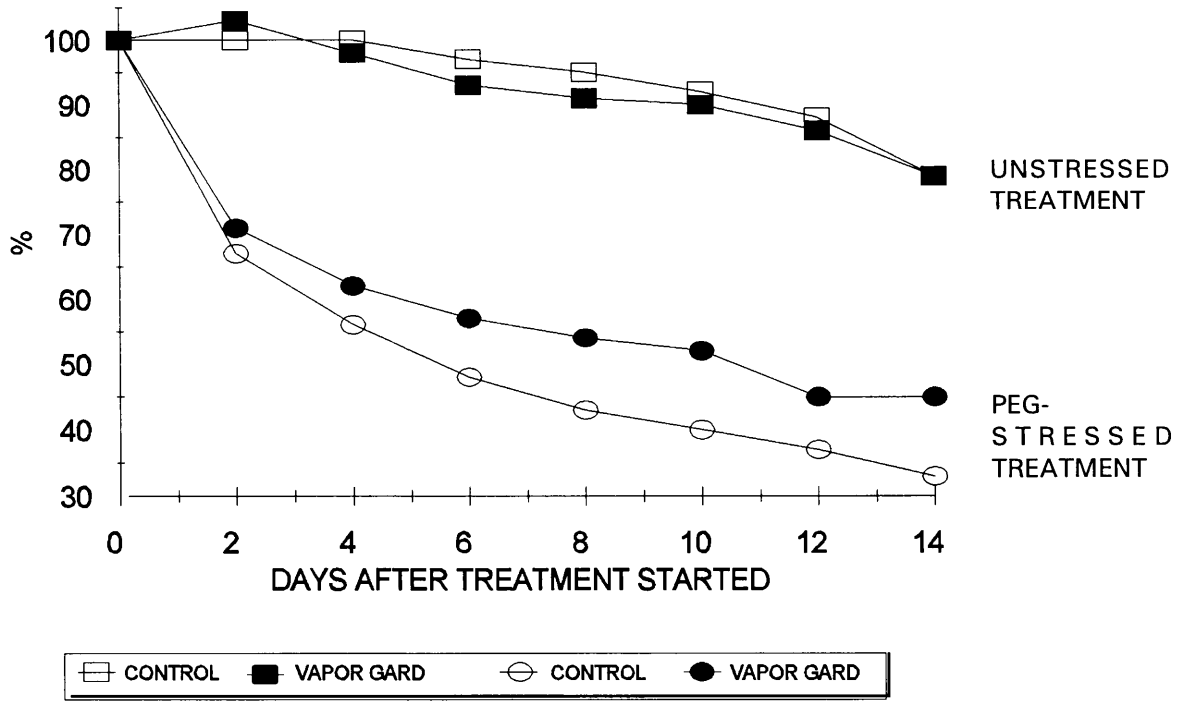

Figure 5.2 Change in relative weight of the plants over time. VG = Vapor Gard, PEG = Polyethylene glycol 6000 (Appendix Table B9).

Unstressed treatment

It is inexplicable why the unstressed plants lost weight, it may be that the nutrient solution did not meet the requirements of the plants as some of the older leaves died back during the observation period, thus losing fresh weight. There was no difference in weight loss between the two treatments.

PEG-stressed treatment

The fresh mass of the plants decreased drastically. It is likely that transpiration was limited to the minimum because the low water potential induced stomatal closure. The decrease in mass of the Vapor Gard treated plants was between 8 and 12 percentage units less than that of the

untreated plants, probably due to better water retention.

The rapid decrease in weight of plants during the first two days (Figure 5.2) could have been the result of osmotic shock to the plant system as the water potential was decreased instantaneously. Krizek (1985) suggested that a membrane system should be used to overcome possible toxic effects of PEG. Toxic impurities in PEG may have contributed to the continuous decrease in the weight of the plants. Solutions of large polymers, such as PEG 6000, tend to foam and this may in addition have created aeration problems (Janes, 1974).

5.3 CONCLUSIONS

The water use of Vapor Gard treated plants of the unstressed treatment was similar to that of the control plants. This suggests that the antitranspirant film did not affect transpiration when plants were not under water stress. The fact that the Vapor Gard treated plants in the PEG-stressed solution used more water than the controls, implies that the control plants were more stressed than the treated plants. This is a tentative indication that Vapor Gard may indeed be advantageous under water stress conditions.

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CHAPTER 6

STOMATAL CONDUCTANCE AND PHOTOSYNTHESIS

6.1 INTRODUCTION

It is important to understand how stomatal responses affect the relationship between the ambient CO_2 concentration (C_o) and the intercellular CO_2 concentration (C_i), since the rate of assimilation in a leaf depends on C_i. Intercellular CO_2 will be equal to C_o when the stomata of a leaf is open and no assimilation is taking place (Raschke, 1986). When photosynthesis commences, the intercellular spaces are depleted of CO_2, and CO_2 diffuses in through the stomatal pores from the atmosphere. A concentration difference that is proportional to CO_2 assimilation (A) and to the resistance (r; see equation 1, p2) develops between the air in the mesophyll and the atmosphere. Only the stomatal component of the resistance is considered. Resistance (r_s) is inversely proportional to conductance (g_s), thus:

$$A = (C_o - C_i)g_s \text{ and}$$

$$C_i = C_o - A/g_s \qquad 6.1$$

Farquhar & Sharkey (1982) developed a simple method of separating stomatal and mesophyll limitations using the A/C_i response. Assimilation rate (A_i), measured at the normal atmospheric CO_2 concentration (C_o = 355 μmol.mol^{-1}), is subtracted from A_o, the rate which would occur if there were no stomatal limitations. The relative limitations (*l*) which the stomata impose, may then be calculated :

$$l = (A_o - A_i)/A_o \qquad 6.2$$

Stomatal limitation (*l*) is the proportionate decrease in A_i that may be attributed to the stomata. The method has the advantage that it makes no assumptions about the shape of the response of A_i to C_i, when calculated graphically (Long & Hällgren, 1993).

If the Vapor Gard film changes transpiration and photosynthesis as a result of a change in the stomatal resistance, it would be detectable from the $A:C_i$ curve. Such curves were drawn for Vapor Gard treated and control plants that were well irrigated.

6.2 MATERIALS AND METHODS

The same plants as in Experiment 3 (p53), where the influence of differential application of Vapor Gard on transpiration and photosynthetic rates were determined, were used to determine the influence of stomatal resistance on photosynthesis. Photosynthetic rates were measured at different CO_2 levels, ranging from 50 to 1 200 $\mu mol.mol^{-1}$ two days after the plants were treated. Only two control and two Vapor Gard treated plants were used, because the procedures were time consuming. The Vapor Gard was applied to the upper and lower leaf surfaces. Photosynthesis: intercellular CO_2 (A:Ci) curves were drawn to determine the extent of stomatal limitation on photosynthesis and whether the antitranspirant treatment had any effect on it.

6.3 RESULTS AND DISCUSSION

Figures 6.1 illustrates the degree to which stomata prevented inward diffusion of CO_2 of control plants, while Figure 6.2 depicts the limitation that stomata placed on CO_2 diffusion when the leaves were treated with Vapor Gard.

By using Eq. 6.2 stomatal limitation (*l*) to photosynthetic rate in Figure 6.1a can be calculated as:
(18.4 - 16)/18.4 = 0.13x100 = 13%. In other words, the degree of stomatal closure was sufficient to limit A by 13% of the potential A, while for the control leaf in Figure 6.1b, *l* = 14%.

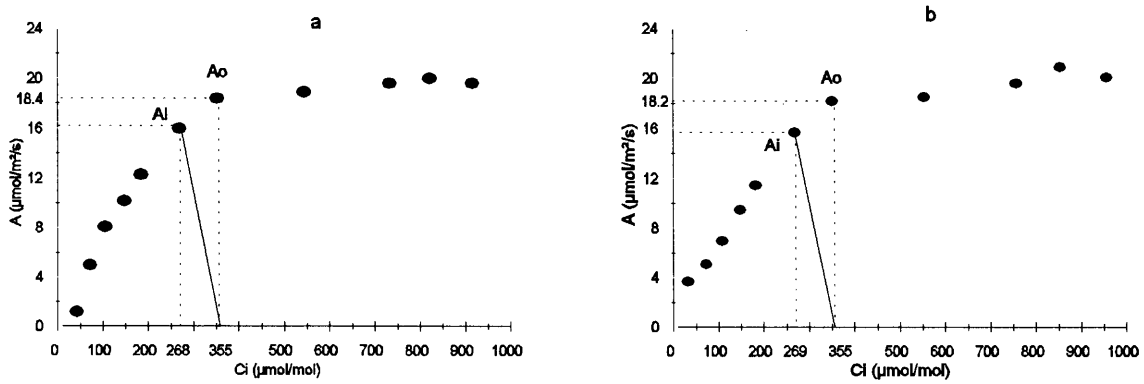

Figure 6.1a,b Response of CO_2 assimilation rate (A) to leaf internal CO_2 mole fraction (C_i). The solid line indicates the "supply line", A_o is the potential rate, A_i is the actual rate (Appendix Table B10).

