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THE MOVEMENT OF LABELLED 2,4-D IN YOUNG BARLEY PLANTS

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Summary. After the 4- or 5-leaf stage in young barley plants, there is a decrease in the translocation of 2,4-D from the leaves to the root system. It is shown that there is no relation between this phenomenon and ear initiation, which occurs at this stage of development. Movement of 2,4-D out of leaves of the tiller in the axils of the 1st and 2nd leaves was also shown to be very small. There is some evidence for a 'block' in the movement of 2,4-D in established grass plants.

La migration du 2,4-D marqué dans l'orge

Résumé. Il est indiqué dans la littérature qu'il existe dans l'orge après le stade 4 à 5-feuilles un ralentissement dans la migration du 2,4-D des feuilles vers le système radiculaire. L'auteur montre dans le présent travail qu'il n'existe aucune corrélation entre ce phénomène et la formation de l'épi qui commence aussi au stade 4 à 5 feuilles. Quand on traite à ce stade les 2 feuilles de la 1re ou 2me talle avec du 2,4-D marqué, on observe peu de migration du 2,4-D vers les racines. On suppose qu'il se produit dans les feuilles développées une adsorption, inhibant la migration du 2,4-D depuis la base des feuilles jusqu'aux racines.

Stoffwanderung von markiertem 2,4-D in jungen Gerstenpflanzen

Zusammenfassung. Nach dem 4- bis 5-Blätter-Stadium hat man eine Abnahme in der Ablagerung von 2,4-D von den Blättern zum Wurzelsystem hin festgestellt. Es wird nachgewiesen, dass dieses Phänomen keine Beziehungen zur Anlage der Ähre hat, welche auch in dieser Entwicklungsstufe stattfindet. Die Ablagerung von 2,4-D aus Blättern am Seitenspross in der Achsel des ersten oder zweiten Blattes ist auch sehr gering. Einiges weist darauf hin, dass der Transport von 2,4-D in ausgewachsenen Gräsern gehemmt wird.

INTRODUCTION

In studies on the penetration and movement of herbicides in plants, experiments with the radioactive isotope ^{14}C occupy a very important place. The incorporation of this isotope in the herbicide molecule allows an easy demonstration of the movement of herbicides or their metabolites. In particular, the macro-autoradiographic technique developed for weed research studies by Crafts (Yamaguchi & Crafts 1958) has contributed greatly to our insight into the behaviour of herbicides in plants.

In the present studies on the movement of herbicides in plants, the autoradiographic technique has been used. Our investigations started from certain questions concerning the movement of radioactive 2,4-D in barley. In the course of these experiments it was realized that 2,4-D could be metabolized in plant tissues and that autoradiographs should therefore be interpreted with caution (cf. Woodford, Holly & McCready 1958). It is believed, however, that for comparing the movement of 2,4-D in plants of various ages or in plants growing under various conditions, labelled 2,4-D may be very useful.

STATEMENT OF THE PROBLEM

The diverse reactions of cereal plants at different stages of growth when treated with 2,4-D and similar auxin type herbicides are generally attributed to differences in the susceptibility of the growth primordium during its vegetative and generative development. In this explanation it is assumed *a priori* that in all growth stages the auxin herbicide can reach the meristematic tissues in sufficient quantity to cause these growth disturbances if the susceptibility is sufficiently great. Several studies point out, however, that the movement of 2,4-D from the leaves of cereal plants to other parts of the plants possesses very peculiar characteristics. Crafts (1959) demonstrated that applications of 0·25 μc of 2,4-D in 0·01 ml droplets to barley leaves resulted in intensive movement of the tracer to the root system if treatment (one droplet) was applied to the 1st, 2nd or 3rd leaf. This was not the case, however, after applications of one droplet to the 4th or 5th leaf. Where the 2nd leaf of plants that had developed four or five leaves were treated, some labelled material was detected in the root system, although there was evidence of less intensive movement than that occurring in plants treated at younger growth stages.

