Effects of High Humidity on Translocation of Foliar-Applied Labeled Compounds in Plants
Part: I. 1, 2
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Introduction
Very little is known about the effects of humidity and water balance on absorption and translocation of foliar-applied materials. Palmquist (11) reported that when a scraped leaf of a water-stressed plant was immersed in fluorescein solution, the solution moved rapidly through and out of the leaf via the xylem. Colwell (5) found that when a solution containing P12 was applied in small amounts covering 10 to 15% of the leaf area, phosphorus was not moved out of the treated leaf in the xylem. When, however, a larger volume of the same solution was applied which covered 40 to 60% of the leaf area, phosphorus moved out of the treated leaf via xylem of the ringed petiole. Went and Carter (14) found that uptake of sucrose by tomato leaves was independent of humidity levels. Other workers reported that high humidity increased the absorption of foliar-applied urea, 2,4-D, and maleic hydrazide (Volk & McAuliff, 13; Zukel et al., 16; Pallas, 10). It has been reported also that plants might absorb water from humid air through the leaves by a reversal of the normal transpiration stream, a process termed “negative transpiration” (Stone et al., 12; Breazeale et al., 2, 3).

Preliminary experiments on translocation of foliar-applied, C14-labeled 2,4-D, amino-triazole and urea, indicated that translocation of these compounds was greatly increased under high humidity conditions. A more detailed investigation was, therefore, desired.

Methods & Materials
Two basically different C14-labeled compounds were used in this work, namely 2,4-D and urea. Experiments (Clor, 4) indicate, furthermore, that urea, after being applied to the leaves, is hydrolyzed and the resulting carbon dioxide is then synthesized into sucrose and translocated. Seedlings of cotton (Gossypium hirsutum L.), grown in Hoagland's solution, were used as test plants. A greenhouse was used, in which the relative humidity during the day usually ranged from 40 to 60%.

C14-labeled 2,4-D of specific activity 6.03 mc per m, was dissolved in 50% ethyl alcohol; urea (sp. activity 1 mc per m) was dissolved in water. Tween 20 was included as surfactant in all treatment solutions. In most experiments a 0.01 ml droplet of a given solution was applied to the surface of a cotyledon or leaf by means of a calibrated micropipette. In experiments where ringing was used, a given portion of the stem or petiole was treated by applying a fine jet of steam for about three minutes; this killed a section around two centimeters in length. The plants were subsequently supported by bamboo sticks.

Radioautographic analysis, similar to that described by Yamaguchi and Crafts (15) was the experimental procedure used in this study.

To provide high humidity conditions the intact plants, growing in cans of soil or jars of solution, were placed in shallow aluminum pans partly filled with water. The pans and plants were enclosed in 0.002 inch polyethylene bags, supported in such a way that the plants were free and exposed to light. By transpiration the bags soon became saturated providing a relative humidity approaching 100%.

Experimental Results

I. Effects of High Humidity on General Pattern of Translocation: Uniform cotton seedlings, 16, at the cotyledonary stage, were selected. Eight plants were treated, each with 0.5 microcurie of 2,4-D, and the other eight each with 0.5 microcurie of urea. The treatment in each case was applied as a 0.01 ml droplet on the upper surface of a cotyledon. Two plants from each group were placed under high humidity conditions and two others were left in the greenhouse. Of the remaining plants, only the treated cotyledons of two plants from each group were placed in high humidity, while in the case of the remaining two plants from each group, whole plants, except the treated cotyledons, were placed in high humidity. After treatment, 3 hours, all the plants were freeze-dried, and subsequently radioautographed. The results are shown in figures 1 and 2.
Fig. 1. Effect of humidity on the translocation of C\textsuperscript{14}-labeled 2,4-D, radioautographs above, mounted plants below. Arrows designate the treated cotyledons, triangles the rings.

A, 2,4-D. The two plants on the left were kept bagged for high humidity conditions; the two on the right were not bagged.

B, 2,4-D. The two plants on the left had their treated cotyledons covered with polyethylene bags; the two on the right were in bags except for their treated cotyledons.

