EFFECTS OF TEMPERATURE AND HUMIDITY ON FOLIAR ABSORPTION AND TRANSLOCATION OF 2,4-DICHLOROPHENOXYACETIC ACID AND BENZOIC ACID [1, 2, 3]

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Little is known concerning the effect of humidity on the absorption of foliarly applied materials or their subsequent translocation. It is generally believed that the higher the humidity at any one temperature, the larger the amount of a foliarly applied material that penetrates the leaf per unit time. Most of the evidence for such an effect has been obtained indirectly. Koontz and Biddulph (16) found that the amount of phosphorus translocated from a given compound seemed to be related to the drying time of the solution on the leaf. By adding glycerine to the treatment solution, the translocation of P^{32} from an application of KH_2PO_4 solution was increased. Other workers have reported increased penetration of foliarly applied materials by additives which increase moisture retention. The recent review by Currier and Dybing (7) covers this work. It has been suggested that thin aqueous films on the leaf surface are important in promoting the absorption of nutrient sprays, the existence of the film depends upon the vapor pressure gradient at the leaf surface (4).

In general, increasing temperature, within physiological limits, has been found to result in increased penetration. Rice (19) using red kidney bean plants studied the absorption of the ammonium salt of 2,4-D. He found that the amount absorbed was positively correlated with temperature over a three-level range of 46 to 58° F, 79 to 82° F and 86 to 92° F. Other workers using soybeans as the test plant found that with increasing temperature, there was an increase in the absorption of the sodium salt of 2,4-D (5,10). Barrier and Loomis (2) reported a temperature effect on the absorption of 2,4-D by soybean seedlings, but no effect upon the absorption of P^{32}. Increased rates of absorption with increasing temperature have been reported for Co^{60} (9) and manganese (18).

Known quantities of maleic hydrazide have been applied on several species of plants; the time course of absorption followed as it was affected by temperature and humidity (21). A variation in temperature at controlled humidities was found to have less effect on the absorption rate than a variation in humidity at controlled temperatures. Either an increase in temperature or humidity gave an increase in absorption of the maleic hydrazide.

The preponderance of evidence to date indicates a maximum in translocation over the range of 20 to 30° C. At temperatures below and above this range translocation is reduced (2, 12, 22).

MATERIALS AND METHODS

Selected seed of *Phaseolus vulgaris* L. var. Red Kidney was used. Seeds were germinated at 25° C in Vermiculite saturated with distilled water. All temperatures reported are accurate within ± 1° C and relative humidities are accurate to ± 3 % as measured by a hair hygrometer calibrated periodically with a hand phychrometer. Forty to 42 hours after sowing, 90 germinated seeds with radicles between 1.5 cm and 1 cm long were selected and planted in 4 inch pots containing Yolo clay loam soil fertilized with 16 ppm N and 6 ppm P on an oven dry weight basis. After subirrigation the pots were transferred to a growth chamber where they remained at 25° C until the 5th day after sowing, when the lights were turned on at 5:30 A.M. At this time the beans were in the crook stage. Subsequent growth conditions were: 15½ hours light, temperature 25° C, relative humidity 60 to 76 % and 8½ hours dark, temperature 15° C, relative humidity 85 to 95 %. Light was obtained from a fluorescent-incandescent source giving 1,100 to 1,300 ft-c at the level of the unifoliate leaves.