The potential photosynthetic rates of Vapor Gard treated plants were decreased by 7% (from 18.2 to 16.9μmol.m^{-2}.s^{-1}) in Figure 6.2a and by 10% (from 16.2 to 14.6μmol.m^{-2}.s^{-1}) in Figure 6.2b, respectively.

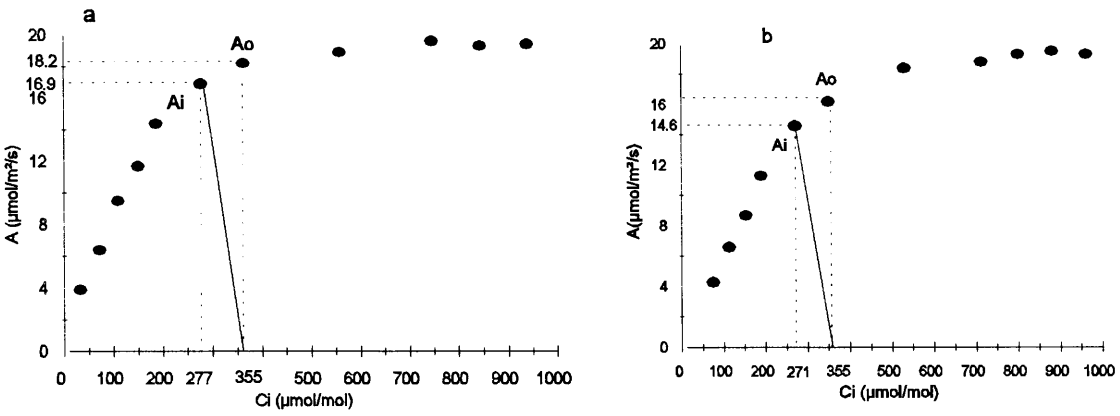

Figure 6.2a,b Response of CO_2 assimilation rate (A) to leaf internal CO_2 mole fraction (C_i) as influenced by a Vapor Gard application. The solid line indicates the "supply line", A_o is the potential rate, A_i is the actual rate (Appendix Table B11).

The first criterion for establishing that stomatal response is dominant in the response of assimilation rate to some interference, is that C_i should change in the same direction as A. If the change is in the opposite direction, the most important change must have been in the mesophyll cells (Farquhar & Sharkey, 1982). The photosynthetic rate of the plant, depicted in Figure 6.2b, was lower than that of the control and other Vapor Gard treated plants. This lower rate was however not accompanied by a lower C_i (271 vs 268 and 269μmol.mol^{-1}) or increased stomatal limitation (*l*). It can not be concluded that stomatal closure was the main cause of the reduced assimilation rate.

The additional mesophyll resistance for CO_2 is the basis for the assumption that partial closure of the stomata will reduce transpiration more than it reduces photosynthesis (Kramer & Boyer, 1995).

6.4 CONCLUSION

The stomatal limitation of both the 5% Vapor Gard treated plants were smaller than that of the control plants, while the C_i was larger than that of the control plants. If a decrease in the assimilation rate of Vapor Gard treated plants would occur, it would probably be a result of reduced mesophyll capacity for assimilation and not due to stomatal closure.

The cracking of the film over the stomata, relatively soon after application, supports this conclusion, since gaseous diffusion can take place normally through the stomata once the film over them has cracked. Cuticular transpiration is still decreased, for the film presents a resistance to water vapour diffusion through the cuticle but when stomata are open, far more water vapour escapes from the leaf along the stomatal pathway than through the cuticle (Gates, 1968).

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CHAPTER 7

EFFECT OF VAPOR GARD ON LEAF TEMPERATURE

7.1 INTRODUCTION

Leaf temperature and transpiration rate are the result of the interaction of several simultaneous environmental factors interacting with a leaf to a degree determined by several plant properties. (Gates, 1968). Transpiration exerts a negative feedback on itself through evaporative cooling which lowers water vapour pressure in the leaf (Solárová, Pospíšilová & Slavík, 1981). Reduction of the cooling effect of transpiration might be expected to cause an increase in leaf temperature (Davies & Kozlowski, 1974).

While antitranspirants of the reflecting type cause reduction in leaf temperature (Abou-Khaled, Hagan & Davenport, 1970) the film-forming types tend to increase leaf temperature by curtailing transpiration rates and thus reducing evaporative cooling. Since thermal emission, rather than evaporative cooling is the most important means of heat dissipation, the increase in leaf temperature under normal conditions is small (Davenport, Hagan & Martin, 1969). Gates (1968) used an energy balance equation to calculate the effect of changes in leaf resistance, caused by antitranspirants, on temperature and transpiration of single leaves. The calculations showed that a relatively low percentage covering by an impervious antitranspirant may cause a significant reduction in water loss, but only a very small increase in leaf temperature. Only when the air is almost still (wind speed = 0.1m.sec^{-1}) would there be a significant, and

possible harmful rise in leaf temperature because of the reduced transpiration.

It is important to examine the effect of antitranspirants on leaf temperature, since most plants have fairly specific temperature thresholds and injury could be expected to occur if these were exceeded (Slatyer & Bierhuizen, 1964).

7.2 MATERIALS AND METHODS

One bean and one butter lettuce plant of each treatment (control, 5% and 10% Vapor Gard) were randomly chosen in the growth chamber experiment (Experiment 1, p45) to measure leaf temperature. The growth chamber was programmed for a 12-hour photoperiod, and a 22°C day/16°C night thermal regime. Copper-constantan thermocouples, used to record leaf temperature, were scotch taped to the abaxial sides of the leaves. Temperatures were measured every 30 min. and recorded by a datalogger over a 24-hour period. Measurements were made one day after the plants were treated and again two weeks later.

A similar trial was conducted at the same time in a glasshouse where the temperature ranged between 20 and 24°C in the day and between 14 and 18°C in the night. Corresponding with the second leaf temperature measurement in the growth chamber, measurements of butter lettuce leaves were made and recorded in the glasshouse in the same manner as described above .

Data were statistical analysed by the Grant Squirrelwise Programme (Grant Instruments, Ltd.,Cambridge, England). Mean, minimum and maximum

temperatures, standard deviations and variances are given in Appendix C.

7.3 RESULTS AND DISCUSSION

Twenty four hours after application

Leaf temperatures of butter lettuce and beans, recorded for a 24-hour period one day after Vapor Gard application, are illustrated in Figures 7.1 and 7.2, respectively. Leaf temperatures changed abruptly with changes in the diurnal growth chamber temperatures.

The leaf temperatures of the three treatments in Figure 7.1 were close to each other when recording started one day after Vapor Gard was applied. Temperatures rose 4°C above ambient. Differences in leaf temperatures were only detected after 21 hours when the leaves of the 5% Vapor Gard treatment was 2.4 and 2.5°C higher than that of the control and the 10% treated leaves, respectively. Leaf temperature of the 10% Vapor Gard treated plant was similar to that of the control plant. Night temperatures were similar for all treatments and were 1°C lower than that of the growth chamber.

In Figure 7.2 it can be seen that leaf temperature of the 10% Vapor Gard treated bean plant was lower than that of the control and 5% treatment for almost the entire 24-hour period. There were no differences between the leaf temperatures during the night. During the morning hours, an elevation of temperature of the 5% treated leaves to a maximum of 4°C above that of the controls were recorded. However, during the hottest noon hours, temperatures of control and 5% treated plants were almost identical.

