TRANSPIRATION AND THE WAXY BLOOM IN *BRASSICA OLERACEA* L.

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[*Manuscript received June* 30, 1969]

Summary

The stomatal and cuticular transpiration rates and quantity of wax per unit area of leaf surface were determined for seven glaucous and non-glaucous sibling lines of *B. oleracea.* There were no statistically significant differences in the stomatal transpiration rates of the glaucous and non-glaucous lines, but there were highly statistically significant differences between the two classes of lines in terms of cuticular transpiration. Rubbing the surfaces of the leaves to remove lightly adhering wax deposits, such as the waxy bloom, significantly increased the cuticular but not the stomatal transpiration rates of both glaucous and non-glaucous plants. There was no appreciable correlation between the quantity of wax per unit area of leaf surface and the loss of water through cuticular transpiration among either the glaucous or non-glaucous lines. It was concluded that the function of cuticular waxes in limiting cuticular transpiration is a product of the architecture of the deposits and the quantity of wax per unit area of leaf surface.

I. Introduction

According to Warth (1956) many species of plants representing many different families are glaucous. Such plants are characterized by the presence of a waxy bloom on the surface of their leaves and stems which imparts a bluish, whitish, or greyish cast to the foliage. The bloom is a result of light scattering from extracuticular wax particles or platelets (Hall *et al.* 1965).

Schieferstein and Loomis (1956) concluded from their work with cabbage (*Brassica oleracea*) plants that waxy blooms are not a factor in moisture conservation in plants; they were of the opinion that a continuous cuticular layer, rather than the bloom *per se*, is the major factor in water conservation. However, these conclusions do not agree with the results of Hall and Jones (1961) with detached clover leaves from which the bloom had been removed with a brush. Hall and Jones found that the removal of the bloom resulted in a greater rate of cuticular transpiration. One could argue that their results apply to detached leaves but not to intact plants, or by physically removing the bloom an underlying continuous wax layer was disturbed, or to both factors. By determining the transpiration rates of genetically glaucous and non-glaucous whole plants, some of which had been rubbed so as to simulate the brushing of Hall and Jones, it was hoped to obtain additional information regarding the possible function of waxy blooms in limiting transpiration.

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II. Materials and Methods

(a) *Inheritance of the Non-glaucous Condition*

Seven sets of glaucous and non-glaucous sibling segregates were selected from seven different F_2 populations. The inheritance of the non-glaucous character has been previously reported for the genes in population BR-2 (Anstey and Moore 1954), BS-1 (North and Priestley 1962), and KA-1 (Thompson 1963). Preliminary breeding studies, based upon limited F_1 and F_2 phenotypic ratios, indicate that in all populations the non-glaucous condition is associated with a single Mendelian factor. In populations BR-1 and CO-1, the non-glaucous condition is dominant to the glaucous; in all other populations the non-glaucous condition is recessive. The possible allelism of the various non-glaucous genes has not been determined. The sources of the various non-glaucous genes are to be found in Section V.

(b) *Plant Growing Conditions*

The seed was planted May 16 and the transpiration experiments were carried out in the period August 11–21, 1967, at Fort Collins, Colorado, U.S.A. The seedlings were first transplanted into small peat pots and when larger into 6-in. clay pots. At each transplanting stage, and before the actual transpiration experiments, rigid selection was done to obtain uniform plants. All plant material was grown in a greenhouse equipped with evapocoolers in order to be able to use fumigants, rather than sprays or dusts, for insect control.

(c) *Transpiration Measurements*

Three days before the actual transpiration measurements were to be made, the old, yellowing, lower leaves on the plants were removed with a razor-blade. One hour before the start of an experiment the soil in the pots was saturated with water and allowed to drain for 30 min. The pots were then wiped dry and wrapped in sheets of black polyethylene in such a way as to form a vapour-tight envelope from which the shoot of the plant protruded. Water loss was measured by weighing the plants, beginning 1 hr after watering and then at the end of the experiment. The cuticular-transpiration experiments were carried out the night preceding the stomatal-transpiration experiments in a completely darkened, ventilated room. The stomatal-transpiration experiments were all carried out in the greenhouse from 9.00 a.m. until approximately 2.00 p.m.; preliminary experiments indicated that a longer period would result in noticeable wilting of some plants. The plants were divided into four groups and each group was measured on a different day. In separate groups were the plants of the broccoli populations (BR-1, BR-2), Brussel sprout populations (BS-1, BS-2), cauliflower populations (CA-1), and the collard and kale populations (CO-1, KA-1).