The beginning of the experimental temperature and humidity regimes coincided with initiation of the light period on the 9th day after planting the dry seeds. Generally, 60 selected plants were treated between 9:00 and 12:00 A.M. The first trifoliate leaves were beginning to unfurl from the bud at this time. Plants were watered before treating; in experiments at lower humidities, they also were watered several hours later. Treatment consisted of applying two 5 µl droplets on each side of a unifoliate leaf, approximately 2/3 cm from the major vein and 1½ cm from the base of the leaf. The treatment solution contained 10 µg acid equivalent of the triethanolamine salt of 2,4-dichlorophenoxyacetic acid or 5.52 µg acid equivalent of triethanolamine benzoate in water with 0.1 % Vatsol OT (sodium dioctyl sulfosuccinate). The C^{14} activity of carboxyl-labelled 2,4-D and benzoic acid was 6.03 mc/mM and 1.0 mc/mM, respectively. Ten replications of every treatment were harvested at 2, 4, 6, and, in two experiments, 8 hours after treatment. The harvest consisted of washing out the root systems and then simultaneously removing two 1 cm sections from the epicotyl by means of a three-bladed knife. The first cut was made as close

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to the junction of the petiole and epicotyl as possible. Remaining plant parts were quick-frozen between blocks of dry ice; when the harvest was completed they were lyophilized. The epicotyl sections were placed in small, numbered culture tubes; 0.5 ml 1 *N* NaOH was added to each, and the tubes were stoppered and placed in an oven at 60 to 65° C for 24 hours for digestion and extraction. A complete extraction of C^{14} was obtained. The extracts were plated, dried, and then counted in a gas flow counter. Due to the hygroscopicity of NaOH, planchets had to be stored at 55 to 60° C to give reproducible counts. The NaOH method is recommended when dealing with an easily subliming compound like benzoic acid since it prevented detectable loss of radioactivity.

To determine the chemical form in which the C^{14} was present at the time of extraction from epicotyls for counting, analyses were made of the fresh petioles of treated leaves of plants treated in experiment No. 2 at 30° C, high humidity since translocation was optimal under these conditions and any metabolic conversion would normally be highest at an elevated temperature. At the time of harvest of epicotyls the petioles were bulked, frozen in dry ice, and remained frozen until extraction. The extraction process consisted of grinding ten petioles in a small tissue grinder to which 3 ml of 0.1 *N* HCl was added. At the completion of grinding 0.5 ml of the unfiltered brei was added to a culture tube and 0.5 ml of 1 *N* NaOH added. Three 3 ml ether extractions were made of the remaining 2.5 ml of brei. After the ether extractions, 0.5 ml of the extracted brei was again added to a culture tube and 0.5 ml 1 *N* NaOH added. The NaOH solutions were placed in the oven at 60 to 65° C for 24 hours, cooled, plated, and counted in the same manner as epicotyl extracts. The ether extract of 2,4-D treated plants was spotted directly on Whatman No. 1 filter paper for chromatography. The ether extract of benzoate-treated plants was further extracted with 3 ml of 0.1 % Na_2CO_3 solution. This additional extraction was found necessary for benzoate-treated leaf petioles since in a preliminary experiment most C^{14} activity was lost upon evaporation of the ether extract. The Na_2CO_3 extract was spotted on Whatman No. 1 filter paper for chromatography. Controls consisted of untreated petioles to which stock radioactive 2,4-D or benzoic acid was added just prior to grinding. Controls were subjected to the same extraction and counting procedures as petioles from treated plants. The Andreae and Good (1) solvent system was used; it consisted of isopropyl alcohol, concentrated ammonium hydroxide, and water (80:10:10, v/v). A co-chromatogram of stock 2,4-D and benzoic acid was run.

Four representative 2,4-D and benzoate-treated, lyophilized plants of every harvest period were selected for radioautography. Since equivalent amounts of chemicals were applied but the activity differed by a factor of six, the benzoate-treated plants were autographed 6 weeks and the 2,4-D treated plants 1 week.

Only one environmental chamber was available; therefore, it was necessary to make successive tests at every selected temperature and humidity. To test the reproducibility of an experiment two tests were made at 25° C high humidity and two tests at 30° C high humidity. There was no significant difference for replications at the 5 % level. Experiments were conducted at 20, 25, 30, and 35° C at high and low humidities (table I).