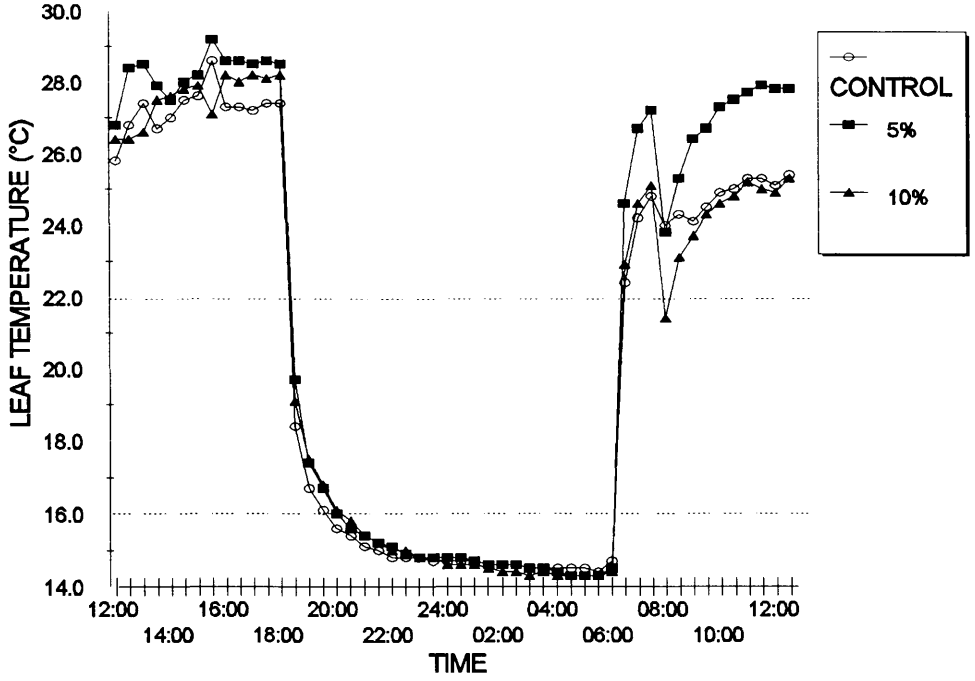

Figure 7.1 Leaf temperatures of butter lettuce recorded for a 24-hour period one day after Vapor Gard application. Horizontal lines indicate ambient temperatures.

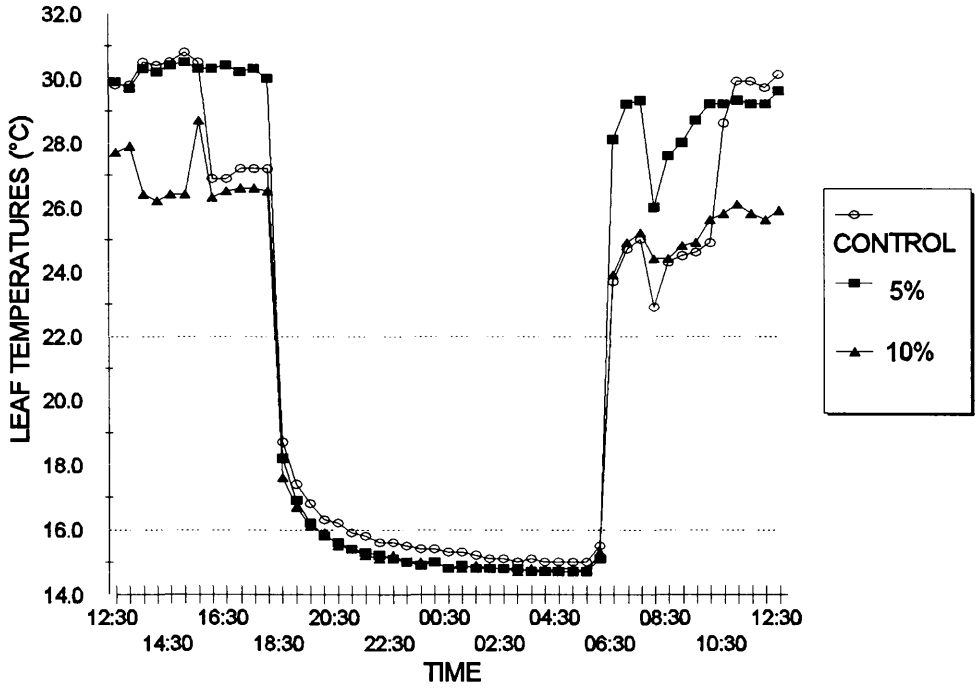

Figure 7.2 Leaf temperatures of beans recorded for a 24-hour period one day after Vapor Gard application. Horizontal lines indicate ambient temperature.

These results are in accordance with those of Gale & Poljakoff-Mayber (1965) who found the same phenomenon in *Beta vulgaris* plants treated with 6% S-789. According to Gale (1961) this may have been due to the midday reduction of transpiration of the control plants as a result of closure of their stomata.

Two weeks after application

Figures 7.3 and 7.4 depict leaf temperatures of the treated and control lettuce and bean plants in the growth chamber, two weeks after Vapor Gard was applied.

It is clear from Figure 7.3 that the leaf temperature of the 10% treated butter lettuce was lower than that of the control and that the temperature of the 5% treatment was higher than that of the control. From Figure 7.4 it is clear that the leaf temperature of the 10% treated bean plant was lower than that of the control and 5% treated plant. The leaf temperature of the 5% treated plant rose higher than that of the control only after 21 hours.

No logical explanation can be offered for the inconsistency of these results.

Figure 7.5 illustrates the leaf temperatures of butter lettuce over a 24-hour period in the glasshouse, two weeks after application. Leaf temperatures of butter lettuce treated with 10% Vapor Gard were higher in day light than the other two treatments and lower than those of the 5% treated plant at night. Differences between the treatments were not great. The largest difference in temperature between the 10% treatment and the other two treatments was 3°C, at 15:00. There were little difference between the treated leaves, untreated leaves and the air temperature.

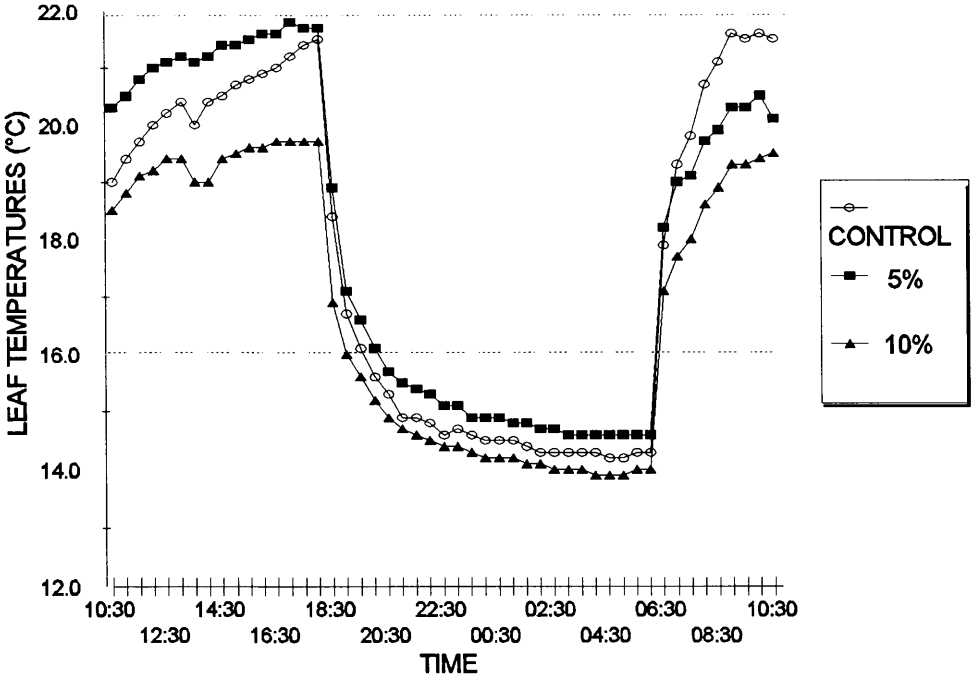

Figure 7.3 Leaf temperatures of butter lettuce two weeks after the Vapor Gard application. Horizontal lines indicate ambient temperatures.