It is seen very clearly that under high humidity conditions translocation of 2,4-D (fig 1A) was greatly increased. It is also seen that there was a tremendous amount of translocation to the opposite untreated cotyledons, while under normal greenhouse conditions there was a limited dispersal of radioactive materials within the treated cotyledon and no translocation to the untreated cotyledon. Evidently the treated leaf, under these conditions, serves as a source of supply for export of the treating solution via both the phloem (increased movement to roots) and via the xylem (movement into the opposite cotyledons).

When only the treated cotyledons were placed in the polyethylene bags, there was considerable more movement to roots via the phloem but evidently not enough free water on the treated cotyledon to allow export via the xylem. There was somewhat more movement within the untreated portion of the treated leaves. When the whole plants, except the treated cotyledons, were enclosed in the polyethylene bags the translocation pattern was the same as that of unbagged plants. Neither phloem nor xylem transport was increased.

In the case of the urea treatments (fig 2) the labeled sucrose, that results from hydrolysis and photosynthesis of the resultant C\textsuperscript{14}-CO\textsubscript{2} has moved freely both downward via the phloem and into the opposite cotyledons via the xylem (fig 2A). In the unbagged plants, movement as in the case of 2,4-D,
of 2,4-D moved into the roots; the lesser movement of 2,4-D is a reflection of the binding of 2,4-D found by Crafts (6) and others.

In the experiment where only the treated cotyledons were placed in the polyethylene bags (fig 2B) the sucrose evidently moved via the phloem to the roots in large quantity; it also went into the opposite cotyledons in traces. Evidently in this particular experiment the humidity was such that the treated leaf served as a source for rapid export via the phloem. There was ready lateral movement in the treated cotyledons. Possibly water stress in the exposed cotyledons and stems resulted in rapid drying of the treatment spot and hence a shorter exposure time to solution of the tracer. As in the case of 2,4-D, when labeled urea was applied to the cotyledon of a cotton seedling having the remainder of the plant in high humidity the picture was the same as in the open greenhouse.

II. Effects of High Humidity as Revealed by Ringing the Epicotyl: Four-week-old cotton plants, grown in clay pots were employed in these experiments to make convenient the ringing of the epicotyls. Twelve plants were selected and the epicotyls of seven of them were steam-ringed about one centimeter above the cotyledonary node. To be sure that the ringing process did not also destroy the xylem, the plants were left untreated for 24 hours to see if wilting occurred. Each plant was treated on one cotyledon with 0.25 microcurie of labeled 2,4-D or urea as indicated. All the plants were harvested 2 days after treatment, freeze-dried, and subsequently radioautographed. The results of this experiment are given in figures 3 to 6.

Fig. 2. Effects of humidity on the translocation of C¹⁴-labeled sucrose within plants treated with C¹⁴-labeled urea; radioautographs above, mounted plants below.
A, urea. The two plants on the left were kept bagged for high humidity conditions; the two on the right were not bagged.
B, urea. The plant on the left is an untreated control; the two on the right had their treated cotyledons (right side) covered with polyethylene bags.
Fig. 3. Effects of humidity and ringing on distribution of C\textsuperscript{14}-labeled sucrose derived from C\textsuperscript{14}-labeled urea and C\textsuperscript{14}-labeled 2,4-D in cotton plants.

A, unringed. Humid (left) vs. dry (right). B, ringed. Dry (left) vs. humid (right). C, ringed (right) vs. unringed. (left) not bagged for high humidity.

Figure 3A shows the contrasting effects of high and low humidity upon the movement of labeled sucrose from C\textsuperscript{14}-urea treated cotton cotyledons. As with the small plants shown in figure 2, the tracer moved basipetally to the roots and high humidity did not increase this movement as it did in the smaller plants of figure 1. In these larger plants acropetal phloem movement from the cotyledons to the growing tip was more pronounced probably due to a higher rate of shoot growth (fig 3A, the plant on the right). Here again high humidity greatly increased movement to the opposite cotyledon and to other mature leaves. This distribution to mature foliage is later shown to occur by xylem.

In figure 3B the experiment was the same as in figure 3A, except that 2 cm of the stem above the cotyledons were steam-ringed. Under greenhouse conditions (low humidity) movement was principally basipetal. On the other hand a faint trace of the label passed through the ring and into the upper leaves. Movement through a ring indicates xylem movement; evidently products of sugar metabolism in the roots leaked into the xylem and were carried upward in the transpiration stream. Maleic hydrazide, dalapon, and phosphorus are known to do this (Crafts 6, Biddulph et al., 1).

