WAX DEPOSITS ON LEAF–SURFACES OF TWO CLONES OF *PANICUM REPENS* GROWN IN CONTRASTING ENVIRONMENTS *)

SOEDHAROEDJIAN RONOPRAWIRO **)

RINGKASAN

Suatu penelitian mengenai besarnya endapan lilin yang terdapat pada permukaan daun *Panicum repens* telah dilakukan pada 2 klon yaitu klon Bogor (Bo) dan klon Rawa Pening (RP) yang tumbuh dalam dua lingkungan hidup yang berbeda.

Dalam keadaan "sejuk" endapan lilin pada permukaan daun ternyata lebih banyak daripada dalam keadaan "panas" dan pada daun yang lebih "tua" terdapat endapan lilin yang lebih besar daripada daun yang "muda".

Perbedaan banyaknya endapan lilin pada permukaan daun muda dan daun tua lebih nyata pada klon RP, dibanding dengan pada klon Bo dan keadaan lingkungan yang sejuk rupanya memperbesar perbedaan ini. Perbedaan banyaknya endapan lilin di permukaan daun pada klon RP dan Bo mungkin lebih ditentukan oleh perbedaan umur sebenarnya daripada daun-daunnya.

SUMMARY

The wax deposits on leaf surfaces of two clones of *Panicum repens*, the Bogor clone (Bo) and Rawa Pening clone (RP), grown in two contrasting environments were studied.

Grown in a "cool" environment there was more wax deposit on the leaf surfaces then if it was grown in a "warm" environment and there was also more wax deposit on the surfaces of "old" leaves as compared to "young" leaves.

The difference in wax deposition on leaf surfaces of "young" and "old" leaves is more striking for the RP clone then for the Bo clone and it seems that a "cool" environment accentuate this difference.

The actual age of the leaves or shoots might be a more important factor for the difference of wax deposit on the leaf surfaces of the RP clone and the Bo clone.

INTRODUCTION

One of the factors that may interfere with herbicide retention, penetration and eventually performance is the cuticle structure of the plant. Wax is an important component of the cuticle, which is embedded within and sometimes exuded over the surface of the cuticle. Their structure, arrangement and amount may vary greatly with each species. The wax bloom of the cabbage leaf (*Brassica oleraceae var capitita*) is due to ubiquitous wax rodlets 1 – 2 um long and 0,5 – 1,0 um in diameter, and the bluish bloom of the leaves of certain varieties of *Eragrotis curvula* is attributed to the effect of wax exudates

*) Presented in the Fifth Indonesian Weeds Conference, at Malang, Indonesia, 1979.

**) Department of Agronomy, Faculty of Agriculture, Gadjah Mada University.

in the form of flat branched ribbons (Leigh & Matthews, 1963 cit Martin and Juniper, 1970). The leaves of the glossy mutants of *Pisum sativum*, cauliflower, *Eucalyptus urnigera* and *Poa colensoi* have wax deposits which are either smooth films on the cuticle or platelets which lie flat on the surface, where as the waxes of the leaves of the normal plants have the shape of rods or filaments growing outwards (Martin and Juniper, 1970).

Environmental conditions are thought to be closely related to the degree of waxiness and cuticle thickness but conflicting reports have been obtained. Mc. Nair (1931) pointed out that more wax-producing plant families are found in the tropics than in temperate zones and Kurtz (1958), reported that the majority of the xerophytes and succulents of S. Arizona (N. America) contain only small amounts of wax (cit. Martin and Juniper, 1970).

Considering that Hulsbruch (1966) has shown that the thickness of cuticle on stems and leaves of *flex integra* increases markedly with age, the amount of wax on young and mature leaves is likely to differ too. This phenomena may play an important role in leaf-wettability, spray retention and herbicide penetration although it must be recognized that many other features of leaf surface, e.g. wax-structure and particularly its chemical nature, may be more important (Holloway, 1969 & 1970).