The application of a single droplet of radioactive 2,4-D cannot be regarded as comparable to an overall spray application in the field; however, applying droplets to all leaves instead of only to a single one approaches field conditions more closely. In experiments similar to those performed by Crafts, Petersen (1959) treated each of the main leaves of barley plants with a droplet of radioactive 2,4-D. Under these conditions a typical change in the distribution pattern could be demonstrated at about the 4 to 5-leaf stage of the barley plants, at which far less 2,4-D moved to the root system than in treatments at earlier stages.

Crafts & Petersen did not indicate whether the cereal used in their studies was a spring or a winter variety. Illustrations given by the authors show barley plants with four leaves, not possessing a well-defined tiller in the axil of the 1st leaf, suggesting that in both experiments spring varieties were being studied. If so, the plants must already have reached the reproductive growth stage when treated. It was therefore considered to be of interest to ascertain whether the phenomenon observed by the two authors could possibly be explained by the change from the vegetative to the generative phase of development. In order to obtain a better insight into the importance of tillers present in the axils of the 1st and 2nd leaf for the movement of radioactive 2,4-D to the root system, the effect of treatments of the leaves on these tillers with radioactive material was also studied.

EXPERIMENTAL METHODS

Seeds of the barley varieties Dea (winter) and Herta (spring) were allowed to germinate in moist, non-sterilized sand. At the early 1-leaf stage they were transferred to a non-aerated Hoagland No. 1 culture solution (Hoagland & Arnon 1950).

The first experiment was carried out in a greenhouse in July 1960 under natural daylengths. The second and third experiments were made in controlled

environment rooms in which plants in vessels with nutrient solutions were placed on a turn-table (two rotations per minute) to allow better standardization of environmental conditions and greater safety in applying the isotope solutions. Light was supplied by four 700 watt Philips high-pressure mercury vapour lamps with fluorescent bulbs (light intensity about $1{\cdot}2 \times 10^5$ ergs cm^{-2} sec^{-1}, $\lambda > 0{\cdot}7\mu$). Due to the high intensity of infra-red radiation, the temperature could not be maintained constant during the 16 hours light–8 hours dark regime and fluctuated between 23° C by day and 20° C by night. Relative humidity fluctuated between 65 and 80%.

A solution of 0·01 M 2,4-D, labelled in the methyl carbon of the acetic acid side chain, was prepared in 50% ethyl alcohol containing 0·1% Tween-20 surfactant. Specific activity was adjusted to 0·5 mC/mM. The dosage generally applied was 0·05 μc ^{14}C (22·1 μg 2,4-D) per droplet of 0·01 ml, unless otherwise indicated. Some applications resulted in contact injury, but the symptoms developed so slowly that no influence on the absorption and movement of the chemical is believed to have occurred.

In the first experiment treatments were carried out on plants of spring and winter barley at comparable stages of development, though not on the same day because of differences in the rate of development in the two varieties.

The second and third experiments were carried out with the winter variety only.

The droplets containing radioactive 2,4-D were applied with an Agla micrometer syringe to the upper surface of the leaves, and plants were harvested 1 day later. Before taking the plants from the vessels the treated spots were covered with small pieces of transparent tape to avoid contamination of the paper during subsequent drying and mounting of the plants.

The roots were rinsed in Hoagland culture solution and dried with Kleenex tissue. In the first experiment, roots and tops were separated and press-dried separately. In the other two experiments the individual leaves were also separated to permit determination of radioactivity in individual leaf sheaths. Because of the press-drying procedure, slight movement of labelled material might have taken place after harvesting the plants, but this will have occurred only within, and of course never between, separated plant parts. It is further realized that part of the radioactivity may have been lost through the cut ends. A freeze-drying apparatus would have allowed more exact experiments, but was not available.