Results and Discussion

It is evident that with an increase in temperature there was an increase in the amount of 2,4-D translocated to the epicotyl (figs 1 and 2). This could have resulted from any or all of three effects: A. Increased absorption, B. Increased translocation, C. A preferential accumulation of the 2,4-D in the epicotyl. The last explanation is the most unlikely of the three, for with increasing temperature, radioautograms showed no lack of activity in other parts of the plant. Rather, with an increase of temperature there was also an increase in activity in the petiole of the treated leaf and the hypocotyl. On the basis of the above, an increase in translocation would most likely account for the increase in activity. However, the increase in translocation probably results from a more rapid rate of absorption under conditions of high humidity. Figure 2 shows that at any given temperature for any given time, there was always less 2,4-D translocated into the epicotyl under a low humidity. In figure 1 it can be seen that the amount of 2,4-D translocated into the epicotyl per unit of time falls off between 4 and 6 hours at both 30 and 35° C. That the translocation of 2,4-D has stopped is not to be regarded as the only possible explanation of the lack of a further increase in activity in the period between 6 to 8 hours at 35° C. Another possible explanation is that the accumulation of 2,4-D by the epicotyl has reached an equilibrium where outflow becomes equal to inflow. The amount of 2,4-D translocated per unit of time at 20 and 25° C appears to be very much the same; however, at these temperatures evidence is lacking of a decrease in translocation between 4 and 6 hours or even between 6 and 8 hours at 25° C. No radioautograms were made of the 8 hour harvest period, so further clarification of this point is not

Table I

Temperature, Humidity, and Vapor Pressure Deficit of Experiments

Temp.	Experiment No.	Relative humidity	Av VPD MM Hg
20° C	1	73 % ± 5 %	4.74
20° C	1	48 % ± 4 %	9.13
25° C	1	70 % ± 5 %	7.13
25° C	2	70 % ± 5 %	7.13
25° C	1	34 % ± 5 %	15.70
30° C	1	70 % ± 5 %	9.56
30° C	2	70 % ± 5 %	9.56
30° C	1	40 % ± 3 %	19.12
35° C	1	74 % ± 5 %	10.98
35° C	2	70 % ± 5 %	12.70
35° C	1	38 % ± 4 %	26.18