Tropical grasses have been neglected in this type of investigation and it was considered valuable to have some information on the quantity of wax and general nature of the leaf surface especially in relation to the investigations on the performance of herbicides.

In this experiment the wax deposits on leaf surfaces of young and mature leaves of two clones of *P. repens* grown in two different environments were investigated.

MATERIALS AND METHODS

Two clones of Panicum repens were used in this experiment, the Bogor clone (Bo) and the Rawa Pening clone (RP).

All the plants were grown in peat from single node stem fragments. The plants were divided into two sets and grown in a temperate and tropical greenhouse, where the temperature and humidity prevailing during the experiment was between $15^{o} - 20^{o}C$, 04 – 60% R.H. and $20^{o} - 28^{o}C$, 55 –70% R.H. respectively.

The plant in the temperate greenhouse were not disturbed until the experiment started and had a dwarfappearance. The stalks were somewhat erect with short internodes and the RP clone was taller than the Bo clone. As the plants in the tropical greenhouse made very luxurious growth, they were cut at about 3 months after planting. The regrowth that developed was rather thin and had long stalks, much longer than the stalks of the plants in the temperate greenhouse.

Two leaf-positions were taken, a) the first fully expanded leaf and b) the fifth fully expanded leaf, which generally is the second leaf from the soil surface for both clones in both environments.

The leaves were taken from each position for each clone in the two different growing conditions to represent one replicate of each sample. Three replicates were taken in this experiment.

Each batch of leaves was first weighed and the leaf areas were then measured by weighing matching graph paper replicas of the leaves and calculating it to total surface area. After wax removal the leaves were dried and weighed.

Each batch of was washed twice in 25 ml of chloroform for 10 – 15 seconds and the two washings were combined in an Erlenmeyer flask for further evaporation in a rotary-evaporator with a water-bath temperature of $35^{o} - 38^{o}C$ until only about 2 ml was left. It was then poured into a 25 ml beaker, and the evaporating flask was twice washed with 2 ml chloroform. The washings were added into

the beaker. The beakers were placed in an incubator at 35°C with open ventilation for further evaporation until dryness.

The difference between the weight of the beaker plus sediment and the clean beaker is considered to be the weight of wax on the leaf surfaces.

RESULTS

The wax deposits on the leaf-surface were expressed as wax per fresh or dry weight in ug/mg and wax per leaf area in ug/cm2. The grand means of the bulk leaves obtained in this investigation were as follows.

a. wax per fresh weight = 2.88 ug/mg
b. wax per dry weight = 13.79 ug/mg and
c. wax per leaf-area = 0,035 ug/cm2, upper and lower surface.

Under cool growing condition the wax deposits on the leaves were significantly greater than those on plants grown in the tropical greenhouse, as shown in table 1.

Table 1. WAX DEPOSITS ON *P. REPENS* LEAVES AS AFFECTED BY WARM AND COOL GROWING CONDITIONS

Units of wax	Warm	Cool	LSD 5%
Wax/fresh weight in ug/mg	2.64	3.13	0.32
Wax/dry weight in ug/mg	12.43	15.14	1.73
Wax/leaf-area in ug/cm2	0.0312	0.0384	0.0039

There was significantly more wax deposit on the basal or older leaves than on the younger leaves (see table 2).

Table 2. WAX DEPOSIT ON APICAL (YOUNG) LEAVES AND BASAL (OLD) LEAVES OF *P. REPENS*.

Wax units	Apical leaf	Basal leaf	LSD 5%
Wax per fresh weight-in ug/mg	2.31	3.45	0.32
Wax per dry weight-in ug/mg	10.99	16.58	1.73
Wax per leaf-area in ug/cm2	0.0276	0.0420	0.0039

The greater amount of wax deposits on the older leaves occurs on both clones, but the difference between the amount on the young and old leaves for the RP clone was more striking than for the Bo-clone (see table 3).

