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WATER UPTAKE FROM FOLIAR-APPLIED DROPS AND ITS FURTHER DISTRIBUTION IN THE OAT LEAF*

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SUMMARY

A fraction (12%) of the water from tritium-labelled foliar-applied drops rapidly penetrates through the cuticle and, after transport through the mesophyll, is distributed in an acropetal direction and probably through the xylem, over the oat leaf. The initial rapid penetration is favourably affected by a water "continuum" that is always formed between tissue and drop, immediately after drop application. It is suggested that the pathways for the creation of this water continuum are the ectodesmata in the guard cells of the stomata. Increasing the calciumchloride concentration of the applied drops has little influence on the water uptake from these drops. Higher salt content of particular parts of the oat leaf increases the water retention by these parts. In the present experimental conditions (60–70% relative humidity) aqueous vapour uptake from the ambient air is small (1,7% of the amounts evaporated), as compared to the uptake from applied drops. Tritium from foliar-absorbed water does not combine with stable compounds (mainly organic) in the leaf.

1. INTRODUCTION

Uptake of water through the leaf surface (dew, mist, rain, sprinkling irrigation) has mainly been studied in respect of its possible contribution to the water economy of plants (KRAUSE 1935; SLATYER 1958; STONE *et al.*, 1956; VAADIA & WAISEL 1963; WAISEL 1958). From such studies and related ones (PLAUT & REINHOLD 1967; WOOD 1925) a rather consistent picture has been obtained of the uptake of foliar-applied water, of its further distribution in the leaf, and of the main factors (cuticle, light, temperature etc.) affecting both processes. Information, however, about, on the one hand, the role of water in the foliar ion uptake process and, on the other hand, the effect of foliar-applied drops and higher mineral content of the leaf on the internal water distribution is scarce.

Therefore, the first purpose of the present study was to measure the amounts of water absorbed from foliar-applied drops of different salt ($CaCl_2$) concentration, as well as its further distribution in the leaf. Furthermore, the influence of the presence of more drops on the leaf surface and of an increased calcium content of the leaf tissue on foliar water uptake and its internal distribution was studied.

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2. MATERIALS AND METHODS

Oat plants (*Avena sativa* L., cv. 'Marne') grown for 20 days on an aerated complete mineral solution (RINGOET *et al.* 1967), were used for the experiments in a climate-controlled growth chamber (35.000 lux, 20 °C, 65 % relative humidity). The air circulation and filtering (VOKES absolute filters) system of the growth chamber assured a continuous removal of the evaporated water from the direct surroundings of the experimental plants.

In all experiments leaves of about 25 cm length and of the same age, according to their position on the plant, were selected (5 or 7 replicates per treatment). The median parts of these leaves were fixed *(fig. 1)* to a polystyrene aluminium-foil covered supporting table. Drops containing 0.01 % Tween 20 were locally applied to the upper surface, over the costa, of the median leaf part. The wetting agent was added to avoid rolling down of the applied drops and to adapt the experimental conditions to the generalized use of surfactants in the spraying-practice. According to preliminary experiments the addition of the wetting agent Tween 20 resulted over the whole experimental period but, of course, mainly during the first hours in a 40 % uptake increase.

In a first series of experiments the time-related uptake and the further distribution of tritiated water in the oat leaf were studied as a function of the $CaCl_2$-concentration of the foliar-applied drops (75 µl; 0,05 µCi ^3H; $CaCl_2$: from 0 up to 10^{-2}M). Samples were taken $\frac{1}{4}$, $\frac{1}{2}$, 1, 2, 3, 4, and 5 hours after the start of the experiment (drop application). This sampling concerned the remaining drop volumes, the fraction of tritiated water adsorbed to the leaf surface (in a limited number of experiments), the ^3H_2O-fraction in the apparent free space (A.F.S.),

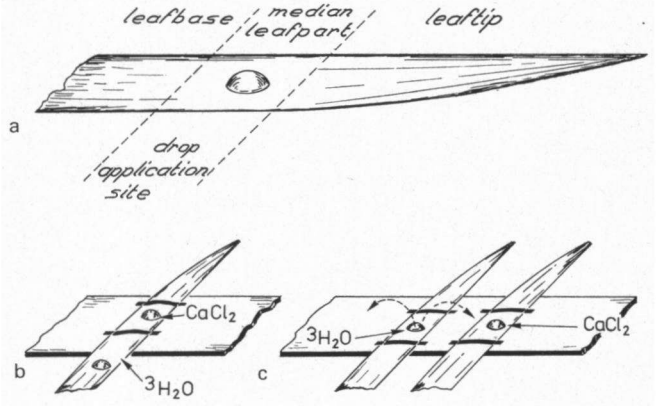

Fig. 1. Experimental set-up; (a) drop application site on median leaf part, leaf parts harvested for ^3H_2O uptake and transport measurements; (b) "double drop on one leaf" set-up; (c) "double leaf" set-up (see text).