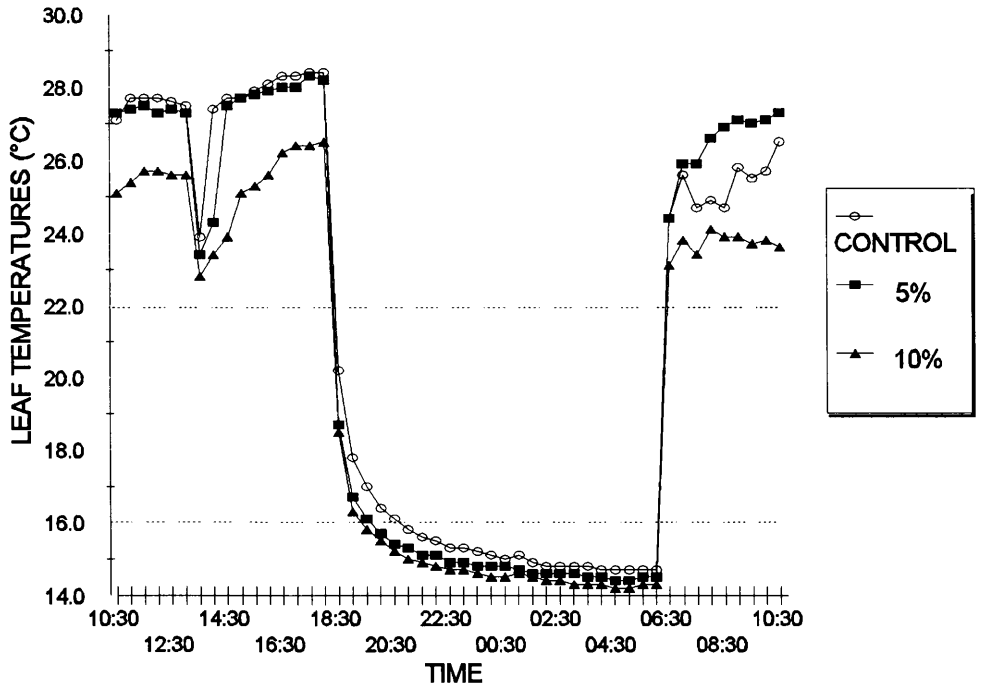

Figure 7.4 Leaf temperatures of bean plants two weeks after the Vapor Gard application. Horizontal lines indicate ambient temperatures.

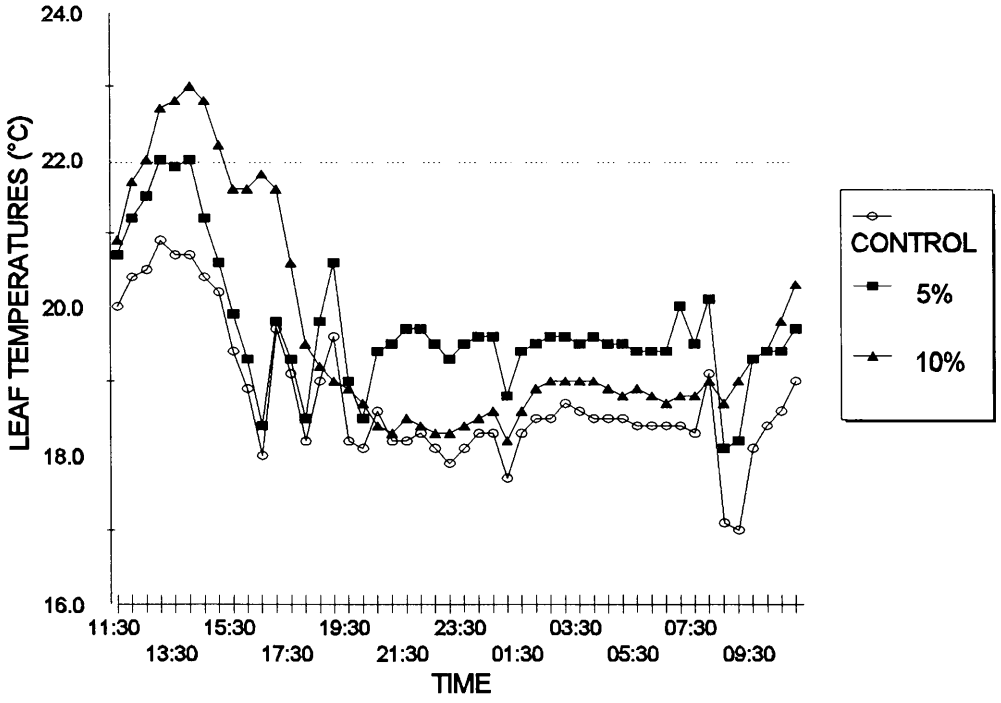

Figure 7.5 Leaf temperatures of butter lettuce in the glasshouse two weeks after Vapor Gard application. Horizontal line indicates maximum air temperature.

These results are in agreement with that of Gale & Poljakoff-Mayber (1965). They measured leaf temperatures of a number of species treated with antitranspirants (all of the plastic emulsion type) and found little difference between treated leaves, untreated leaves and air temperature. It appears that only under extreme conditions of high incident radiation and very low wind velocity would leaf temperatures be significantly raised by reduction of transpiration (Gale & Hagan, 1966).

7.4 CONCLUSION

Little difference between leaf temperatures of control and treated plants were found. The rise in leaf temperature of the 10% treated plant in the glasshouse might be the result of Vapor Gard that changed the albedo of the leaf by trapping long wave radiation, or by modifying the convective sensible cooling of the air by changing the leaf surface texture. Increases in leaf temperatures by the Vapor Gard treatment were insignificant in this preliminary trial, but much more data are required before any reliable conclusions can be drawn.

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CHAPTER 8

GENERAL DISCUSSION

Antitranspirants have been used on a variety of crops to reduce water use or to improve the water potential of the crop. Film-forming antitranspirants add additional resistance to the water loss pathway by blocking or partially covering stomata (Gale & Hagan, 1966; Davenport, Fisher & Hagan, 1972; Solàrovà, Pospíšilovà & Slavík, 1981). A concurrent reduction in photosynthesis may occur, depending on the permeability of the film to CO_2, which is often one-fourth of that for water vapour (Davies & Kozlowski, 1974).

The literature contains many discrepancies over the effect of antitranspirants on transpiration and photosynthetic rates. It is important to define environmental conditions when recommending antitranspirant treatments or reporting research results. Most results showed that film-forming antitranspirants decreased transpiration with a concurrent decrease in photosynthesis (Davies & Kozlowski, 1974; Davenport, Uriu & Hagan, 1974; Olofinboba, Kozlowski & Marshall, 1974).

Quantifying the effects of Vapor Gard on plants under controlled environmental conditions was in many respects an ideal research topic. The particular antitranspirant was reported to greatly improve the performance of transplanted seedlings, especially lettuce, under experimental and commercial conditions in South Africa. At the same time little was known about the specific effects it had on plants, especially on the

rates of transpiration and photosynthesis. This presented opportunities of getting acquainted with pot experiments in controlled environments, getting exposure to the power of electron microscopy, operating a modern Ciras-1 photosynthesis-system, and in the process gaining a better understanding of assimilation and plant water relations.

Surprisingly no clear effects of the Vapor Gard film on plant processes could be detected in numerous trials, some not even reported in this manuscript. This project was a fascinating introduction to the principles and practices of scientific research, as well as the pleasure and pain associated with it. Distinct results included:

1. Clear micrographs illustrating the nature of the film on the leaf surface.
2. Indisputable evidence that under the experimental conditions a Vapor Gard film did not affect the rate of transpiration.
3. No indication was found that the presence of a Vapor Gard film impaired gas exchange and photosynthesis.
4. The amount of water transpired by lettuce plants over a 14 day period was not reduced by Vapor Gard application, on the contrary the treated plants transpired more water than the control.
5. Leaf temperature was not increased to such an extent that it could affect plant processes.

Additional observations were made on the incidence of powdery mildew. It was observed that the incidence on Vapor Gard treated lettuce plants under humid conditions was much lower than on untreated plants. A Vapor Gard application seemed to influence the plant-host interactions.

However a number of aspects remains unclear and should be investigated in more detail.

1. This study sheds no light on the physiological explanations for the reported improvement in the performance of transplanted seedlings.
2. Micrographs of leaf surfaces treated with a range of Vapor Gard concentrations (1, 5 and 10%), applied when stomata are open and when they are closed, should reveal the cause and extent of cracking of the film at the stomatal sites.
3. The possibility that Vapor Gard treatment will reduce transpiration under conditions of high evaporative demand was not properly addressed. Future research should attempt to measure transpiration over a range of vapour pressure deficits.
4. Experiments to quantify the possible effects of Vapor Gard under field conditions will aid in the extrapolation of results achieved under controlled environmental conditions. The summer of 1996/97 was exceptionally wet in Pretoria, resulting in failure of the field trials.
5. A pot experiment with a range of PEG-concentrations will shed more light on the advantages of Vapor Gard where the water supply is limited.