The plants were mounted, pressed and autographed as indicated by Yamaguchi & Crafts (1958). After mounting, the plants were covered with a thin layer of mylar (Melinex) to avoid damage to the plants and chemical influences on the film emulsion. The standard film exposure time was 6 weeks. The X-ray film used was Kodak No-Screen X-ray Film, an individually-wrapped, double emulsion film. During exposure excessive pressure was avoided. The films were developed according to the standard procedure for X-ray film for normal contrast.

In the first experiment, determinations were made using a dissecting microscope of the developmental stage of the growing point of the main shoots of plants at growth stages comparable to those treated. The stages recognized

corresponded to the following description of the shoot apex, taken from Bonnett (1936) and also presented by Esau (1953).

A, B and C ..... Various stages in the elongation of the shoot apex before spikelet initiation.
D ..... Elongated shoot tip just before spikelet initiation.
E ..... Early double-ridge stage.
F ..... Double ridges of equal size.
G, H ..... Spikelet primordia developing from upper members of the pairs of ridges.

From other plants in the first experiment the fresh and dry weights of the tops and dry weights of roots were determined. In the second and third experiments no determination of the stage of development nor of fresh or dry weight were made.

RESULTS

Autoradiographs of plants treated at the 1-leaf stage showed almost no radioactivity in the young primary root system. In this first experiment, appreciable movement of labelled material into the roots occurred, after treatment of the 1st and 2nd leaf at the 2-leaf stage of the plants; no differences between the spring and the winter variety of barley were observed. Similar observations were made after treatment of the 1st, 2nd and 3rd leaf at the 3-leaf stage of the plants.

After treatments at the 4-leaf stage in the spring variety (all leaves treated) the entire root system could still be traced on the autoradiographs, but in the more leafy winter variety the intensity in the root system was much lower and only in certain of the very young new roots could appreciable radioactivity be detected. After treatment at the 5-leaf stage, only traces of radioactivity could be detected in the longest roots, and only the young roots showed greater activity. Although the winter variety at this stage had more tillers than the spring variety, there were no great differences between the autoradiographs of the two.

For the first experiment the stage of development of the growing point of the plants at the moment of applying the radioactive product is indicated in Fig. 1, which represents the root/shoot dry weight ratio at the various times of treatment. Fig. 2 gives the increase in total and shoot dry-matter production in both varieties.

In the second experiment, carried out in the controlled environment room, an investigation was made of whether giving increased amounts of radioactivity to only the 4th or the 5th leaf of plants at the 4- or 5-leaf stage of growth would result in movement of the tracer to the root system. It appeared that applications of three 0·01 ml droplets each containing 0·05 μc of 2,4-D to the 4th leaf did not lead to distinct autoradiographs of the root system. Even where the 5th leaf of winter barley plants was treated with 0·04 ml (four droplets with 0·05 μc of 2,4-D each) almost no radioactivity could be detected in the roots (Plate 7).