the leaf part directly underneath the application site of the drop, different parts of the leaf tip and base *(fig. 1a)*. The adsorbed ^3H_2O-fraction was obtained by surface washing with demineralized water for 10 sec. The A.F.S.-fraction was measured by the amount of tritiated water removed from the leaf part underneath the application site by its immersion for 2 to 3 min in demineralized water. To avoid washing-out from the inner tissues, the cut edges of this leaf part were coated with paraffin wax.

In a second series of experiments the influence of the presence of other non-labelled drops of different $CaCl_2$ concentration and the effect of an increased calcium content of particular leaf parts on the uptake of ^3H_2O from foliar-applied drops and on its internal distribution after absorption were studied.

According to the results of the first series of experiments, a similarity most probably exists – after the initial penetration through the mesophyll – between the internal pathways of root and foliar absorbed water. Therefore the high amounts of the tritium label that would have been needed when in the second series of experiments tritiated water was applied to the roots, could be avoided by the use of a 'double drop on one leaf' technique. One drop, near the leaf base, ^3H-labelled (1 μCi ^3H), and another of varying calciumchloride concentration (from 0 up to 5.10^{-2}M), were either simultaneously or at a certain time interval (3 to 4 hours) applied to the upper surface of the median leaf part (4 cm distance; *fig. 1b*). Application of $CaCl_2$-drops indeed results after 3 to 4 hours in a considerable increase of the calcium content of the leaf tissue underneath the drop and in the tip (RINGOET *et al.* 1967). Sampling was done as in the first series of experiments.

Direct absorption of tritiated aqueous vapour, produced by evapotranspiration, from the leaf surrounding atmosphere may interfere with the measurement of water uptake and further distribution from drops. Water vapour absorption was estimated by experiments using 'a double leaf technique' *(fig. 1c)*. One leaf received a drop of tritiated water and was sampled as described before. A second leaf was situated on the same supporting table in such a position that parts of leaf tip and base could be representatively sampled for estimating the ^3H_2O-vapour absorption.

The samples of 'washing' liquid collected in counting vials were directly measured by liquid scintillation ß-spectrometry, using a dioxane-cellosolve scintillation liquid similar to the one proposed by BRUNO & CHRISTIAN (1961). The plant material rapidly cut into small pieces and the drops absorbed by small strips of kleenex tissues were treated according to the MAHIN & LOFBERG (1966) technique.

Counting efficiencies, 70 and 13 % respectively in the dioxane-cellosolve and toluene-cellosolve scintillation liquids, were rather low, but the balance of all countings with respect to the total amount of ^3H applied was generally correct within the 10 % limits.

Because of the mass-differences between hydrogen and tritium the results are considered to give essentially a qualitative picture of the water uptake and distribution process in oat leaves.

3. RESULTS

3.1. Time-related uptake and further distribution of tritiated water in the oat leaf

3.1.1. Time-related ^3H_2O uptake from foliar-applied drops

Evapotranspiration, uptake by the leaf from the drop and remaining drop volume, in relation to time after application and in percentages of the total amount initially applied, are represented by the graph in *fig. 2*. The experimental points are average values calculated from different experiments with various treatments (e.g. salt concentration of the drop), which will later be considered more in detail.