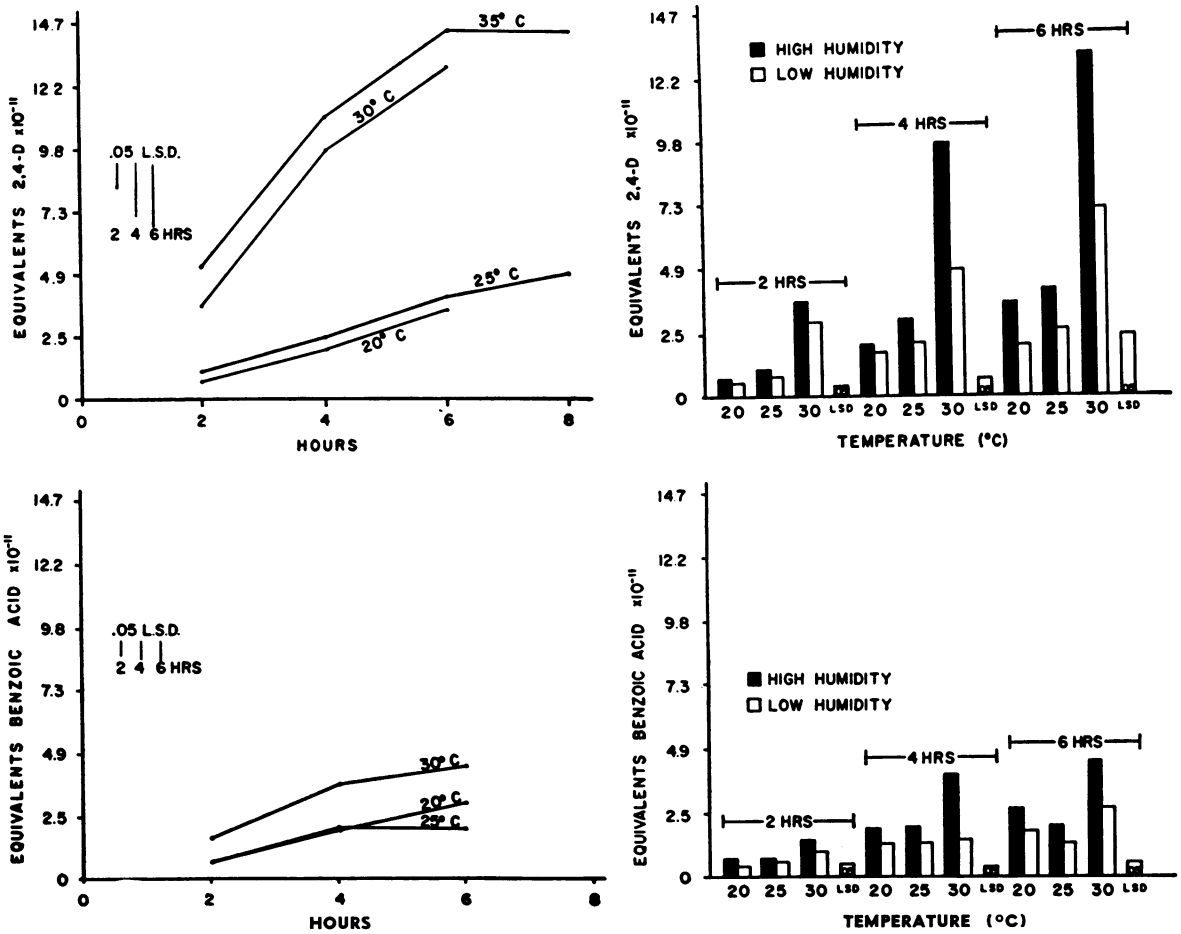

FIG. 1 (*upper left*). The relationship of the amount of 2,4-D absorbed and translocated to the epicotyl at indicated temperatures (high humidity) with increasing time.

FIG. 2 (*upper right*). The relationship of the amount of 2,4-D absorbed and translocated to the epicotyl at high and low humidities at indicated temperatures and time.

FIG. 3 (*lower left*). The relationship of the amount of benzoic acid (or a metabolic derivative) absorbed and translocated to the epicotyl at indicated temperatures (high humidity) with increasing time.

FIG. 4 (*lower right*). The relationship of the amount of benzoic acid (or a metabolic derivative) absorbed and translocated to the epicotyl at high and low humidities at indicated temperatures and time.

possible. Day (8), Rice (19), and Weaver and DeRose (24) found that under greenhouse conditions the translocation of 2,4-D into the stem was essentially completed 4.5, 4, and 5 hours, respectively, after treatment.

Figure 3 indicates that translocated activity in benzoate-treated plants did not vary between 20 and 25° C, but a marked increase was noted at 30° C. At any given temperature and time, the amount of benzoic acid translocated into the epicotyl is less at a low humidity than at a high humidity (fig 4).

Leaves of plants treated at 35° C (experiment No. 1) showed necrosis at the point of application, especially under the more humid conditions. The effect was more pronounced in 2,4-D treated plants. At this temperature high coefficients of variation were found; activities of epicotyl extracts were less than 30° C experiments. A rerun at the higher humidity did give the expected trend for 2,4-D (fig 1); but treated areas of leaves still showed considerable necrosis resulting from a surfactant effect.

No recovery data for 2,4-D are shown in table II as 95 % of the C^{14} was found to be ether extractable after a six hour treatment period. Chromatograms indicated the bulk of the labelled ether-soluble material was 2,4-D. This finding is in accord with the literature (11, 13, 14, 15, 25).

