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Effect of Stage of Seedling Development on Absorption of Selected Pre-Emergent Herbicides

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EFFECT OF STAGE OF SEEDLING DEVELOPMENT ON ABSORPTION OF SELECTED PRE-EMERGENT HERBICIDES

by

Pairoj Suchinda

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of the requirements for the degree

of

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Pairoj Suchinda

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ABSTRACT

Effect of Stage of Seedling Development on Absorption

of Selected Pre-Emergent Herbicides

by

Pairoj Suchinda, Master of Science

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Major Professor: Dr. J. LaMar Anderson
Department: Plant Science

Using radioautography ^{14}C simazine, ^{14}C EPTC, ^{14}C 2,4-D, ^{14}C DCPA and ^{14}C pyrazon absorption by seeds of bean (Phaseolus vulgaris L.), squash (Cucurbita maxima), corn (Zea mays), onion (Allium cepa) and oat (Avena sativa) was studied. Simazine, EPTC, 2,4-D and pyrazon were absorbed through the seed coat of bean, squash, corn and onion, the amount increasing with time. Little DCPA was absorbed by seeds of bean, squash, onion and oat. Distribution and translocation of ^{14}C simazine, ^{14}C EPTC and ^{14}C pyrazon occurred in the young plants of bean, squash, corn and onion. Simazine and pyrazon accumulated in the leaf margins of bean, EPTC and pyrazon were found in the cotyledon margins of squash, and pyrazon was found in the coleoptile tips and leaf tips of corn. The translocation of ^{14}C 2,4-D in bean, onion and squash showed a characteristic fixation along the path of the translocation. Very little translocation or distribution of ^{14}C DCPA were found in bean, squash, onion or oat.

In bean leaf, ^{14}C pyrazon was absorbed and moved acropetally in the apoplast. Basipetal movement of ^{14}C pyrazon was limited.

Microradioautography was used to determine the tissues involved in the translocation of ^{14}C simazine, ^{14}C EPTC, ^{14}C 2,4-D and ^{14}C pyrazon. Simazine and EPTC were found in the vascular bundle tissues of bean leaf. EPTC was found in the cortex of squash root. 2,4-D was found in the vascular tissues and surrounding the vascular bundle of squash cotyledon and onion hypocotyl. Pyrazon was found intercellularly and intracellularly in bean cotyledon.

(63 pages)

INTRODUCTION

Most of the experimental work with recently developed herbicides has been aimed at evaluating their effectiveness as herbicides. Little is known concerning the stage of development at which absorption of these herbicides takes place. Since soil applied herbicides come in contact with seeds, the herbicide may enter the seed. It is, therefore, the purpose of this investigation to study in detail the effect of stage of seedling development on absorption of 5 soil applied herbicides, simazine, EPTC, 2,4-D, pyrazon and DCPA.

Bean (Phaseolus vulgaris L.), squash (Cucurbita maxima), corn (Zea mays), onion (Allium cepa) and oat (Avena sativa.) seeds and seedlings were used in this study. Attention was given to the absorption of each herbicide to determine if it enters through the seed coat, epicotyl, hypocotyl, radicle or root. Particular attention was given to the translocation and accumulation of each herbicide within seedlings.

Understanding of absorption, translocation and accumulation of these herbicides will aid in understanding damage from the residual herbicides on germinating seedlings and subsequent seedling growth.

REVIEW OF LITERATURE

Herbicides can be divided into several categories on the basis of translocation characteristics (Leonard, 1963): (1) those that do not translocate, (2) those that translocate only in the symplast, (3) those that translocate either in symplast or apoplast, and (4) those that translocate only in the apoplast.

Simazine

Leonard, Linder and Glenn (1966) found that the Thompson Seedless (Sultanima) roots absorbed simazine and translocated it only in the xylem or cell walls (apoplast).