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SUMMARY

The possibility of reducing transpiration, thus saving water and alleviating the adverse effects of water stress on plant growth will be advantageous in a country where scarce water resources create pressure for effective use. Methods of reducing transpiration date back to 300 B.C.. Unfortunately it has been observed that these methods often affect photosynthesis negatively. It was postulated that film-forming antitranspirants may be able reduce transpiration without a concomitant decrease in photosynthesis. Vapor Gard, a β-pinene polymer, is often used in South Africa, but no locally published research on its effectiveness is available. This study was conducted to determine the effects of Vapor Gard on basic physiology. Experiments were performed to measure transpiration and photosynthetic rates, leaf water content and leaf temperatures.

Microscopic study of the film on leaf surfaces

The Vapor Gard film was clearly visible on micrographs taken shortly after application. Although most of the stomata were covered, the film was cracked over a few pores. Micrographs taken two weeks after application showed that the film was still evident on the surface, but that the incidence of cracking had increased.

Transpiration and photosynthetic rates

Transpiration and photosynthetic rates were measured with a Ciras-1 portable photosynthesis system. Growth chamber and glasshouse experiments with lettuce and bean plants showed that Vapor Gard did not affect the rates of these processes though the transpiration rate of bean

plants was decreased in a glasshouse experiment when measurements were made in full sun light. Whether Vapor Gard was applied on both sides or just one side of the leaf made no difference to transpiration and photosynthetic rates.

Water relations

Water potential of the nutrient solution was lowered by adding polyethylene glycol (PEG). Evaporation was prevented by covering the individual pots with polystyrene disks. The loss of water and change in weight of plants were recorded every second day. Vapor Gard treated plants seemed to use more water than control plants, but there was an indication that such plants retained more water when under water stress conditions.

Stomatal conductance

The influence of stomatal limitation on photosynthesis of Vapor Gard treated plants was compared to that of untreated plants by measuring photosynthesis under increasing CO_2 concentrations. The film on treated plants did not increase stomatal limitation and intercellular CO_2 concentrations were increased.

Leaf temperature

Leaf temperatures of neither butter lettuce nor bean plants were increased to such an extent that they could affect plant processes or cause increased transpiration rates.

OPSOMMING

Die moontlikheid om transpirasie te beperk en sodoende water te bespaar en negatiewe effekte van waterstremming op plantgroei te verlig wanneer die transpirasietempo water opname oorskry, sal voordelig wees in 'n land waar skaars water hulpbronne druk plaas op effektiewe water verbruik. Metodes om transpirasie te beperk dateer sover terug as 300vC., maar hierdie metodes beïnvloed fotosintese dikwels negatief. Dit word beweer dat film-vormende antitranspirante moontlik transpirasie kan beperk sonder meegaande beperking van fotosintese. Vapor Gard, 'n β-pineen polimeer word dikwels in Suid-Afrika gebruik, maar geen plaaslike navorsingsverslae oor die effektiwiteit daarvan is beskikbaar nie. Die studie was onderneem om die effek van Vapor Gard op basiese fisiologiese prosesse te ondersoek. Eksperimente is uitgevoer om transpirasie- en fotosintese tempo's, blaarwaterinhoud en -temperature te meet.

Mikroskopiese studie van die film op blaaroppervlaktes

Die Vapor Gard film was duidelik sigbaar op mikrograwe wat geneem is kort na toediening. Alhoewel meeste van die huidmondjies bedek was met die film, het dit reeds begin kraak oor die porieë. Mikrograwe wat twee weke na toediening geneem is, het getoon dat die film nog aanwesig was, alhoewel daar 'n toename in krake voorgekom het.

Transpirasie- en fotosintese tempo's

Transpirasie- en fotosintese tempo's is gemeet met 'n Ciras-1 draagbare fotosintese sisteem. Groeikabinet- en glashuisproewe met slaai en boontjies het getoon dat Vapor Gard nie die tempo's beïnvloed het nie. Geen verskil is gemeet wanneer Vapor Gard aan een of beide kante van die

blaaroppervlak gespuit is nie. Transpirasietempo's van boontjies is wel verlaag wanneer metings in volson gemaak is.

Waterverhoudings

Waterpotentiaal van die voedingsoplossing is verlaag met poli-etileen glikol (PEG). Verdamping vanaf die oppervlak is voorkom deur individuele potte te bedek met polistereen skywe. Waterverlies en gewigsveranderings van die plante is elke tweede dag aangeteken. Dit het voorgekom asof Vapor Gard behandelde plante meer water verbruik het as kontrole plante, maar daar was 'n aanduiding dat sulke plante meer water terughou tydens droogte stremming.

Huidmondjie geleiding

Die invloed wat huidmondjie beperking op die fotosintese van Vapor Gard behandelde plante het, is vergelyk met dié van onbehandelde plante, deur fotosintese te meet by toenemende CO_2 konsentrasies. Die film op die behandelde plante het egter nie fotosintese beperk deur huidmondjie weerstand te vergroot nie en intersellulêre CO_2 konsentrasies van behandelde plante is verhoog.

Blaartemperatuur

Blaartemperature is nie sodanig deur 'n Vapor Gard toediening verhoog dat dit plantprosesse kon beïnvloed of transpirasie tempo's verhoog nie.

ACKNOWLEDGEMENTS

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APPENDIX A

ANOVA FOR EXPERIMENT 1 - GROWTH CHAMBER TRIAL

SOURCE	DEGREES OF FREEDOM (DF)
MODEL	2
ERROR	21
TOTAL	23

Table 1A Modified ANOVA Table of transpiration rates over a 20-day period of butter lettuce, cabbage lettuce and bean plants treated with 5% and 10% Vapor Gard

DAYS	0	1	5	6	8	11	14	17	20
BUTTER LETTUCE									
R^2	0.04	0.02	0.05	0.01	0.02	0.09	0.02	0.11	0.04
C.V. [a]	35.3	42.7	29.2	29.3	35.7	59.7	47.9	77.5	92.5
Pr>F	0.71	0.83	0.57	0.87	0.81	0.38	0.77	0.31	0.85
RMSE[b]	0.77	0.95	0.79	0.75	0.99	1.00	0.95	0.72	1.22
CABBAGE LETTUCE									
R^2	0.09	0.16	0.06	0.05	0.14	0.07	0.03	0.18	0.02
C.V.	41.8	32.3	51.7	28.8	25.8	44.1	56.8	66.1	164
Pr>F	0.41	0.16	0.53	0.57	0.27	0.48	0.74	0.12	0.90
RMSE	1.09	1.02	2.34	1.11	0.84	1.24	1.78	1.08	1.79
BEANS									
R^2	0.17	0.04	0.04	0.02	0.03	0.01	0.05	0.01	0.37
C.V.	51.2	48.6	32.0	23.5	33.4	23.3	52.9	42.7	91.8
Pr>F	0.20	0.63	0.65	0.79	0.74	0.91	0.60	0.92	0.12
RMSE	1.17	1.26	1.65	1.25	1.54	1.23	2.33	1.65	2.93

[a] Coefficient of variance (%)

[b] Root mean square error

Table 2A Modified ANOVA Table of photosynthetic rates over a 20-day period of butter lettuce, cabbage lettuce and bean plants treated with 5% and 10% Vapor Gard

DAYS	0	1	5	6	8	11	14	17	20
BUTTER LETTUCE									
R^2	0.22	0.13	0.05	0.08	0.14	0.02	0.07	0.01	0.24
C.V.	23.7	21.6	35.5	22.1	30.1	40.8	80.7	45.9	360
RMSE	1.03	1.06	2.09	1.28	1.56	1.60	2.75	1.17	1.32
Pr > F	0.11	0.22	0.57	0.42	0.26	0.80	0.48	0.90	0.29
CABBAGE LETTUCE									
R^2	0.05	0.02	0.22	0.06	0.03	0.01	0.05	0.09	0.04
C.V.	49.5	40.8	59.0	40.0	50.0	58.1	48.2	38.6	59.0
RMSE	0.71	0.63	1.21	0.69	1.06	0.80	1.24	0.25	0.99
Pr > F	0.62	0.82	0.79	0.52	0.76	0.91	0.56	0.36	0.85
BEANS									
R^2	0.07	0.07	0.04	0.09	0.20	0.15	0.04	0.02	0.45
C.V.	53.6	45.9	54.1	41.4	40.5	44.2	49.3	58.4	35.5
RMSE	0.47	0.30	0.90	0.81	0.84	0.72	1.28	0.83	0.85
Pr > F	0.61	0.47	0.64	0.38	0.14	0.18	0.65	0.81	0.07

ANOVA FOR EXPERIMENT 2 - GLASSHOUSE TRIAL

Table 3A Anova Table of transpiration rates of bean plants in the glasshouse over a 3-day period