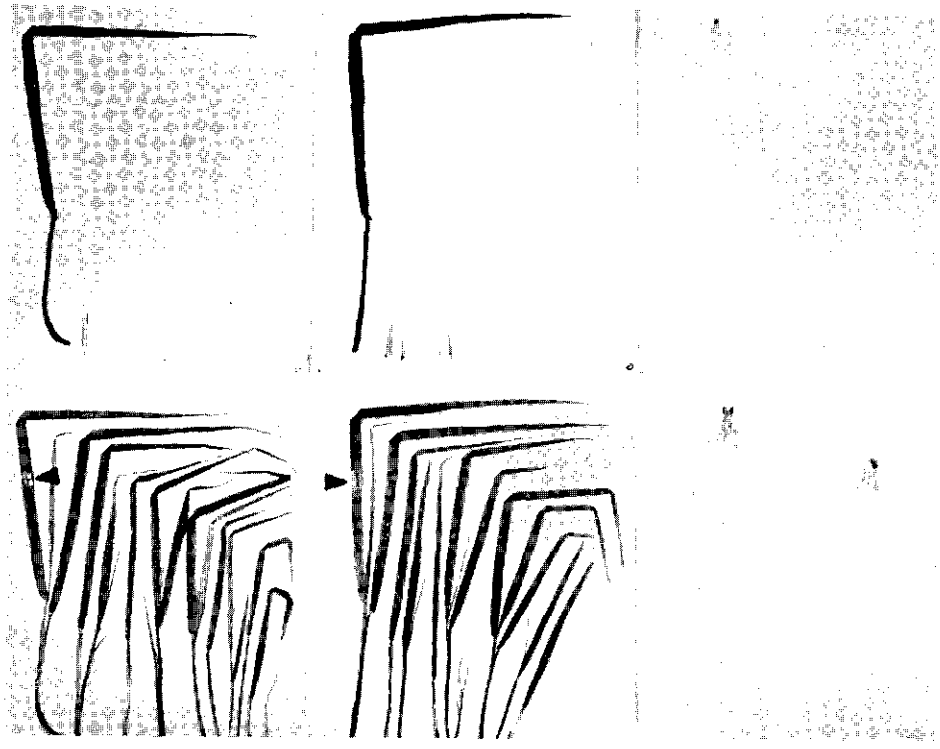

PLATE 7. Barley plants of the winter variety Dea treated at the 5-leaf stage on the 5th leaf with 4 0.01 ml droplets containing 0·01 M 2,4D. Dosage was 0·2 μc per leaf. Sp. activity 0·5 mc/mM.

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In both treatments (to the 4th and to the 5th leaf) some radioactive tracer was noted in all non-treated leaves, with decreasing intensity from the 3rd and 2nd leaf and their tillers, to the 1st leaf and its tiller.

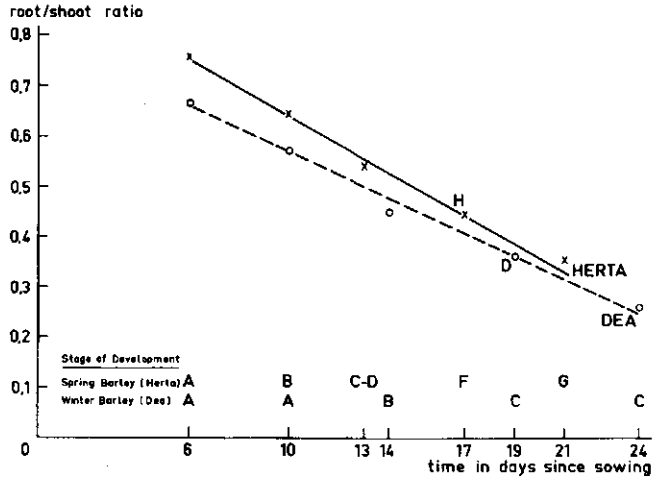

FIG. 1. Root/shoot dry-weight ratio of barley plants harvested at (from left to right) the 1-, 2-, 3-, 4- and 5-leaf stage.
Herta = spring variety, Dea = winter variety. Stage of development of the growing point: symbols described in text.

In the third experiment the tillers of the 1st leaf of plants at the 4- and 5-leaf stage were treated in various ways, but treatment even of both leaves of the