Crafts (1961) observed that simazine apparently penetrates the cuticle readily but subsequently moves only in an acropetal direction by diffusion along cell walls and does not enter readily the living symplast. Hilton, Jansen and Hull (1963) stated that movement of simazine in the phloem, if it occurs at all, seems negligible.

Crafts (1966) found that the bean leaf absorbs simazine and moves it acropetally in the apoplast forming a wedge-shaped pattern; no simazine enters or moves in the symplast. Bean roots absorb simazine rapidly and transport it to tops where it accumulates in high concentration. Barley leaves also absorb and transport simazine via the apoplast; none moves to roots via the symplast. Simazine enters barley roots rapidly and moves freely to the foliage; there is no evidence for recirculation.

Davis et al. (1959) and Sheets (1961) have shown that ^{14}C simazine is readily absorbed from nutrient solutions and moves with the transpiration stream to the leaves, and accumulates first at the leaf tips of oats or leaf margins of cucumber. But the ^{14}C label (probably in the degradation products) is distributed throughout the entire leaf of maize (Davis et al., 1959), a simazine tolerant species.

Radioactive tracer techniques showed that simazine readily entered the root of one-month-old red pine seedlings and moved rapidly from roots to stems and needles, and the accumulation of the simazine was highest in roots, intermediate in stems and lowest in needles (Dhillon, Byrnes and Merritt, 1966).

EPTC

Recent research by Dawson (1963) has shown that leaf tissue is the main site of EPTC uptake in barnyard grass as well as the prime site of injury. Exposing the seed and primary root to EPTC did not lead to injury, whereas exposing the shoot or only the coleoptile did.

Appleby, Furtick and Fang (1965) found that in Victory oat seedlings (Avena sativa), both roots and coleoptiles readily absorbed EPTC from soil and that measurable amounts translocated both upward and downward.

The result obtained by Appleby and Furtick (1962) indicated that the primary route of entry of EPTC appears to be through the developing epicotyl of grass seedlings. Fang and Theisen (1959) and Yamaguchi (1961)

concluded that root absorption occurs readily but seems of secondary importance to effective action of the herbicide.

2,4-D

In studies on translocation it has always been observed that 2,4-D distribution in any plant is more limited than that of amitrole or maleic hydrazide (Yamaguchi, 1965). Yamaguchi (1965) found that concentration in and around the translocating vascular tissues near the point of application is commonly high but steeply declines with increasing distance from the application point. Often translocation of 2,4-D is limited, it may not reach the buds or root tips. Crafts and Yamaguchi (1958) found that the gradient is usually steep under conditions attending a slow rate of growth. The view that active absorption by the vascular and cortical parenchyma depletes the 2,4-D being carried by the seive tubes, agrees with the subsequent observation that 2,4-D applied to the culture solution at a concentration of 10^{-6} M or less does not move upward into the shoots of the plant, except in extremely small concentrations. It is retained in the root. Yamaguchi (1965) concluded that as long as 2,4-D is absorbed by the symplast there can be no 2,4-D in the transpiration stream. Any translocatory activity of the phloem would presumably carry root-applied 2,4-D toward the root tips (where growth is occurring).

Crafts (1967) studying the transport mechanism in soybean seedlings (Glycine max)using ^{14}C labeled tracers, found that 2,4-D movement is largely restricted to the phloem.

Rincón (1966) who investigated the translocation of 2,4-D in groundnut, tomato and rice plants, found that 2,4-D applied to leaves moves toward regions of rapid growth through the phloem. When applied to the culture solution it accumulates in the roots with very little of it moving to the foliage.

Pyrazon

Stephenson and Ries (1967) applied pyrazon labeled with tritium in the phenyl ring to both the root and shoot of seedlings of red beet, German millet and tomato. They found that the greatest root absorption and translocation to the shoot occurred in tomato and the least in red beet, while millet was intermediate. Applications to the first true leaf indicated that pyrazon was absorbed by the foliage of all three but that it was not transported basipately. They concluded that pyrazon was transported primarily in the xylem.