DAY 0

SOURCE	DF	SUM SQUARES	MEAN SQUARE	F VALUE	Pr > F
MODEL	9	53.177	5.909	2.13	0.098
ERROR	14	38.754	2.768		
TOTAL	23	91.931			
	R^2	C.V.	ROOT MSE		MEAN
	0.578	16.858	1.664		9.870
SOURCE	DF	TYPE III SS	MEAN SQUARE	F VALUE	Pr > F
CONC	2	24.193	12.097	4.37	0.034
BLOCK	7	28.984	4.141	1.50	0.247

DAY 1

SOURCE	DF	SUM SQUARES	MEAN SQUARE	F VALUE	Pr > F
MODEL	9	54.470	6.052	11.44	0.0001
ERROR	14	7.403	0.529		
TOTAL	23	61.873			
	R^2	C.V.	ROOT MSE		MEAN
	0.880	6.871	0.727		10.583
SOURCE	DF	TYPE III SS	MEAN SQUARE	F VALUE	Pr > F
CONC	2	4.123	2.062	3.90	0.0451
BLOCK	7	50.346	7.192	13.60	0.0001

DAY 2

SOURCE	DF	SUM SQUARES	MEAN SQUARE	F VALUE	Pr > F
MODEL	9	18.905	2.101	2.07	0.107
ERROR	14	14.183	1.013		
TOTAL	23	33.087			
	R^2	C.V.	ROOT MSE		MEAN
	0.571	10.978	1.006		9.168
SOURCE	DF	TYPE III SS	MEAN SQUARE	F VALUE	Pr > F
CONC	2	9.349	4.674	4.61	0.0289
BLOCK	7	9.556	1.365	1.35	0.2999

DAY 3

SOURCE	DF	SUM SQUARES	MEAN SQUARE	F VALUE	Pr > F
MODEL	9	42.063	4.674	1.30	0.317
ERROR	14	50.176	3.584		
TOTAL	23	92.238			
	R^2	C.V.	ROOT MSE		MEAN
	0.456	18.539	1.893		10.211
SOURCE	DF	TYPE III SS	MEAN SQUARE	F VALUE	Pr > F
CONC	2	11.413	5.707	1.59	0.238
BLOCK	7	30.649	4.378	1.22	0.354

Table 4A ANOVA Table of photosynthetic rates of bean plants in the glasshouse over a 3-day period

DAY 0

SOURCE	DF	SUM SQUARES	MEAN SQUARE	F VALUE	Pr > F
MODEL	9	62.003	6.889	0.68	0.719
ERROR	14	142.772	10.198		
TOTAL	23	204.775			
	R^2	C.V.	ROOT MSE		MEAN
	0.303	27.108	3.193		11.781
SOURCE	DF	TYPE III SS	MEAN SQUARE	F VALUE	Pr > F
CONC	2	35.281	17.641	1.73	0.213
BLOCK	7	26.722	3.817	0.37	0.902

DAY 1

SOURCE	DF	SUM SQUARES	MEAN SQUARE	F VALUE	Pr > F
MODEL	9	63.129	7.014	0.74	0.668
ERROR	14	132.420	9.459		
TOTAL	23	195.549			
	R^2	C.V.	ROOT MSE		MEAN
	0.322	29.903	3.075		10.285
SOURCE	DF	TYPE III SS	MEAN SQUARE	F VALUE	Pr > F
CONC	2	36.195	18.097	1.91	0.184
BLOCK	7	26.934	3.048	0.41	0.883

DAY 2

SOURCE	DF	SUM SQUARES	MEAN SQUARE	F VALUE	Pr > F
MODEL	9	115.445	12.827	7.23	0.001
ERROR	14	24.821	1.773		
TOTAL	23	140.266			
	R^2	C.V.	ROOT MSE		MEAN
	0.823	1.449	1.332		11.631
SOURCE	DF	TYPE III SS	MEAN SQUARE	F VALUE	Pr > F
CONC	2	1.965	0.982	0.55	0.587
BLOCK	7	113.481	16.212	9.14	0.003

DAY 3

SOURCE	DF	SUM SQUARES	MEAN SQUARE	F VALUE	Pr > F
MODEL	9	228.540	25.393	3.11	0.028
ERROR	14	114.211	8.158		
TOTAL	23	342.751			
	R^2	C.V.	ROOT MSE		MEAN
	0.667	21.966	2.856		13.003
SOURCE	DF	TYPE III SS	MEAN SQUARE	F VALUE	Pr > F
CONC	2	22.157	11.078	1.36	0.289
BLOCK	7	206.384	29.483	3.61	0.020

ANOVA FOR EXPERIMENT 3 - DIFFERENTIAL APPLICATION ON UPPER AND LOWER LEAF SURFACES

Table 6A ANOVA Table of transpiration rates of butter lettuce treated on either or both sides on days 3, 8 and 16

DAY 3

SOURCE	DF	SUM SQUARES	MEAN SQUARE	F VALUE	Pr>F
MODEL	3	1.159	0.386	0.83	0.494
ERROR	20	9.328	0.466		
TOTAL	23	10.487			
	R^2	C.V.	ROOT MSE		MEAN
	0.110	12.261	0.683		5.570

DAY 8

SOURCE	DF	SUM SQUARES	MEAN SQUARE	F VALUE	Pr>F
MODEL	3	4.752	1.584	0.39	0.758
ERROR	20	72.427	4.014		
TOTAL	23	76.999			
	R^2	C.V.	ROOT MSE		MEAN
	0.062	60.057	2.003		3.336

DAY 16

SOURCE	DF	SUM SQUARES	MEAN SQUARE	F VALUE	Pr>F
MODEL	3	3.921	1.307	0.33	0.806
ERROR	20	67.934	3.996		
TOTAL	23	71.855			
	R^2	C.V.	ROOT MSE		MEAN
	0.055	47.492	1.999		4.210

Table 7A ANOVA Table of photosynthetic rates of butter lettuce treated on either or both sides on days 3, 8 and 16

DAY 3

SOURCE	DF	SUM SQUARES	MEAN SQUARE	F VALUE	Pr>F
MODEL	3	20.253	6.751	1.47	0.252
ERROR	20	91.680	4.584		
TOTAL	23	111.933			
	R^2	C.V.	ROOT MSE		MEAN
	0.181	15.788	2.141		13.561

DAY 8

SOURCE	DF	SUM SQUARES	MEAN SQUARE	F VALUE	Pr>F
MODEL	3	53.153	17.718	0.71	0.559
ERROR	18	449.331	24.963		
TOTAL	21	502.484			
	R^2	C.V.	ROOT MSE		MEAN
	0.106	52.930	4.996		9.439

DAY 16

SOURCE	DF	SUM SQUARES	MEAN SQUARE	F VALUE	Pr>F
MODEL	3	46.441	15.480	0.66	0.586
ERROR	17	397.032	23.355		
TOTAL	20	443.472			
	R^2	C.V.	ROOT MSE		MEAN
	0.105	41.165	4.833		11.740

ANOVA FOR POLYETHYLENE GLYCOL TRIAL

SOURCE	DEGREES OF FREEDOM
MODEL	1
ERROR	10
TOTAL	11

Table 8A ANOVA Table of water used by butter lettuce plants over a 14-day period

	DAY	2	4	6	8	10	12	14
UNSTRESSED	R^2	0.03	0.03	0.02	0.01	0.01	0.03	0.04
	C.V.	36.14	44.61	47.84	51.71	48.78	52.61	66.0
	RMSE	71.96	63.87	78.07	105.5	76.72	71.74	92.7
	Pr > F	0.623	0.641	0.648	0.736	0.723	0.587	0.56
PEG-STRESSED	R^2	0.02	0.16	0.08	0.09	0.15	0.2	0.10
	CV	87.77	76.47	80.43	63.29	72.27	72.88	97.2
	RMSE	34.74	17.52	18.10	15.56	9.94	10.02	10.5
	Pr > F	0.657	0.192	0.361	0.331	0.220	0.144	0.30

Root Mean Square Error / Root Replicates = Standard Error of the Mean

APPENDIX B

Table 1B Mean transpiration rates of butter lettuce, cabbage lettuce and bean plants

Butter lettuce

DAYS	CONTROL	5%	10%
0	2.372	2.164	2.032
1	2.051	2.283	2.32
5	2.648	2.519	2.93
6	2.444	2.576	2.636
8	2.882	2.84	2.564
11	1.444	2.078	1.489
14	2.002	1.813	2.162
17	0.715	1.25	0.815
20	1.275	1.1	1.6