Free benzoic acid is reported in some plants (3); however, it is rapidly metabolized in incubated pea epicotyl sections (1). The initial translocation of the C^{14} in benzoate-treated bean plants occurred primarily as benzoic acid or a dissociable salt thereof. With increasing time from treatment, it is apparently metabolized into other compounds possibly both before and after translocation. This deduction is made on the basis of ether solubility of the radioactive compounds

TABLE II

% ETHER-SOLUBLE RADIOACTIVE COMPOUNDS IN PETIOLES OF LEAVES TREATED WITH TRIETHANOLAMINE SALT OF BENZOIC ACID

TREATMENT TIME	EXTRACTION	CPM/10 PETIOLES	% ETHER SOLUBLE
2 hrs	Brei before extraction	580	78.1 %
	Ether extracted brei	127	
4 hrs	Brei before extraction	1,035	76.8 %
	Ether extracted brei	240	
6 hrs	Brei before extraction	2,220	67.2 %
	Ether extracted brei	728	
Control	Brei before extraction	6,200	96.5 %
	Ether extracted brei	218	

from petiole extracts (table II) and the identification of the translocate from chromatograms.

There is an apparent difference in mobility of the two compounds when applied to the bean leaf. The amount of benzoic acid translocated to the epicotyl at any given time, temperature, and humidity was