As expected, the major fraction of the tritiated water directly evaporates from the drop. After the first half hour about 12% of the total amount applied has been taken up by the leaf. Further uptake is increasingly compensated for

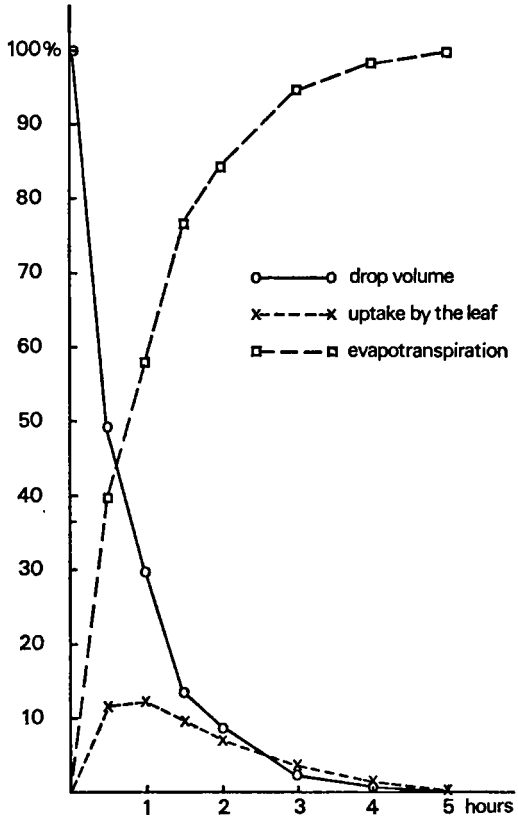

Fig. 2. Evaporation, remaining drop volume, and uptake by the leaf in relation to time after application (in percentages of the total amount initially applied)

by evapotranspiration from the leaf. After 5 hrs only trace-amounts of ^3H_2O are left over in the leaf.

Experimental data point to an immediate start of the water uptake by the leaf at a rate which – neglecting evapotranspiration from the leaf during this early stage after the drop application – is apparently rather high.

3.1.2. Distribution of tritiated water in the leaf

3.1.2.1. Foliar-applied drops of pure tritiated water.

The graph in *fig. 3* represents the distribution of tritiated water in different parts of the oat leaf (% of the total amount absorbed) in relation to the time after the application of the drop. Uptake is maximal in the median leaf part directly under the application site. Uptake in the leaf tip is delayed with respect to the absorption by the median part, suggesting internal (xylem) transport to the tip after initial penetration and movement through the leaf mesophyll.

Transport to the leaf base is negligible; the small amounts found in that leaf part may be due to direct absorption from the ^3H_2O-vapour atmosphere surrounding the experimental leaf (see below).

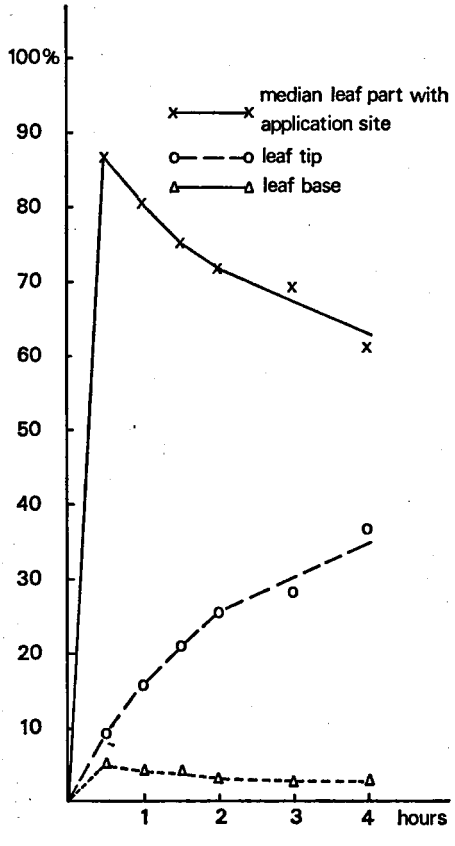

Fig. 3. Distribution of tritiated water in different parts of the oat leaf in relation to the time after the application of the drop (percentages of the total amo untabsorbed).

After the first half hour the relative ^3H_2O-content of the median leaf part rapidly decreases. This decrease is due to evapotranspiration and to transport to the leaf tip. The increase of the ^3H_2O tip-fraction shows that translocation to the tip is relatively more important than the evapotranspiration from this leaf part.

3.1.2.2. Foliar-applied drops of different $CaCl_2$-concentration.
During the first hours of the experiment no influence of drop concentrations of 2.10^{-4} up to 3.10^{-2}M $CaCl_2$ on the uptake and leaf distribution of tritiated water was observed.