In the case of the high humidity conditions there was a large movement of the tracer across the ring and into the upper leaves. The high accumulation in young leaves does not necessarily signify phloem movement across the ring; rather it probably represents retranslocation from the fully developed leaves to the younger leaves via the phloem above the ring.

In figure 3 C are shown two 2,4-D treated plants kept under greenhouse conditions, and the plant on the right was ringed above the cotyledons. Move-
ment in the unringed plant was normal for 2,4-D in cotton, namely both acropetal and basipetal, with fully expanded leaves being bypassed; this is phloem movement. In the ringed plant basipetal movement was the same; acropetal movement was essentially lacking.

In figure 4 A are shown two 2,4-D treated plants, both under high humidity conditions; the plant on the left was ringed above the cotyledons. In the unringed plant on the right the 2,4-D moved downward, as would be expected from a lower leaf; it also moved upward to the mature and to the developing leaves and bud. Labeling of the opposite cotyledon indicates movement across the cotyledonary node. As shown by the plant on the left, ringing apparently did not reduce upward movement; the distribution of labeling was somewhat more general. Here the ringing indicates that the acropetal movement of 2,4-D from a lower leaf in high humidity can be accounted for possibly by condensation on the treated spot and attendant movement of 2,4-D via xylem into the region above the cotyledons.

In figure 4 B are shown two 2,4-D treated plants having the treated cotyledons enclosed in polyethylene bags. Evidently this treatment is quite different from having the entire plant in the bag because little or no xylem movement is shown. The light labeling of the upper portions again may be the result of leakage of 2,4-D or its metabolites into the transpiration stream. Figure 4 C shows on the left a ringed, 2,4-D treated plant, on the right a ringed, urea-treated plant; in both cases the entire plants except for the cotyledons were in polyethylene bags. Phloem movement into roots was normal and the slight labeling of young leaves represents tracer that has traversed the steam ring in each instance. This again may represent metabolites of the original tracers that have leaked into the xylem. Note the heavy labeling of root tips by sucrose, light labeling of roots as contrasted with the hypocotyl by 2,4-D.

![Fig. 4. Effects of bagging (high humidity) and ringing on distribution of labeled sucrose and labeled 2,4-D in cotton plants. A, 2,4-D humid ringed (left) vs. unringed (right), both bagged. B (center), 2,4-D ringed (left) unringed (right), treated cotyledons only bagged. C, (right) 2,4-D (left) vs. urea (right) plants except the treated cotyledons bagged, both ringed.](image-url)
Fig. 5. Effects of humidity and ringing on distribution of labeled sucrose in cotton plants. A, urea, ringed (right) below the cotyledons vs. unringed (left), both humid. B, urea, ringed (right) below the cotyledons vs. unringed (left), both dry. C, urea, unringed, humid (left) vs. dry (right).

III. Effect of High Humidity as Revealed by Ringing the Hypocotyl: Three-week-old cotton seedlings, grown in Hoagland’s solution, were used. Four uniform plants were selected and steam ringed on the hypocotyl about one centimeter below the cotyledonary node. Two ringed and two non-ringed plants were treated each with 0.25 microcurie of labeled urea. The tracer in all cases was added on the upper surface of one cotyledon of each plant. One ringed and one non-ringed plant of each treatment were bagged to obtain high humidity, the remaining equivalent two plants were kept under greenhouse conditions. All the plants were harvested 30 hours after treatment, freeze-dried and radioautographed. The results for the urea treatments are shown in figures 5A and B.

Figure 5A shows two urea treated plants, both bagged during the treatment period; it is evident that the bulk of the basipetal movement takes place via the phloem for when this was killed no tracer appeared below the ring. Under greenhouse conditions (fig 5 B) the total movement was very much less and as would be expected from strictly phloem movement there was no distribution into the opposite cotyledons or to mature green leaves. Here too, ringing completely inhibited basipetal movement.