The removal of the waxy blooms, or the simulated removal in the case of the non-glaucous plants, was accomplished by gently rubbing the leaf surfaces with wads of cheesecloth; the glaucous plants appeared non-glaucous following the treatment. No readily observable lesions were observed on any of the leaves following the rubbing operation.

(d) *Atmospheric Conditions*

The greenhouse ventilating fans were turned off for the period of each stomatal-transpiration experiment. Wind was absent from both the stomatal and cuticular experiments. The sky was sunny and clear for all the stomatal experiments; cloud cover was very sparse. During the four stomatal-transpiration experiments the temperature varied between 24 and 36°C and the relative humidity between 26 and 57%. In the cuticular-transpiration experiments the temperature varied between 22 and 34°C and the relative humidity between 43 and 54%.

(e) *Leaf Measurements and Wax Extraction*

The surface area of the leaves of each plant was determined by tracing the outlines of the leaves on uniform sheets of paper, cutting out the tracings, and weighing together those cutouts which represented a particular plant. The condition of the stomatal aperatures during the daytime and night-time experiments was determined by using the Silicone rubber impression technique of Zelitch (1961).

The wax was extracted from the surface of the leaves by dipping them for 10 sec in each of three successive beakers of diethyl ether. This method of extraction has been reported by Purdy and Truter (1963) to remove better than 90% of the cuticular wax without also extracting sub-epidermal cell materials. The contents of the three beakers were combined, filtered (Whatman No. 1), reduced in volume in a rotary evaporator, and reduced to dryness in an evaporating dish following several days in an air stream. The resulting dry weights of ether-extracted material was used to calculate the average quantity of wax per unit area of leaf surface for the non-rubbed plants. In the case of the rubbed plants, only the remaining wax on the BS-1 glaucous leaves was determined.

(*f*) *Experimental Design*

The experimental design was a series randomized complete blocks with five replications.

III. Results

Impressions of the stomatal apertures of three randomly selected plants in each daytime experiment were determined and found to be fully open. Similar sampling procedures carried out on the plants in the night-time experiments demonstrated that the stomatal apertures were closed. This supports the assumption that the major loss of water in the daytime experiments was through stomatal transpiration, while in the night-time experiments it was through cuticular transpiration.

Table 1

STOMATAL AND CUTICULAR TRANSPIRATION IN RELATION TO THE PRESENCE OR ABSENCE OF A WAXY BLOOM AND THE QUANTITY OF WAX IN GENETIC LINES OF *BRASSICA OLERACEA*

N, non-rubbed; R, rubbed

Line	Treatment	Av. Water Loss in Stomatal Transpiration (mg/cm^2)		Av. Water Loss in Cuticular Transpiration (mg/cm^2)		Average Quantity of Wax (μg/cm^2)	
		Glaucous	Non-glaucous	Glaucous	Non-glaucous	Glaucous	Non-glaucous
BR-1	N	78·2	92·3	18·5	23·0	37·8	6·8
BR-1	R	80·9	89·0	18·2	27·7	—	—
BR-2	N	73·3	75·5	15·4	17·2	47·4	41·0
BR-2	R	80·8	76·4	15·8	19·2	—	—
BS-1	N	48·9	53·4	6·1	8·6	30·6	10·8
BS-1	R	50·6	56·6	6·4	10·3	—	—
BS-2	N	58·7	55·6	4·6	8·1	25·4	11·3
BS-2	R	56·2	61·1	6·1	10·0	—	—
CA-1	N	50·6	55·6	11·4	16·5	31·1	12·2
CA-1	R	57·2	57·6	13·4	19·5	—	—
CO-1	N	54·6	62·3	10·9	16·7	27·9	8·0
CO-1	R	65·0	64·1	10·5	19·9	—	—
KA-1	N	59·5	61·6	10·7	18·6	32·8	5·3
KA-1	R	57·6	—	10·4	—	—	—
Av.	N	60·5	65·2	11·1	15·5	33·3	13·6
Av.	R	64·0	67·5	11·5	17·8	—	—

(*a*) *Stomatal Transpiration*

There was a trend for the non-glaucous plants to have a slightly greater rate of stomatal transpiration than the glaucous (Table 1), but the differences were not statistically significant (5% level). The rubbed plants also appeared to lose more water through stomatal transpiration than the non-rubbed (Table 1), but, again, the differences were not statistically significant. There was no appreciable correlation ($r = 0 \cdot 32$) between the stomatal transpiration and the average quantity of wax per unit area of leaf surface.