The data in *table 1* show that after 3 or 4 hrs relatively more ^3H_2O is retained by drops of intermediate $CaCl_2$ concentration, which appear to dry out more slowly (see below). Low concentration ($\pm 10^{-4}$M) does not affect the retention. Reduced retention at initial concentrations of 10^{-1} to 10^{-2}M $CaCl_2$ may result from necrosis of the leaf surface by the high salt concentration of almost dried-out drops (RINGOET *et al.* 1967).
As after 3 and 4 hrs only small amounts of water are left, the overall effect of increased calcium concentration of the drops on ^3H_2O uptake by the leaf is negligible.

3.1.3. Uptake of tritiated aqueous vapour from the air surrounding the leaf

Evaporation of the ^3H_2O-drops creates a tritiated aqueous vapour atmosphere in the surroundings of the plant and particularly of the experimental leaf. Direct absorption of ^3H_2O-vapour that may interfere with the ^3H_2O-uptake and distribution data (3.1.1. and 3.1.2.), has been measured by the 'double-leaf' technique described above. In the environmental conditions existing in the growth chamber (frequent air renewal) and on the average for the whole experimental period (5 hrs), about 1.7% of the evaporated water is re-absorbed by the tip and base of the treated leaf. In some preliminary experiments with leaves in a perfectly closed system it was shown on the contrary that as much as 20% of the tritiated aqueous vapour may be re-absorbed by the oat leaf surface during an experimental period of 5 hours. This absorption rapidly decreases at larger distances from the drops; e.g. absorption by tips of non-treated leaves has been estimated at 0.1% of the tritiated aqueous vapour

Table 1. Tritiated water retention by foliar-applied drops of different $CaCl_2$-concentration (in % of the total amount of ^3H_2O present in the drop and in the underlying tissue at 3 and 4 hrs after the start of the experiment).

	Drop without calcium	$CaCl_2$-concentrations of the applied drops			
		2.10^{-4}M	3.10^{-3}M	7.10^{-3}M	3.10^{-2}M
after 3 hrs	57 ± 4	56 ± 4	67 ± 5	76 ± 6	53 ± 4
after 4 hrs	33 ± 2	34 ± 3	63 ± 4	74 ± 5	49 ± 3

available. Although small as compared to the water uptake from the drops, ^3H_2O-vapour absorption is important enough to make data about basipetal transport of foliar-absorbed water at least doubtful (see 3.1.2.1.).

Absorption of H_2O-vapour increases with the Ca-content of the leaf tissue (see below). Water drops on the leaf surface also absorb tritiated aqueous vapour from the surrounding air and, taking into account the exposed surfaces, this absorption is even higher than that by the leaf. A 100 µl drop at 3–4 cm distance from the ^3H-labelled drop absorbs up to 0,9% of the available ^3H_2O-vapour. Absorption, probably by exchange with water molecules of the non-labelled drop, of course decreases with the volume of the drop, but, unlike what holds for the leaf, is not affected by its $CaCl_2$ concentration.

3.2. Influence of other surface-applied drops and higher foliar Ca-content on the tritiated water distribution in the oat leaf

As mentioned before, the second purpose of this study was to measure the influence of two factors directly related to the foliar application of drops of different $CaCl_2$-concentration on the distribution of tritiated water in the leaf. The first factor is the mere presence of drops on the leaf surface. The second one is the increase of the calcium content of a particular leaf part as a result of absorption from a foliar-applied drop of maximum, non-toxic calcium concentration. For reasons stated before, a "double drop on one leaf" technique was applied in these experiments.

3.2.1. H_2O- or $CaCl_2$-drops applied simultaneously with the ^3H_2O drop

Although expected from the presence of a supplementary external water source and from the resulting reduction of the evaporating surface, no decrease of the amount and rate of tritiated water uptake was observed in these experiments.

The ^3H_2O-distribution patterns being quite characteristic of each treatment, the data in *fig. 4* have been averaged over the 5 hrs experimental period. No supplementary effect of increased $CaCl_2$ concentration of the non-labelled but simultaneously applied drops on tritiated water distribution in the leaf was found (see 3.1.2.2.). Therefore, results at all different salt concentrations (0 up to 5.10^{-2}M $CaCl_2$) again were averaged.

Comparing the results of the treatments with and without a supplementary H_2O or $CaCl_2$-solution drop in *fig. 4*, it appears that relatively more tritiated water moves through the leaf part between the applied drops to the tissue underneath the second drop. In this leaf part ^3H_2O is almost equally distributed between the tissue itself, the apparent free space of the cuticle, and the applied, initially non-labelled drop. To a limited extent and mainly during the first hours of the experiment (see 3.1.3.) tritium labelling of this drop is due to absorption of tritiated aqueous vapour from the leaf surrounding atmosphere.