IV. Effects of High Humidity as Revealed by Ringing the Petioles: The above experiments indicate that under high humidity conditions materials moved out of the treated leaf via both xylem and phloem. To substantiate this point the following experiment was designed. Four-week-old cotton plants, grown in Hoagland’s solution, were used. Six uniform plants were selected and the petiole of one cotyledon of each of four of these plants was steam-ringed at midpoint. The four ringed plants were divided into two groups of two plants each. In group A the treatment was applied on the cotyledons, the petioles of which were ringed, whereas in group B the treatment was applied on the opposite cotyledons, the petioles...
oles of which were left unringed. The two unringed plants constituted the third group C. Each plant was treated with 0.5 microcurie of labeled urea, applied on the upper surface of the specified cotyledon. One plant from each group was placed under high humidity conditions, while the other plants of each group were left under greenhouse conditions. All six plants were harvested 24 hours after treatment; freeze-dried and radioautographed as usual (figs 5 C & 6).

As shown in figure 5 C, (unringed controls) high humidity resulted in the complete labeling of the cotton seedling whereas in the drier conditions of the greenhouse less movement takes place and it is entirely limited to the phloem. Figure 6, left (radioautograph) shows that in high humidity, ringing the petiole of the treated cotyledon has no effect on the distribution of the tracer in an acropetal direction; basipetal movement, namely that part which is effected by phloem translocation, is materially reduced. This movement across a steam ring in the petiole provides strong evidence for reversal of the transpiration stream in the cotyledons of cotton plants under conditions of high humidity. Without the high humidity (fig 6 right) there was no movement of the label out of the cotyledon across the steam ring. Ringing the petiole of the cotyledon opposite the treated one had no appreciable effect; the autographs resembled those of figure 5 C.

**Discussion & Conclusions**

Under normal conditions of the greenhouse, the translocation of foliar applied 2,4-D and sucrose occurs through the phloem; it is dominantly downward from the treated cotyledons of cotton seedlings; some movement via the phloem to the shoot tip and young expanding leaves may also take place. Occasionally one may observe a light labeling of mature leaves above a treated leaf when amino triazole or maleic hydrazide is used as a tracer; this results from migration of the tracer from phloem to xylem. In the experiments with 2,4-D and urea described here, the mature leaves were bypassed by the tracers in their movement to shoot tips; this is taken to indicate phloem movement and results from the fact that the mature leaves, like the treated cotyledon or leaf, are exporting foods and hence the assimilate stream is moving out rather than into these leaves.

When the plants were placed under very high humidity conditions, the translocation pattern of foliar applied substances was drastically changed; export of the tracers was greatly increased and they moved into opposite cotyledons and into all mature as well as young leaves. This general distribution could have resulted only from simultaneous transport from the treated leaf via both phloem and xylem.

Ringing of the stem above the treated cotyledon did not materially alter the distribution pattern in plants in high humidity; tracers went to the roots and to the opposite cotyledon and to all leaves above the treated cotyledon. Ringing the stem below the treated cotyledon prevented basipetal translocation via phloem. Even placing a ring on the petiole of the treated cotyledon failed to hinder movement to the shoot tip and mature leaves. These ringing experiments prove that movement in normal plants under high humidity conditions takes place simultaneously.

![Fig. 6. Labeled urea treatment as in figure 5, but the petioles of the treated cotyledons were steam-ringed.](https://example.com/fig6.png)
through phloem (basipetal movement to roots, acropetal movement to young leaves) and xylem (acropetal movement to mature leaves). Apparently this latter represents a reversal of the normal movement of the transpiration stream in the case of the treated leaf.

Experiments carried out many years ago (Curtis, 8) proved that it is virtually impossible to stop transpiration from leaves, even when they are surrounded by a saturated atmosphere, so long as they are in the light. Careful temperature measurements proved that such leaves, by converting absorbed light to heat, are able to maintain a slight temperature differential and hence to transpire moisture into a saturated atmosphere.

In the above described experiments it was observed that soon after the polyethylene bags were placed around the plants in the high humidity treatments, moisture started to condense inside the bags on the polyethylene surface. This process continued so long as the plants were maintained in the light, and after 24 hours a very appreciable volume of water occurred as drops on the inside surface of the bags.