Table 3. WAX DEPOSITS ON APICAL AND BASAL LEAVES OF TWO CLONES OF *P. REPENS.*

Wax units	RP clone		Bo clone		LSD 5%
	Apical leaf	Basal leaf	Apical leaf	Basal leaf	
Wax/f.wt in ug/mg	2.14	3.69	2.48	3.21	0.46
Wax/dr.wt in ug/mg	9.80	18.36	12.81	14.81	2.44
Wax/leaf-area ug/cm2	0.0246	0.0444	0.0306	0.0396	0.0056

The same trend was true for both clones grown under warm and cool condition. There was no significant difference of wax deposition on the apical and basal-leaves of the Bo-clone under warm condition and under cool condition this difference was just significant. The RP clone showed a significant difference in wax deposition on young and old-leaves under both growing condition. An interesting point here is that the young leaves of the Bo-clone had a greater wax deposition than the young leaves of the RP clone. This difference was significant under cool condition but not so for the warm condition (see table 4).

Table 4. WAX DEPOSITION ON APICAL AND BASAL LEAVES OF TWO CLONE OF *P. REPENS* GROWN UNDER WARM AND COOL CONDITION (WAX /LEAF AREA - UG / CM^2)

Clone	Warm		Cool		LSD
	Apical leaf	Basal leaf	Apical leaf	Basal leaf	
RP	0.0262	0.0354	0.0230	0.0534	0.0079
Bo	0.0292	0.0339	0.0320	0.0453	

D I S C U S S I O N

Lee and Priestly (1924) (cit Martin and Juniper, 1970) concluded that species growing under highlight intensities or with reduced moisture contents develop thick cuticles and plants indigenous to arid and hot regions have been believed to have waxy cuticles. Based on these it was expected that the leaves of the plants from the tropical greenhouse will have more wax than the leaves form the plants in the temperate greenhouse, but in this experiment a greater amount of wax deposit was obtained for the leaves of plants grown in the temperate greenhouse. It seems in this case, that there is an interaction between the environment and age of the shoots. The actual age the shoots of plants grown in the tropical

greenhouse were only about three months old. However, the shoots had comparable numbers of leaves, so while leaf position was similar, leaf-age was different. It would be more accurate to say in this case, that the age of the shoots, had to be accounted for to this result, more than to the environment. It might be that the cuticle and wax development of *P. repens* has the same tendency as *Ilex integra* (Hulsbruch, 1966, cit Martin and Juniper, 1970), where the thickness of the cuticle on leaves increases markedly with age.

It is clear that wax deposition on older leaves was much more than on younger leaves in both clones. Especially the older leaves of the RP clone under cool environment had a distinct high wax deposition value, while the younger leaves wax deposition value was quite low. In the tropical greenhouse the same clone did not show a very great difference in wax deposition between the older and the younger leaves although the difference is still significant.

The Bo-clone showed the same tendency, but the difference was less pronounced, and on the young shoots grown in the tropical greenhouse this difference was not significant. This indicated that the wax deposition on the Bo-clone, from young to older leaves on a shoot has less gradient than on the RP clone.

Another interesting point is that the wax deposition on young leaves of the Bo-clone was greater than on the RP clone and this difference was even significant on the older plant grown in the temperate greenhouse. The possible consequence that arise from this situation is that the younger parts of the Bo-clone are covered with more wax than the RP clone which in turn may interfere with spray retention, and herbicide performance.

ACKNOWLEDGEMENT

This work has been done at the W.R.O., Oxford England and the author is very much indebted to Dr. J.C. Caseley at the Environmental Laboratory for his guidance and corrections. Also to all staff members of the Environmental Lab. the author wishes to express his gratitude for their cooperation.

REFERENCES

Holloway, P.J. (1969) Chemistry of Leaf waxes in relation to weetting. *J. Sci Fd Agric.* 20,124–128.

Holloway, P.J. (1970) Surface factors affecting the weeting of leaves. *Pestic. Sci.* 1. 156–163.

Martin, J.T. and Juniper, B.E. (1970) The cuticles of Plants. Edward Arnolds (Publishers).