(*b*) *Cuticular Transpiration*

In every case the non-glaucous siblings lost more water through cuticular transpiration than their glaucous counterparts (Table 1); the differences were statistically highly significant (1% level). The rubbing of the leaf surfaces to remove or disturb the bloom had a statistically highly significant effect on increasing cuticular transpiration (Table 1). However, there did not seem to be an overall close association between the average quantity of wax per unit area of leaf surface and cuticular transpiration ($r = 0 \cdot 15$) (Table 1). Interestingly both the non-glaucous and glaucous plants had an average cuticular water loss that represented approximately 9% of the average stomatal transpiration for comparable plants.

IV. Discussion

(*a*) *Stomatal Transpiration*

The trend for the non-glaucous plants to have a higher rate of stomatal transpiration than their glaucous siblings would seem to be of interest—particularly since the difference was almost statistically significant at the arbitrary 5% level. A similar trend was observed in the case of the rubbed plants; they appeared to have a higher rate of stomatal transpiration as a result of the rubbing treatment. The trend was almost statistically significant at the 5% level. One wonders if the cuticle may play a greater role in stomatal transpiration in *B. oleracea* than has been hitherto realized.

(*b*) *Cuticular Transpiration*

It is clear from the results of the cuticular-transpiration experiments that the presence of a waxy bloom is associated with a reduction in the rate of water loss by intact plants. The observed increase in the cuticular transpiration of glaucous plants upon removal of their waxy bloom by rubbing with cheesecloth extends the earlier observations of Hall and Jones (1961) with detached clover leaves to whole plants. However, the cuticular transpiration rate increase was also observed in the case of the non-glaucous plants which were rubbed. It is known that the non-glaucous line BS-1 does not have a waxy bloom (North and Priestley 1962) and it would seem to be the case with the other non-glaucous lines as well. If this is so, then it appears that the rubbing operation is responsible for doing more than just removing the bloom, and that experiments of this type are incapable of separating out the effect of the waxy bloom *per se* on transpiration.

The results of Daly (1964), which suggested that the waxy bloom in populations of the grass *Poa colensoi* was associated with the aridity of the field environment, are supported by the results of the cuticular-transpiration experiments. Under conditions of high night-time moisture stress, one would expect the glaucous phenotype to have greater survival and reproductive potential than the non-glaucous phenotype. But this is not to say that the only difference between the glaucous and non-glaucous plants is the presence and absence, respectively, of a waxy bloom.

Possingham *et al.* (1967) suggests that the surface wax, which consists of overlapping platelets that are hydrophobic in nature, may be important in controlling cuticular transpiration, while Schieferstein and Loomis (1956) contend "—that a continuous cuticular layer, rather than scattered surface deposits, is the major factor in water conservation by epidermal cells." The relative contribution of the two wax deposits to limiting water loss through the cuticle will probably require detailed physical analysis of the layers together with associated transpiration measurements.

V. Acknowledgments

The author wishes to thank the following for supplying the various non-glaucous genetic lines used in the study: Dr. D. R. Sampson, Ottawa Research Station, Canada Department of Agriculture, Ottawa, Canada, for line BR-2; Dr. J. D. Atkins, Keystone Research Department, Hollister, California, for lines BR-1, CO-1; Dr. D. Dolan, USDA Plant Introduction Station, Geneva, New York, for line CA-1; Dr. C. North, Scottish Horticultural Research Institute, Mylnfield, Invergowrie, Dundee, Scotland, for lines BS-1, BS-2; and Dr. K. F. Thompson, Plant Breeding Institute, Maris Lane, Trumpington, Cambridge, England, for line KA-1.

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