The most plausible explanation for the strong movement of tracer solution out of the treated leaves under the conditions of the high humidity experiments seems to be that very soon after the plants were placed inside the bags, condensation occurred at the treated spot thus converting this to a source of moisture at atmospheric pressure. This would maintain a supply of tracer in solution for ready uptake into the phloem and strong basipetal movement via phloem to roots. At the same time tracer solution was evidently absorbed by the xylem and exported to all transpiring leaves via xylem. In plants having mature leaves above the treated cotyledon, acropetal phloem movement also occurred into the shoot tip and young expanding leaves. Under greenhouse conditions the mature leaves were bypassed (fig 5B); under high humidity conditions the mature leaves were strongly labeled (fig 5C, left; fig 6, left). Even the untreated portions of the treated leaves became strongly labeled under high humidity whereas under low humidity they remained relatively free of label (fig 5C, left vs right). Ringing of the petiole of the treated leaf (fig 6) proved that the heavy export took place in the xylem. Basipetal movement of tracer to roots in the case of the plant with the ringed petiole (fig 6, left) must have taken place via phloem from the opposite cotyledon after the tracer had entered it. Ringing the hypocotyl, on the other hand, destroyed the phloem and prevented such movement (fig 5A).

The increased phloem movement from treated leaves under high humidity conditions may be explained partly by an increased absorption, as reported by several workers (Volk & McAuliff, 13; Zulkel et al., 16; Pallas, 10; Dybing, 9). It is also likely that the increased phloem transport results from the greater turgor of the leaf cells and phloem conduits at the source in the treated leaf (Crafts, 7). Although the phloem is adapted for conduction of foods under a wide range of water stress in the plant, transport is probably greatest under low stress and optimum conditions for photosynthesis.

The increased transport and extensive distribution of tracer resulting from the high humidity conditions employed in these experiments have important implications with respect to the use of growth regulators and herbicides. The growth inhibitor maleic hydrazide has proven to be effective in the suppression of shoot growth in topped tobacco plants in a limited region that is known to have high humidity for at least a portion of the diurnal cycle for many days during the growth period. As an inhibitor of grass growth this compound is more effective under conditions of a humid climate than under dry conditions.

Likewise the grass killer dalapon has proved much more effective in the control of perennial grasses in humid regions than under dry climates. And 2,4-D is more effective for field bindweed control in the humid coastal fogbelt of California, than in the dry interior valley. All of these effects probably relate to the enhanced uptake of these compounds and more thorough distribution via the phloem.

The increased absorption and reverse movement in the xylem found in these experiments probably resulted from a differential condensation of moisture on the treated spots, resulting from the inclusion of a hygroscopic surfactant (Tween 20) in the treatment solution. Possibly also, condensation from a supersaturated atmosphere might take place on a water surface (treated spot) in contrast with the waxy cuticle of the untreated portion of a leaf.

Under conditions of field application where all foliage is occupied, at least partially by spray droplets, this effect would be expressed only as an increased local uptake and distribution, and not by a reversal of transpiration as observed in the above experiments where only one leaf received treatment. Nevertheless, such increased local uptake (as in treated cotyledons in humid vs dry treatments in fig 5C & 6) might well result in increased phloem transport, and thus account for the advantage noted above for use of herbicides and regulators in humid as contrasted with dry climates.

Finally the experiments described here may point the way to new technics for studying the effects of climate on the effectiveness of pesticides. And they explain some of the discrepancies experienced in the field in the testing work with new materials.

Summary

The effects of high humidity conditions on translocation of foliar-applied labeled 2,4-D and urea were studied in cotton seedlings, using the radioautographic method. Under normal conditions, C14-labeled 2,4-D and sucrose (resulting from urea application) moved out of the treated leaves in the phloem going basipetally to roots and acropetally to growing shoots.
Under high humidity conditions translocation of the compounds was greatly increased. Experiments with steam-ringing of various portions of the stem and petioles showed that under high humidity conditions the radioactive compounds moved out of the treated organs and ascended acropetally throughout the shoot mainly in the xylem. At the same time there was a basipetal phloem movement to the roots. Thus the tracers may attain a complete distribution in the plant in appreciably greater amounts than occurs from phloem movement alone under low humidity conditions. Possibly condensation on the treated spot enables it to serve as a source for both phloem and xylem transport.

**Literature Cited**