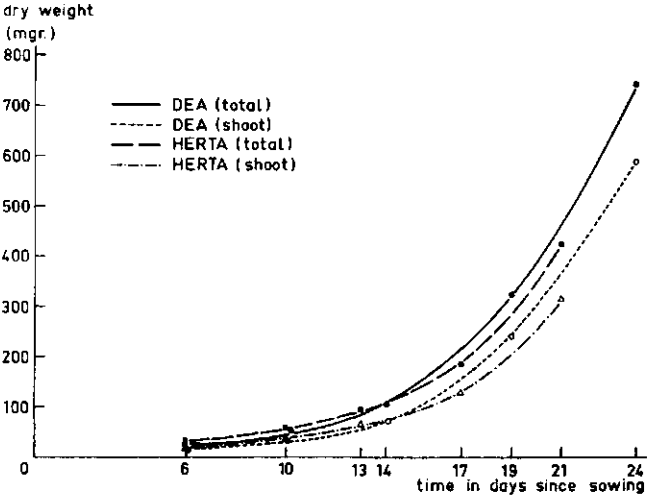

FIG. 2. Total dry matter production and dry matter production of the shoot of plants harvested at (from left to right) the 1-, 2-, 3-, 4- and 5-leaf stage of barley plants.
Herta = spring variety, Dea = winter variety.

tillers with droplets containing 0·05 μc of 2,4-D did not result in any appreciable activity in other parts of the plants (Plate 8, A). This same observation was made in studies on the movement of 2, 4-D from tillers of the second leaf (Plate 8,

B, C and D). In this experiment, treatment of both leaves of this tiller did not result in any radioactive tracer in the root system and only very weak activity in other leaves of the plants, mainly in the 2nd tiller of the 2nd leaf.

DISCUSSION

It is generally agreed that for the movement of 2,4-D and similar phenoxyacetic acids from leaves to other plant parts, translocation of photosynthates must take place (Crafts 1961). It is also known that the adsorption of auxin type herbicides by plant constituents may be a very important factor in understanding the behaviour of these products in plants (Brian 1960). Taking both points of view into consideration, the amount of radioactive tracer present in a specific tissue will be the total of the locally adsorbed molecules and the molecules in process of being moved through the tissue at the moment of harvesting.

In the first experiment, the importance of photosynthate movement for 2,4-D translocation is not apparent, since radioactive tracer is absent from the roots of young barley plants treated at the 1-leaf stage. This absence can be understood, however, by assuming that at this stage the seed dominates the early development of the root system. The function of the young leaf in supplying the root system with photosynthates is entirely overshadowed by the supply of reserve material by the seed. It is realized, however, that adsorption processes in the root-shoot interzone could also account for the lack of movement to the roots.

At the 2- and 3-leaf stage the seed is exhausted and consequently the foliage has become the only source of respiration substrates and material required for the further development of the root system. At these stages, good movement of 2,4-D to the root system can be observed. The importance of photosynthesis for this movement is indicated by other experiments of the present author, the results of which will be the subject of a future paper. In these experiments with plants at the 2-leaf stage of development, adding small amounts of simazine, atrazine or monuron to the nutrient solutions 1 day prior to 2,4-D treatment of the leaves inhibited the movement of 2,4-D to the root system under conditions in which control treatments on normal culture solutions showed considerable transport of the labelled 2,4-D. The addition of these herbicides to the culture solution is known to inhibit specific steps in the photosynthesis process (Crafts 1961).

In the first experiment at the 3-leaf stage, both the winter and the spring varieties demonstrated intensive movement of 2,4-D to the root system. At this stage the winter variety was still in the vegetative and the spring variety already in the reproductive stage of primordium development. In the treatments carried out at the 4- and 5-leaf stage, the winter barley was still in a vegetative growth stage, while the spring barley had already a well-developed ear primordium. It appears, therefore, that the change in the distribution pattern of 2,4-D is independent of the important phenomenon of ear initiation.

In the second experiment, unfortunately, increasing the amount of radioactive tracer also entailed an increase in the amount of growth regulator applied. This may have resulted in a greater morphological influence of the treatment on connecting tissues, thus influencing the movement of the tracer