DCPA

The mobility of DCPA through xylem in some plants such as bermudagrass is very low. Bingham (1967) found that when DCPA was placed at various depths of soil, it did not affect the rooting of stolons above the level of the herbicide but reduced it at lower levels.

Nishimoto et al. (1967) used oat seeds to find the site of uptake of DCPA and concluded that the herbicide was active on oat primarily through xylem in some plants.

MATERIALS AND METHODS

Seeds

Tendercrop snap bean (Phaseolus vulgaris L.), warted hubbard squash (Cucurbita maxima), corn (Zea mays), white sweet spanish onion (Allium cepa) and oat (Avena sativa) seeds were selected as the experimental material.

Herbicides

The following ^{14}C labeled herbicides were used:

2,4-D (2,4-dichlorophenoxyacetic acid) with a specific activity of 11.1 μc/mg,

Simazine (2, chloro-4,6-bisethylamino-1,3,5-triazine) with a specific activity of 7.8 μc/mg,

EPTC (S-ethyl NN-diprophylthiolcarbamate) with a specific activity of 1.33 mc/m mole,

Pyrazon (5-amino-4-chloro-2-phenyl-3-pyridazone) with a specific of 19.2 μc/mg,

DCPA (dimethyl 2,4,5,6-tetrachloroterephthalate) with a specific activity of .137 μc/mg.

Concentrations were adjusted to the following:

Herbicides	Concentrations (ppm)
2,4-D	2.2
Simazine	20

EPTC	189.3
Pyrazon	100
DCPA	25

Seed treatment

Four uniform seeds with intact seed coats, of each species (bean, squash, corn and onion) were placed in plastic boxes (4.5 x 4.5 x 1, 1 inch high and 4.5 inches wide). Each box containing an aqueous solution of ^{14}C 2,4-D, ^{14}C EPTC, ^{14}C pyrazon or ^{14}C DCPA. Each species were soaked in those aqueous solutions for varying periods of time (1 hour, 2 hours, 4 hours, 8 hours and 20 hours) at the constant temperature of 75° F. Oat seeds were soaked in aqueous solution of ^{14}C DCPA and the treatment times were 1 hour, 2 hours, 4 hours, 8 hours, 20 hours and 48 hours.

The seeds of each exposure time were then washed in running tap water for an hour. Following this the seeds of each treatment were planted one-half inch deep in pots containing water-saturated vermiculite in growth chambers. The growth chambers used for growing treated plants were programmed for a relative humidity of 40 to 50 per cent and alternating 12-hour periods of light at 75° F and dark at 65° F. The plants were watered every other day. Plants were harvested 12 to 25 days after treatment. Harvested plants were mounted on paper and pressed. The dried plants were mounted on white paper and exposed to Kodak Medical Royal Blue X-ray film for 1 month. Standard procedures were used to develop the film.

Plant treatment

Bean seeds were planted in pots containing water-saturated vermiculite and then placed in a growth chamber at an approximate day temperature of 75° F and night temperature of 65° F and relative humidity of 40 to 50 per cent.

The leaf applications were made at the margins of the unifoliate leaves by dipping them into a 25 ppm solution of ^{14}C pyrazon. Plants were harvested 8 and 48 hours after treatment and mounted on paper and pressed. The dried plants were mounted on white papers, pressed and placed against Kodak Medical Royal Blue X-ray film, for 2 weeks exposure. Standard procedures were used to develop the film.

Micro-radioautography

The collection and processing of all tissues used for micro-radioautography was carried out in the following manner. The roots, leaves, stems and cotyledons of the experimental seedlings were collected. All material collected was frozen in iso-pentane with liquid nitrogen. The frozen tissues were sectioned to 16 microns on a microtome cryostat, CTD, were placed on microscope slides, and were freeze-dried in a thermovac freeze dryer for 10 hours.