These results suggest a rapid mixing of water from the tissue and from the applied drops; a water continuum is thus created between the tissues and the surface-applied drops (CRAFTS 1961).

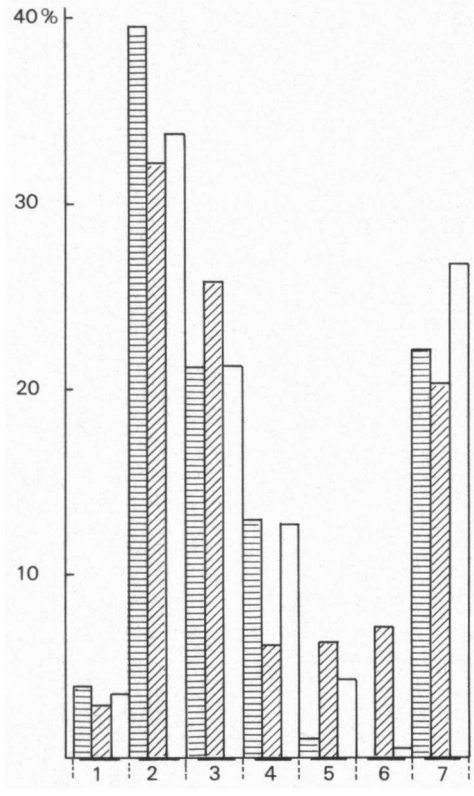

Fig. 4. Influence of the presence of a supplementary drop and of increased Ca content of the leaf on the internal distribution of the absorbed tritiated water (average values over 5 hours in % of the total amount absorbed)
horizontally hatched columns without supplementary drop;
obliquely hatched columns second drop of varying Ca concentration (0–5.10^{-2}M) applied simultaneously;
non-hatched columns second drop (5.10^{-2}M $CaCl_2$) applied 3–4 hrs prior to ^3H_2O drop.
1. leaf base;
2. tissue underneath ^3H_2O drop;
3. leaf part between drops (see fig. 1b);
4. tissue underneath supplementary H_2O or $CaCl_2$ drop;
5. A.F.S. of cuticle under H_2O or $CaCl_2$-drop;
6. H_2O or $CaCl_2$ drop;
7. leaf tip.

3.2.2. Increased calcium content of the leaf tissue

From earlier but similar experiments it was known that after an initial period of slow penetration calcium is rapidly absorbed from foliar-applied drops and that this absorption reaches a maximum (80–90% of the amount applied) 6 or 7 hours after the application (RINGOET *et al.* 1967).

In the present experiments, after a 3 to 4 hours pretreatment period with a 5.10^{-2}M $CaCl_2$-drop the calcium content increases, on the average by 25 μg

in the leaf part underneath the drop and by 16 μg in the tip. A few hours later uptake maxima of about 64 and 42 μg Ca, respectively, in the tissue under the application site and in the tip were observed.

The data in *table 2* show that relatively more tritiated water is taken up and retained by high-calcium oat leaves. The ratios between 'high' and 'normal' calcium leaves show that ^3H_2O-retention increases with the time-lag after the drop application. As mentioned before, absorption of tritiated aqueous vapour also increases at high calcium content of the tissue. According to the results in *fig. 4* concerning the tritiated water distribution in calcium pretreated leaves, preferential water accumulation is found in the leaf tip and in the tissue underneath the drying-out $CaCl_2$-solution drop. These observations suggest that the increased calcium contents of the leaf part underneath the $CaCl_2$-solution drop and of the leaf tip are mainly responsible for the higher tritiated water retention.

4. DISCUSSION AND CONCLUSIONS

Oat leaves not only absorb water from drops on their surface but also aqueous vapour from the surrounding air. In the present experimental conditions (60–70% relative humidity), however, aqueous vapour uptake from the ambient air is small as compared to the water uptake from the drop and its transport to the leaf tip (WAISEL 1958; VAADIA & WAISEL 1963).