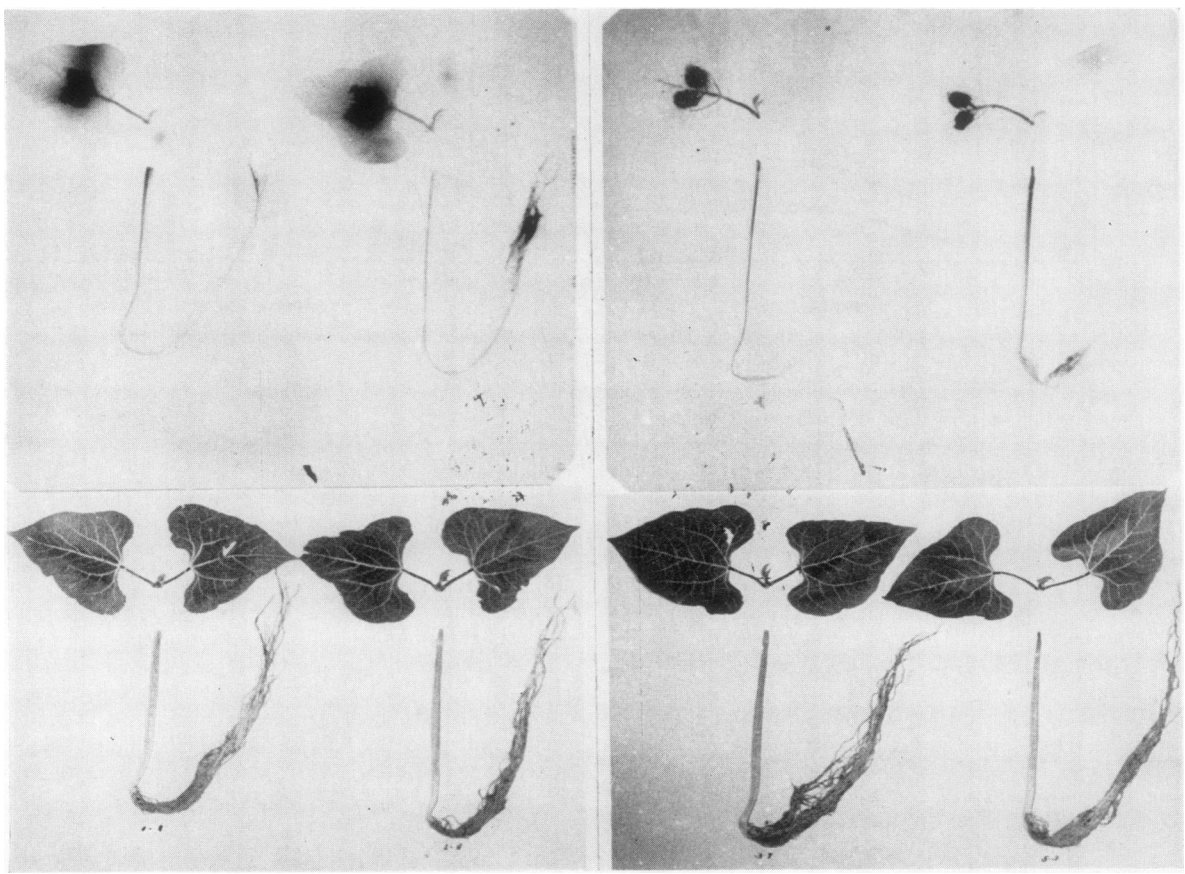

FIG. 5 (*left*). Distribution of benzoic acid or a metabolic derivative in bean plants treated at 30° C, low humidity for 4 hours. Upper section is radioautogram of treated plants shown in lower section. Missing epicotyl of plants was used in C^{14} analysis. The intensely dark area of roots on the radioautogram is associated with the adherence of soil particles.

FIG. 6 (*right*). Distribution of 2,4-D in bean plants treated at 30° C, low humidity for 4 hours. Upper section is radioautogram of treated plants shown in lower section.

always less than for a comparable 2,4-D treatment (compare fig 2 with fig 4). The more diffuse movement of benzoic acid in the treated leaf (fig 5) is in contrast to the polarized movement of 2,4-D (fig 6); the indication is that benzoic acid moves not only in the assimilate stream but spreads throughout the treated leaf. The diffuse spread of activity in the benzoate-treated leaf could be a movement via the symplast and/or apoplast (7) of either benzoic acid and/or a metabolic derivative. This movement is similar to the movement of the more water-soluble herbicides (6). Benzoic acid is much more soluble in water than 2,4-D.

A highly significant increase in epicotyl activity of 2,4-D treated plants was found upon an increase of temperature from 25 to 30° C at a high humidity (figs 1 and 2). A discrepancy in the results might be suggested, had not reruns been made at both temperatures. When a comparison is made of the activity in the epicotyl of plants treated with 2,4-D at temperatures of 20° C (high humidity) with those similarly treated at 30° C (high humidity) a Q_{10} of about four is apparent for the 4 hour treatment period. This is too high to be accounted for by an enzymatic reaction let alone a diffusional one. In the case of benzoic acid, a Q_{10} of about two is apparent from a comparison of the 20° C experiment with the 30° C experiment at a 4 hour harvest period (fig 3). The difference between the two Q_{10}'s may be accounted for by the loss of activity due to diffused movement in benzoate-treated plants. At even short periods of harvest there was a large difference between the activity in the epicotyl of plants treated at a high or a low humidity (figs 3 and 4). In general, little difference was noted in the speed of drying of applied droplets under the various humidities and temperatures, at least there was not enough difference to explain the results.

It was noted in several experiments that stomata were more frequently closed and their apertures smaller at lower humidities than at higher humidities. Plants were grown as previously described to study the degree of stomatal closure as it affected the uptake and translocation of 2,4-D. The experiment was run at 30° C, relative humidity 30 % ± 3 % and the plants were harvested 4 hours after treatment. Stomata of the plants were observed in situ under a compound microscope before treatment. Treatment solutions were applied as two 5 µl drops to areas previously described. Treatments consisted of:

I. 10 µg 2,4-D triethanolamine and 0.1 % Vatsol OT in 10 µl of distilled water.

II. 10 µg 2,4-D triethanolamine, 0.1 % Vatsol OT and 0.1 % glycerine as a humectant.

III. 10 µg 2,4-D triethanolamine and 0.1 % Vatsol OT; 10 minutes after application to the leaf the areas of treatment were rewet with 5 µl of water.