All subsequent steps in the process of micro-radioautography were carried out under light filtered through a Wratten Safelight, series 2 filter. Ilford L-4 emulsion in gel form was diluted 1:2 with distilled water, and heated at 55° C until dissolved. The specimen slides were coated by means

of a fine wire loop which was dipped into the emulsion and then slowly withdrawn leaving a thin layer of the diluted emulsion in the loop. The emulsion was then placed in the specimen slide by passing the loop down over the stationary slide.

After coating the slides were placed in a light-tight box and exposed for 10 days.

The radioautograms were then developed for 5 minutes in Eastman Kodak D-19 developer, rinsed in tap water, fixed in acid fixer for 5 minutes and thoroughly rinsed in running tap water for 30 minutes.

RESULTS

Radioautography

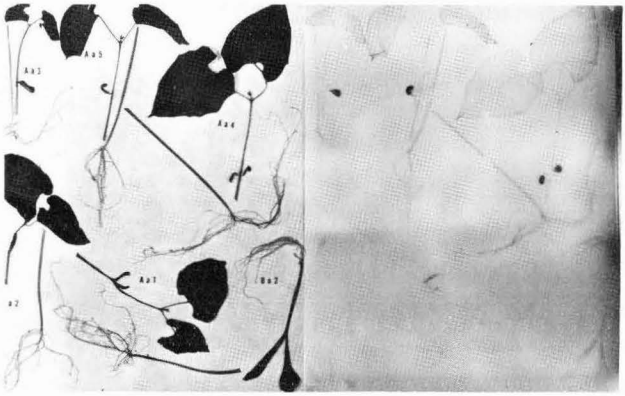
Seeds of bean treated with ^{14}C simazine. The distribution of ^{14}C simazine following its imbibition by dry bean seed is shown in Figure 1. The cotyledons contained most of the ^{14}C activity in every treatment. The label in the roots and aerial portions gave a very clear image on the radioautograms; moreover, the density increased greatly from the 1 and 2 hour treatments through the 4, 8 and 20 hour treatments. This indicated that with longer treatment time more simazine entered the seed or developing seedling. The accumulation of radioactivity in the leaf margins became strongly evident.

Seeds of squash treated with ^{14}C simazine. Plant mounts and radioautographs in Figure 1 and Figure 2 show the distribution of ^{14}C in squash seedlings. The image of the seedlings on the radioautographs following 1, 2 and 4 hour exposures to the labeled herbicide show similar distribution patterns. In these there was little radioactivity in any part of the seedlings. Following 8 and 20 hour treatments accumulation in the roots and leaves became strongly evident as in the case of simazine treated beans. There was evidence of herbicide accumulation in the leaf margins.

Seeds of corn treated with ^{14}C simazine. Mounted plants and radioautographs in Figure 3 show the distribution of ^{14}C simazine in corn seedlings. In the 2 and 4 hour (Ca2 and Ca3) treatments, the label in the seedlings gave moderately clear images on the radioautograms and show a uniform

Figure 1. Distribution of ^{14}C simazine from treated seeds of bean (Aa. .) and squash (Ba. .). Treatment time for beans, 1, 2, 4, 8 and 20 hour (Aa1, Aa2, Aa3, Aa4, and Aa5), and for squash, 2 hour (Ba2). Radioautographs are at right, mounted plants left.

Figure 2. Distribution of ^{14}C simazine from treated seeds of squash (Ba. .) and onion (Da. .); distribution of ^{14}C EPTC from treated seeds of onion (Db. .). Treatment time for squash, 1, 4, 8 and 20 hour (Ba1, Ba3, Ba4 and Ba5), for onion, 1, 2, 4, 8 and 20 hour (Da1, Da2, Da3, Da4 and Da5) and for onion, 1, 2, 4, and 8 hour (Db1, Db2, Db3 and Db4). Radioautographs are at right, mounted plants left.