As of course might be expected, water of a drop on the leaf surface mainly evaporates. Aqueous vapour of the surrounding air exchanges with water molecules in the drop. A relatively small fraction of the available water (at the maximum 12%) is absorbed by the leaf and almost immediately after drop application this uptake starts at a rather high rate. The overall effect of increased $CaCl_2$ concentration of the applied drops on water uptake from these drops is negligible (see, however, below). A striking difference exists between the uptake pattern for water and that for calcium ions. The latter is characterized by a relatively rapid but small initial absorption. The main uptake occurs when the applied drop has almost dried out (RINGOET *et al.* 1967). Apparently water and calcium uptake processes are, at least to a certain extent, independent of each other.

Table 2. Uptake and retention of ^3H_2O by the oat leaf, at normal and high calcium content respectively, in relation to time (percentages of the total amount of ^3H_2O initially applied).

	½ h	1 h	1½ h	2 h	3 h	4 h
Normal calcium content (N)	9.4 ± 0,7	12.6 ± 1,1	8.6 ± 0,7	5.3 ± 0,5	1.8 ± 0,3	0.8 ± 0,1
High calcium content (H)	12.9 ± 1,3	14.0 ± 1,5	11.3 ± 1,1	10.1 ± 0,9	6.0 ± 0,4	3.6 ± 0,3
Ratio ($\frac{H}{N}$)	1.37	1.11	1.32	1.90	3.35	4.29

Information concerning the possible mechanism and pathway of the initial water penetration from foliar-applied drops comes from the experiments with supplementary water or $CaCl_2$-drops. It was indeed observed that always water from the underlying tissue moves to the drop on the leaf surface (CRAFTS 1961). The presence of drops on this surface eliminates cuticular and stomatal transpiration as possible driving forces for the outward movement. In the literature, however, the guard cells of the stomata are mentioned, not only as the sites of peristomatal transpiration but also of water and solutes exchange between the leaf epidermis and the environment (MAERCKER 1965; FRANKE 1967; KOEPPNER 1970; RASCHKE & KUHL 1969). It has been suggested that ectodesmata, which are particularly abundant in the guard cells, are important pathways for foliar penetration and excretion (FRANKE 1967; RINGOET *et al.* 1971). As shown by autoradiographs (MAERCKER 1965), the sites of excretion of tritiated water in fact coincide with the distribution of ectodesmata in the guard cells (FRANKE 1967). Through these ectodesmata a water continuum may be created between the drop and the underlying tissue and thus a pathway for rapid 'mixing' of water from both sources becomes available. Such a water continuum would certainly improve and intensify all processes of water (e.g. the rapid initial penetration of ^3H_2O from drops) and solutes exchange (penetration and excretion) between the leaf epidermal cells and their outer surface (CRAFTS 1961).

After its penetration, water absorbed from foliar-applied drops most probably moves through the mesophyll cells to the conducting tissue. This pathway of translocation through the leaf is analogous to that followed by foliar-absorbed cations (RINGOET *et al.* 1967 and 1971). According to the experimental results movement to the other leaf parts in the apparent free space of the cuticle or through the epidermal cells is less likely. The small fraction of absorbed water that might be transported along this pathway immediately evaporates from the median leaf part (with the drop application site) and therefore could not be observed in the present experiments. Further transport of the water absorbed from foliar-applied drops and translocated through the mesophyll in the conducting vessels is mainly directed to the leaf tip. No basipetal transport was observed (BIDDULPH & CORY 1957; PLAUT & REINHOLD 1967; VAADIA & WAISEL 1963). In the xylem the foliar absorbed water is mixed with the transpiration stream and then follows the usual evapotranspiration pattern. During the latter process in the tip part most of the recently absorbed water again disappears from the leaf.

The experimental data also give some information about secondary factors that may affect the leaf water distribution. Increased mineral (e.g. calcium) content of specific leaf parts favours water movement to and retention by these parts, probably for maintaining acceptable osmotic conditions within the cells that have absorbed the calcium (DREW 1967). Furthermore, and as mentioned before, foliar-applied drops while certainly reducing evapotranspiration from the drop-covered surfaces promote movement of tissue water to these drops. Higher salt (hygroscopic calcium chloride) concentration of the applied drops favours the outward water movement. This observation at least partly explains

their apparent slower – as compared to pure water drops – drying-out. According to the available results, higher salt content of the applied drops does not affect evaporation or aqueous vapour absorption by these drops.

Finally, according to the ^{3}H-counting in the different leaf parts at the end of the 5-hour experiments, which is never higher than the general background-counting, tritium from foliar-absorbed water does not combine with stable organic coumpounds in the leaf.

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