Cabbage lettuce

DAYS	CONTROL	5%	10%
0	2.567	3.026	2.236
1	3.542	3.375	2.579
5	4.631	5.135	3.81
6	3.546	3.908	4.138
8	3.583	2.845	3.352
11	3.223	2.758	2.469
14	2.735	3.354	3.317
17	1.925	0.969	2.031
20	0.925	0.925	1.425

Bean plants

DAY	CONTROL	5%	10%
0	2.802	1.64	2.425
1	2.942	2.446	2.383
5	5.335	4.708	5.404
6	5.493	5.088	5.431
8	4.755	4.224	4.807
11	5.115	5.358	5.34
14	3.933	5.083	4.219
17	4.05	3.773	3.756
20	2.975	5.688	0.9

Table 2B Mean photosynthetic rates of butter lettuce, cabbage lettuce and bean plants

Butter lettuce

DAYS	CONTROL	5%	10%
0	4.983	3.774	4.212
1	5.314	4.392	5.06
5	5.873	6.431	5.315
6	5.977	6.138	5.319
8	5.981	4.929	4.626
11	3.679	3.904	4.21
14	2.806	4.375	3.052
17	2.475	2.713	2.481
20	-0.237	1.25	0.088

Cabbage lettuce

DAYS	CONTROL	5%	10%
0	1.609	1.425	1.239
1	1.49	1.666	1.499
5	1.818	2.221	2.13
6	1.55	1.946	1.701
8	2	1.975	2.355
11	1.279	1.404	1.45
14	2.281	2.483	2.942
17	0.756	0.59	0.606
20	1.85	1.45	1.713

Bean plants

DAY	CONTROL	5%	10%
0	0.862	0.751	1.013
1	0.676	0.737	0.551
5	1.422	1.734	1.838
6	2.098	1.637	2.171
8	1.629	2.005	2.563
11	1.487	1.36	2.017
14	2.341	2.525	2.923
17	1.392	1.302	1.565
20	1.95	3.35	1.938

Table 3B Mean transpiration rates of bean leaves in the glasshouse

DAY	CONTROL	5%	10%
0	10.67	10.48	8.45
1	11.16	10.39	10.2
2	10.04	8.64	8.82
3	11.17	9.89	9.58

Table 4B Mean photosynthetic rates of bean leaves in the glasshouse

DAY	CONTROL	5%	10%
0	12.24	12.98	10.12
1	10.7	11.54	8.62
2	11.4	11.46	12.03
3	11.66	13.48	13.87

Table 5B Mean transpiration/photosynthesis ratios of bean seedlings

DAY	CONTROL	5%	10%
0	0.87	0.81	0.83
1	1.04	0.90	1.18
2	0.88	0.75	0.73
3	0.96	0.73	0.69

Table 6B Mean transpiration rates of butter lettuce treated on either or both sides

DAY	CONTROL	BOTH SIDES	ADAXIAL	ABAXIAL
3	5.52	5.26	5.88	5.62
8	3.91	3.47	2.67	3.28
16	4.21	3.49	4.64	4.5

Table 7B Mean photosynthetic rates of butter lettuce treated on either or both sides

DAY	CONTROL	BOTH SIDES	ADAXIAL	ABAXIAL
3	13.36	13.15	15.09	12.65
8	10.75	8.26	7.8	11.6
16	10.29	12.98	13.58	10.39

Table 9B Change in relative weight of butter lettuce plants over 14 days

	DAY0	DAY2	DAY4	DAY6	DAY8	DAY10	DAY12	DAY14
UNSTRESSED CONTROL	100	100	100	97	95	92	88	79
UNSTRESSED WITH VAPOR	100	103	98	93	91	90	86	79
STRESSED CONTROL	100	67	56	48	43	40	37	33
STRESSED WITH VAPOR GARD	100	71	62	57	54	52	45	45

Table 10B Response of CO_2 assimilation rate to leaf internal CO_2 mole fraction

Figure 6.1a	
A	Ci
1.2	42
5	72
8.1	105
10.2	148
12.3	184
16	269
18.4	352
18.9	543
19.6	732
20	821
19.6	916
20.4	1002

Figure 6.1b	
A	Ci
3.7	32
5.1	72
7	108
9.5	147
11.5	182
15.7	268
18.2	350
18.5	553
19.6	757
20.9	854
20.1	956
21.4	1052

Table 11B Response of CO_2 assimilation rate to leaf internal CO_2 mole fraction as influenced by a Vapor Gard application

Figure 6.2a	
A	Ci
3.9	33
6.4	72
9.5	109
11.7	149
14.4	186
16.9	277
18.2	363
18.9	558
19.6	747
19.3	844
19.4	939
20.1	1030

Figure 6.2b	
A	Ci
-2.6	0
4.3	75
6.6	113
8.7	152
11.3	189
14.6	271
16.2	350
18.4	531
18.8	713
19.3	801
19.5	882
19.3	962

APPENDIX C

Statistics - Butter lettuce and Bean plants in the growth chamber one week after Vapor Gard application

Recording interval : 00:30:00

Butter lettuce

TREATMENT	CONTROL	5%	10%
MINIMUM TEMPERATURE	14.40	14.30	14.30
MAXIMUM TEMPERATURE	28.60	29.20	28.20
MEAN TEMPERATURE	20.68	21.57	20.72
STANDARD DEVIATION	5.56	6.26	5.61
VARIANCE	30.99	39.13	31.41

Bean plant

TREATMENT	CONTROL	5%	10%
MINIMUM TEMPERATURE	15.00	14.70	14.70
MAXIMUM TEMPERATURE	30.80	30.50	28.70
MEAN TEMPERATURE	21.87	22.61	20.85
STANDARD DEVIATION	6.25	7.13	5.47
VARIANCE	39.08	50.81	29.96

Figure 7.1

TIME	CONTROL	5%	10%
12:00	25.8	26.8	26.4
12:30	26.8	28.4	26.4
13:00	27.4	28.5	26.6
13:30	26.7	27.9	27.5
14:00	27.0	27.5	27.6
14:30	27.5	28.0	27.8
15:00	27.6	28.2	27.9
15:30	28.6	29.2	27.1
16:00	27.3	28.6	28.2
16:30	27.3	28.6	28.0
17:00	27.2	28.5	28.2
17:30	27.4	28.6	28.1
18:00	27.4	28.5	28.2
18:30	18.4	19.7	19.1
19:00	16.7	17.4	17.5
19:30	16.1	16.7	16.8
20:00	15.6	16.0	16.1
20:30	15.4	15.6	15.8
21:00	15.1	15.4	15.4
21:30	15.0	15.2	15.2
22:00	14.8	15.1	15.0
22:30	14.8	14.9	15.0
23:00	14.8	14.8	14.8
23:30	14.7	14.8	14.8
24:00	14.7	14.8	14.6
00:30	14.7	14.8	14.6
01:00	14.7	14.7	14.6
01:30	14.6	14.6	14.5
02:00	14.6	14.6	14.4
02:30	14.6	14.6	14.4
03:00	14.5	14.5	14.3
03:30	14.5	14.5	14.4
04:00	14.5	14.4	14.3
04:30	14.5	14.3	14.3
05:00	14.5	14.3	14.3
05:30	14.4	14.3	14.3
06:00	14.7	14.5	14.4
06:30	22.4	24.6	22.9
07:00	24.2	26.7	24.6
07:30	24.8	27.2	25.1
08:00	24.0	23.8	21.4
08:30	24.3	25.3	23.1
09:00	24.1	26.4	23.7
09:30	24.5	26.7	24.3
10:00	24.9	27.3	24.6
10:30	25.0	27.5	24.8
11:00	25.3	27.7	25.2
11:30	25.3	27.9	25.0
12:00	25.1	27.8	24.9
12:30	25.4	27.8	25.3