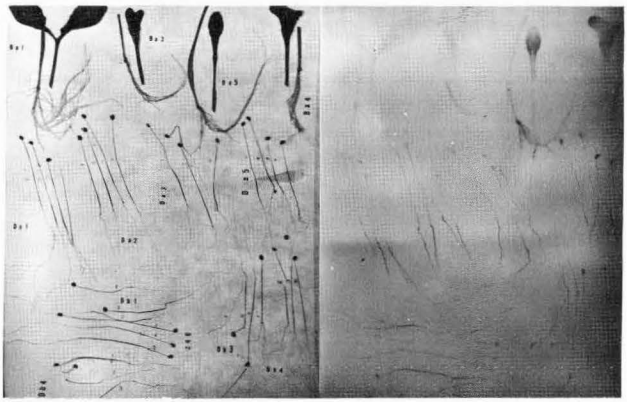
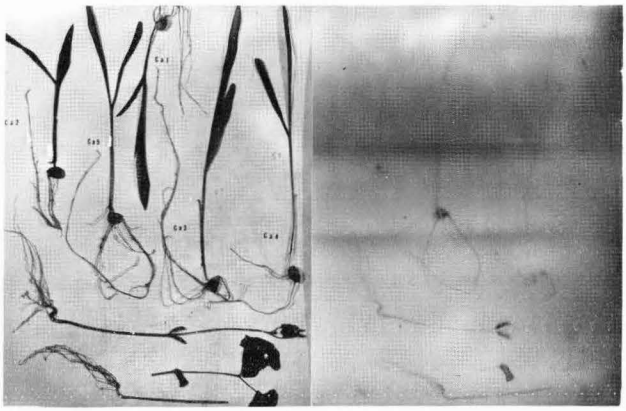
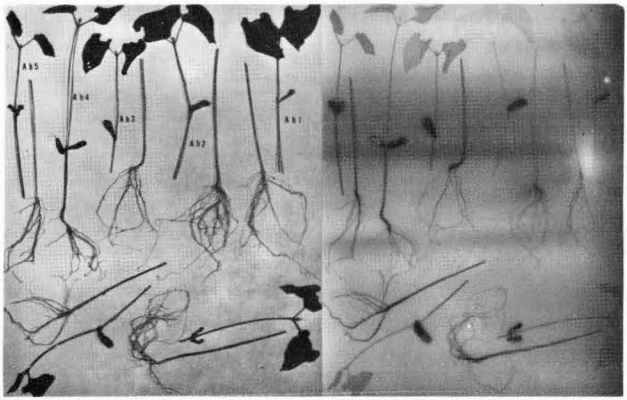


Figure 3. Distribution of ^{14}C simazine from treated seeds of corn (Ca. .). Treatment time, 1, 2, 4, 8 and 20 hour (Ca1, Ca2, Ca3, Ca4 and Ca5). Radioautographs are at right, mounted plants at left.

Figure 4. Distribution of ^{14}C EPTC from treated seeds of bean (Ab. .). Treatment time, 1, 2, 4, 8 and 20 hour (Ab1, Ab2, Ab3, Ab4 and Ab5). Radioautographs are at right, mounted plants at left.


Gx1
Gx2
Gx3
Gx4


Ab5
Ab4
Ab3
Ab2
Ab1

distribution throughout the plants. In the 8 and 20 hour (Ca4 and Ca5) treatments the accumulation of radioactivity throughout the plants was even more evident. There was some evidence of accumulation at the leaf tips, but accumulation was not as pronounced as in treated seedlings of bean or squash.