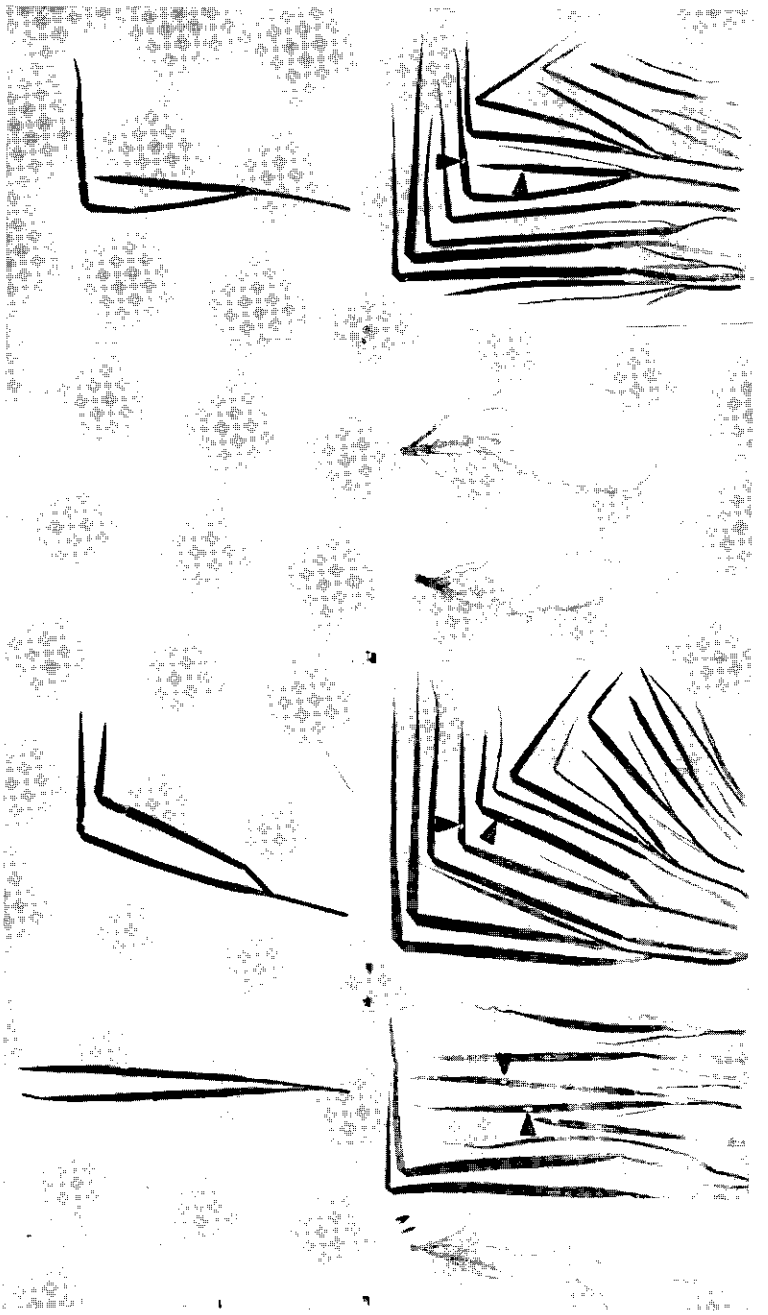

PLATE 8. Barley plants of the winter variety Dea treated on both leaves of the tiller of the 2nd leaf of the main stem. In A the plant was treated at the 4-leaf stage, in B and D at the 3-leaf stage. C = roots of plants B and D. Dosage was 0·05 μc per leaf. Sp. activity 0·5 mc/mM.

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through the plant. It is very difficult to decide whether this influence did really occur. It can only be stated that our results were in agreement with those of Crafts (1959), in whose experiments a dosage of 0·25 μc (sp. activity 5 mc/mM) to the 4th leaf and of 0·05 mc (sp. activity 0·5 mc/mM) to the second leaf of plants at the 4-leaf stage of growth did not result in any appreciable amount of tracer being moved to the root system.