Figure 7.2

TIME	CONTROL	5%	10%
12:00	26.6	29.3	27.3
12:30	29.8	29.9	27.7
13:00	29.8	29.7	27.9
13:30	30.5	30.3	26.4
14:00	30.4	30.2	26.2
14:30	30.5	30.4	26.4
15:00	30.8	30.5	26.4
15:30	30.5	30.3	28.7
16:00	26.9	30.3	26.3
16:30	26.9	30.4	26.5
17:00	27.2	30.2	26.6
17:30	27.2	30.3	26.6
18:00	27.2	30.0	26.5
18:30	18.7	18.2	17.6
19:00	17.4	16.9	16.7
19:30	16.8	16.2	16.1
20:00	16.3	15.8	15.9
20:30	16.2	15.6	15.5
21:00	15.9	15.4	15.4
21:30	15.8	15.3	15.2
22:00	15.6	15.2	15.1
22:30	15.6	15.1	15.2
23:00	15.5	15.0	15.0
23:30	15.4	14.9	15.0
24:00	15.4	15.0	15.0
00:30	15.3	14.8	14.8
01:00	15.3	14.9	14.8
01:30	15.2	14.8	14.9
02:00	15.1	14.8	14.8
02:30	15.1	14.8	14.8
03:00	15.0	14.8	14.7
03:30	15.1	14.7	14.8
04:00	15.0	14.7	14.7
04:30	15.0	14.7	14.8
05:00	15.0	14.7	14.8
05:30	15.0	14.7	14.7
06:00	15.5	15.1	15.3
06:30	23.7	28.1	23.9
07:00	24.7	29.2	24.9
07:30	25.0	29.3	25.2
08:00	22.9	26.0	24.4
08:30	24.3	27.6	24.4
09:00	24.5	28.0	24.8
09:30	24.6	28.7	24.9
10:00	24.9	29.2	25.6
10:30	28.6	29.2	25.8
11:00	29.9	29.3	26.1
11:30	29.9	29.2	25.8
12:00	29.7	29.2	25.6
12:30	30.1	29.6	25.9

Statistics - Butter lettuce and Bean plants in the growth chamber two weeks after Vapor Gard application

Recording interval : 00:30:00

Butter lettuce

TREATMENT	CONTROL	5%	10%
MINIMUM TEMPERATURE	14.20	14.60	13.90
MAXIMUM TEMPERATURE	21.90	21.80	19.70
MEAN TEMPERATURE	17.99	18.21	17.02
STANDARD DEVIATION	2.99	2.82	2.40
VARIANCE	8.96	7.97	5.74

Bean plants

TREATMENT	CONTROL	5%	10%
MINIMUM TEMPERATURE	14.70	14.40	14.20
MAXIMUM TEMPERATURE	28.40	28.30	26.50
MEAN TEMPERATURE	21.69	21.60	20.21
STANDARD DEVIATION	5.74	6.01	4.97
VARIANCE	32.98	36.13	24.68

Figure 7.3

TIME	CONTROL	5%	10%
10:00	18.6	20.4	18.3
10:30	19.0	20.3	18.5
11:00	19.4	20.5	18.8
11:30	19.7	20.8	19.1
12:00	20.0	21.0	19.2
12:30	20.2	21.1	19.4
13:00	20.4	21.2	19.4
13:30	20.0	21.1	19.0
14:00	20.4	21.2	19.0
14:30	20.5	21.4	19.4
15:00	20.7	21.4	19.5
15:30	20.8	21.5	19.6
16:00	20.9	21.6	19.6
16:30	21.0	21.6	19.7
17:00	21.2	21.8	19.7
17:30	21.4	21.7	19.7
18:00	21.5	21.7	19.7
18:30	18.4	18.9	16.9
19:00	16.7	17.1	16.0
19:30	16.1	16.6	15.6
20:00	15.6	16.1	15.2
20:30	15.3	15.7	14.9
21:00	14.9	15.5	14.7
21:30	14.9	15.4	14.6
22:00	14.8	15.3	14.5
22:30	14.6	15.1	14.4
23:00	14.7	15.1	14.4
23:30	14.6	14.9	14.3
24:00	14.5	14.9	14.2
00:30	14.5	14.9	14.2
01:00	14.5	14.8	14.2
01:30	14.4	14.8	14.1
02:00	14.3	14.7	14.1
02:30	14.3	14.7	14.0
03:00	14.3	14.6	14.0
03:30	14.3	14.6	14.0
04:00	14.3	14.6	13.9
04:30	14.2	14.6	13.9
05:00	14.2	14.6	13.9
05:30	14.3	14.6	14.0
06:00	14.3	14.6	14.0
06:30	17.9	18.2	17.1
07:00	19.3	19.0	17.7
07:30	19.8	19.1	18.0
08:00	20.7	19.7	18.6
08:30	21.1	19.9	18.9
09:00	21.6	20.3	19.3
09:30	21.5	20.3	19.3
10:00	21.6	20.5	19.4
10:30	21.5	20.1	19.5

Figure 7.4

TIME	CONTROL	5%	10%
10:00	27.3	26.8	24.7
10:30	27.1	27.3	25.1
11:00	27.7	27.4	25.4
11:30	27.7	27.5	25.7
12:00	27.7	27.3	25.7
12:30	27.6	27.4	25.6
13:00	27.5	27.3	25.6
13:30	23.9	23.4	22.8
14:00	27.4	24.3	23.4
14:30	27.7	27.5	23.9
15:00	27.7	27.7	25.1
15:30	27.9	27.8	25.3
16:00	28.1	27.9	25.6
16:30	28.3	28.0	26.2
17:00	28.3	28.0	26.4
17:30	28.4	28.3	26.4
18:00	28.4	28.2	26.5
18:30	20.2	18.7	18.5
19:00	17.8	16.7	16.3
19:30	17.0	16.1	15.8
20:00	16.4	15.7	15.5
20:30	16.1	15.4	15.2
21:00	15.8	15.3	15.0
21:30	15.6	15.1	14.9
22:00	15.5	15.1	14.8
22:30	15.3	14.9	14.7
23:00	15.3	14.9	14.7
23:30	15.2	14.8	14.6
24:00	15.1	14.8	14.5
00:30	15.0	14.8	14.5
01:00	15.1	14.7	14.6
01:30	14.9	14.6	14.5
02:00	14.8	14.6	14.4
02:30	14.8	14.6	14.4
03:00	14.8	14.6	14.3
03:30	14.8	14.5	14.3
04:00	14.7	14.5	14.3
04:30	14.7	14.4	14.2
05:00	14.7	14.4	14.2
05:30	14.7	14.5	14.3
06:00	14.7	14.5	14.3
06:30	24.4	24.4	23.1
07:00	25.6	25.9	23.8
07:30	24.7	25.9	23.4
08:00	24.9	26.6	24.1
08:30	24.7	26.9	23.9
09:00	25.8	27.1	23.9
09:30	25.5	27.0	23.7
10:00	25.7	27.1	23.8
10:30	26.5	27.3	23.6

Statistics - Butter lettuce in the glasshouse two weeks after Vapor Gard application

Recording interval : 00:30:00

Butter lettuce

TREATMENT	CONTROL	5%	10%
MINIMUM TEMPERATURE	17.00	18.10	18.20
MAXIMUM TEMPERATURE	20.90	22.00	23.00
MEAN TEMPERATURE	18.78	19.74	19.69
STANDARD DEVIATION	0.90	0.88	1.46
VARIANCE	0.82	0.79	2.12

Figure 7.5

TIME	CONTROL	5%	10%
11:30	20	20.7	20.9
12:00	20.4	21.2	21.7
12:30	20.5	21.5	22
13:00	20.9	22	22.7
13:30	20.7	21.9	22.8
14:00	20.7	22	23
14:30	20.4	21.2	22.8
15:00	20.2	20.6	22.2
15:30	19.4	19.9	21.6
16:00	18.9	19.3	21.6
16:30	18	18.4	21.8
17:00	19.7	19.8	21.6
17:30	19.1	19.3	20.6
18:00	18.2	18.5	19.5
18:30	19	19.8	19.2
19:00	19.6	20.6	19
19:30	18.2	19	18.9
20:00	18.1	18.5	18.7
20:30	18.6	19.4	18.4
21:00	18.2	19.5	18.3
21:30	18.2	19.7	18.5
22:00	18.3	19.7	18.4
22:30	18.1	19.5	18.3
23:00	17.9	19.3	18.3

TIME	CONTROL	5%	10%
23:30	18.1	19.5	18.4
24:00	18.3	19.6	18.5
00:30	18.3	19.6	18.6
01:00	17.7	18.8	18.2
01:30	18.3	19.4	18.6
02:00	18.5	19.5	18.9
02:30	18.5	19.6	19
03:00	18.7	19.6	19
03:30	18.6	19.5	19
04:00	18.5	19.6	19
04:30	18.5	19.5	18.9
05:00	18.5	19.5	18.8
05:30	18.4	19.4	18.9
06:00	18.4	19.4	18.8
06:30	18.4	19.4	18.7
07:00	18.4	20	18.8
07:30	18.3	19.5	18.8
08:00	19.1	20.1	19
08:30	17.1	18.1	18.7
09:00	17	18.2	19
09:30	18.1	19.3	19.3
10:00	18.4	19.4	19.4
10:30	18.6	19.4	19.8
11:00	19	19.7	20.3