IV. One unifoliate leaf was bagged with a small polyethylene bag at the initiation of the light period. During and after treatment of the bagged leaf with 10 µg 2,4-D triethanolamine and 0.1 % Vatsol OT the bag was opened and remained open.

Adding a hygroscopic agent or rewetting of the treated area appears to be better than applying straight droplets at a low humidity (although not statistically significant at the tested levels) (table III). A rather large increase in absorption and translocation of 2,4-D is evident when the stomata were open at the time of treatment. That the droplet remained moist for a longer time, thus increasing uptake, was ruled out since the leaf was subjected to the low humidity upon treating and thereafter. It is believed that the greater activity in the epicotyl at higher humidities is at least partially the result of an increase in stomatal penetration. It has been reported that stomata open under a high humidity (23). Wilson (26) found that a greater percentage of stomata are open in most species he studied at higher temperatures than at lower ones. If the stomata were open more at the higher temperatures with a high humidity, this would account for the difference in absorption and translocation at the higher temperatures as found.

The hypothesis that temperature and humidity control the amount translocated by their effect on stomatal opening is further strengthened by the results shown in the low humidity experiments (fig 2). At low humidities with stomata frequently closed, penetration would be mainly rate-limited by the cuticle, thus giving a Q_{10} of 1.5 or higher if cuticular diffusion was associated with some mass movement through stomata. At the 4 hour harvest period, when a comparison of the activity in the epicotyl at 20 and 30° C is made, a Q_{10} of about two is derived.

Even if stomatal penetration is important, ectodesmata that have been found directly below the cuticle

TABLE III

STOMATAL OPENING AS RELATED TO PENETRATION AND TRANSLOCATION

CHEMICAL APPLICATION	FURTHER TREATMENT	AV CPM* **	STOMATA OPEN	OPENING WIDTH
Triethanolamine salt of 2,4-D and 0.1 % Vatsol OT	None	141	25 %	1/3
"	Treated area rewet	195	25 %	1/3
"	0.1 % glycerin to treatment solution	209	25 %	1/3
"	Treated leaf bagged	449	75 %	3/4-full

* $LSD_{0.05} = 94$; $LSD_{0.01} = 126$

** cpm of 0.1 ml of 0.5 ml 1 *N* NaOH extract of bean epicotyls. To convert cpm to equivalents of 2,4-D per 2 cm section of epicotyl, multiply cpm by 2.5×10^{-13}.

(17) should be given due consideration (20). The direct involvement of these ectodesmata in the penetration of foliarly applied herbicides or nutrients has not as yet been shown; they may function more efficiently at higher humidities in absorption phenomenon.

Summary and Conclusions

Radioautograms were used as a qualitative estimate and counting as a quantitative estimate of the absorption and translocation of C^{14}-labelled 2,4-D and benzoic acid applied to red kidney bean plants for 2, 4, 6, and 8 hours. The compounds were applied as the triethanolamine salts.

With increasing temperatures from 20 to 30° C increased absorption and translocation of both compounds were found. At temperatures of 20, 25, and 30° C less 2,4-D or benzoic acid was absorbed and translocated at low humidities (34–48 %) than at high humidities (70–74 %). The increased absorption and translocation at a higher humidity was correlated with the degree of stomatal opening.

The movement of 2,4-D in the leaf, as indicated by radioautograms, was confined in general to the vascular bundles and followed the route of the assimilate stream out of the leaf and into the stem, bud, and roots. The benzoate-treated plants showed not only a similar movement out of the leaf but also a diffuse movement away from the treated areas to all parts of the treated leaf.

Studies involving extraction of the radioactivity and partitioning in solvents coupled with chromatography showed the bulk of the radioactivity was being translocated as free 2,4-D, and benzoic acid or dissociable salts thereof.

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