It was originally thought that during the further development of the plants, great importance might have to be attached to the tillers in the axils of the youngest leaves in supplying the root system with photosynthates. This theory was not supported by the results of the third experiment. Studies with labelled urea, CO_2 or carbohydrates would be necessary to demonstrate whether carbohydrates are translocated in great amounts from the tillers to the main stem and the root system, but the absence in our experiment of any evidence of 2,4-D movement to the root system does not seem to be compatible with the theory that in such a movement the translocation of photosynthates is the only important factor. Even to a much stronger degree than in treatments of the 4th and 5th leaf with radioactive 2,4-D there seems to be 'block', preventing 2,4-D distribution to parts of the plant other than those treated.

Fig. 1 and 2 clearly show that in barley the changing root/shoot ratio reflects a change in the distribution of organic matter during the development of the plant. This could support the suggestion of Crafts (1959) that the gradual change in photosynthate movement is the cause of changes in 2,4-D distribution. Our results are, however, also in agreement with the opinions of Andersen (1958). In *Cyperus rotundus*, he observed a definite 'block', preventing movement of labelled 2,4-D from the treated leaves to points beyond their base. The same observation could be made for 2,4,5-T. Amitrole and maleic hydrazide, on the other hand, moved through the entire plant after being applied in a manner similar to 2,4-D in the present experiments. Our data do not bring any supporting evidence to the suggestion of Andersen that the phenomenon of intercalary growth is in some way connected with the 'blocking effect'. Microautoradiographic studies should help in elucidating further the degree of movement through the tissues connecting leaf sheaths and main stem and the translocation of auxin herbicides to the meristematic stem-tip region.

The difference in behaviour of various herbicides observed by Andersen and others (Crafts 1961) points to the important fact that in the control of monocotyledonous plants with foliage-applied systemic herbicides, these chemicals should not possess highly peculiar adsorption characteristics which prevent them reaching the root system in sufficient amounts.

In dicotyledonous plants, also, there are some indications of restricted movement of 2,4-D from the shoot to the root system. The bio-assay data of Hay and Thimann (1956) on restricted movement of 2,4-D in *Phaseolus* beans beyond the stem base was confirmed with labelled 2,4-D by the present author, only where the 2,4-D was applied to beans growing in non-aerated culture solutions (Van der Zweep, unpublished). Under aerated conditions, good autoradiographs of the root system were always obtained. This seems to be in good agreement with the data of Brouwer (1960), indicating a great susceptibility of *Phaseolus* growth to aeration of the root system.

The recent publication of Canny & Markus (1960) on the movement of 2,4-D in *Vicia faba* var. *minor* beans points to a possible fixation of 2,4-D in the top part of the root system preventing movement of the chemical to the root system and preceding intensive breakdown. This phenomenon could be of a nature similar to that observed in monocotyledons. It is of importance to study whether in grasses also a higher rate of breakdown of 2,4-D in the roots than in the shoots could account for absence of tracer in autoradiographs of root systems. However, from our third experiment, in which the leaves of auxiliary tillers showed very restricted movement of 2,4-D beyond the leaf base, one could also conclude that adsorption of the tracer is taking place in tissues above the top part of the root system.

There are several indications that also other phenoxy-herbicides will also show translocation characteristics similar to those observed for 2,4-D and 2,4,5-T. In the phenoxypropionic acids mecoprop and 2,4,5-TP, the consistent absence after leaf applications of the development of typical 'onion'-leaves and similar auxin symptoms in cereals and other grasses (cf. Sen 1960) could be due to an extreme degree of adsorption or trapping of the chemical, thus not allowing the chemical to reach the stem tips.

In conclusion, we can state that in barley plants after the 3- to 4-leaf stage, the decrease in the degree of movement of radioactive 2;4-D to the root system is not determined by the change from the vegetative to the reproductive growth stage. The function of the tillers in translocating to the root system 2,4-D applied to their leaves is very problematical. The function of these tillers in supplying photosynthates to the root system needs further investigation. There is some evidence of a 'block' at the base of older leaves, preventing 2,4-D from moving to other plant parts.

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