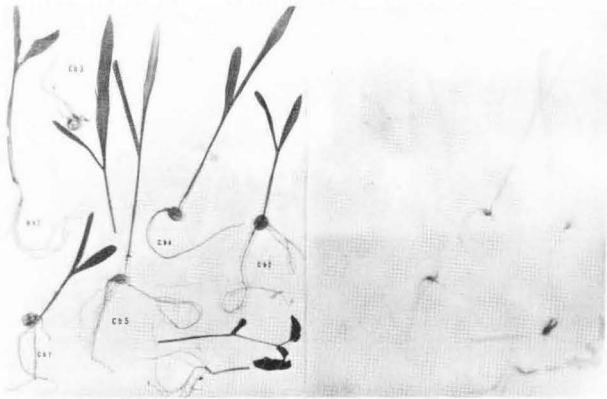
Seeds of onions treated with ^{14}C simazine. Plant mounts and radioautographs in Figure 2 (Da1, Da2, Da3, Da4 and Da5) show the distribution of ^{14}C simazine in onions following 1, 2, 4, 8 and 20 hour seed treatments. The accumulation of the radioactivity in the roots of all seedlings is very evident on the radioautogram. In the 8 and 20 hour treatments (Da4 and Da5), the label in the shoots gave very clear images on the radioautogram indicating a greater accumulation of the isotope.

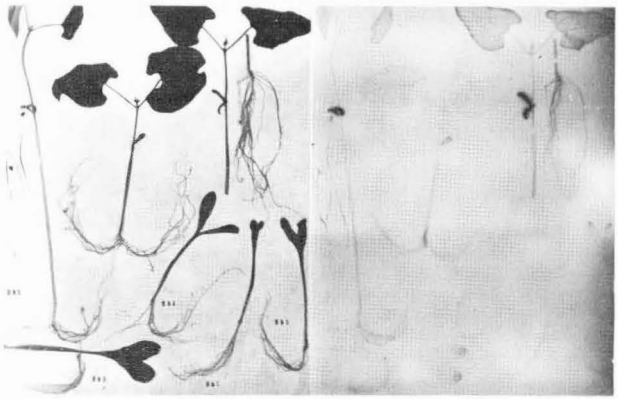
Seeds of bean treated with ^{14}C EPTC. Plant mounts and radioaugtograms in Figure 4 (Ab1, Ab2, Ab3, Ab4 and Ab5) show the distribution of ^{14}C EPTC in bean seedlings following 1, 2, 4, 8 and 20 hour seed treatment, respectively. In the 1 and 2 hour treatments (Ab1 and Ab2) the radioactivity was distributed throughout the plants and gave a light image on the radioautogram. With more time, 4, 8 and 20 hour treatments (Ab3, Ab4 and Ab5), the image of the isotope in the seedlings were somewhat darker than Ab1 and Ab2, and the accumulation of the radioactivity was primarily in the hypocotyls and in the roots.

Seeds of squash treated with ^{14}C EPTC. Mounted plants and radioautographs in Figure 6 (Bb1, Bb3, Bb4 and Bb5) and in Figure 5 (Bb2) show the distribution of ^{14}C EPTC in squash seedlings following seed treatment. Following 1 hour (Bb1) seed treatment the label distribution was spread

Figure 5. Distribution of ^{14}C EPTC from treated seeds of corn (Cb. .) and squash (Bb. .). Treatment time for corn, 1, 2, 4, 8 and 20 hour (Cb1, Cb2, Cb3, Cb4 and Cb5) and for squash 2 hour (Bb2). Radioautographs are at right, mounted plants at left.

Figure 6. Distribution of ^{14}C EPTC from treated seeds of squash (Bb. .) and onion (Db. .). Treatment time for squash, 1, 4, 8 and 20 hour (Bb1, Bb3, Bb4 and Bb5) and for onion, 20 hour (Db5). Radioautographs are at right, mounted plants at left.





throughout the plant. Seedlings from 4, 8 and 20 hour (Bb3, Bb4 and Bb5) treated seeds showed a greater accumulation of radioactivity, judging from the image density. The ^{14}C EPTC tended to accumulate in the leaf tips (Bb3 and Bb5).

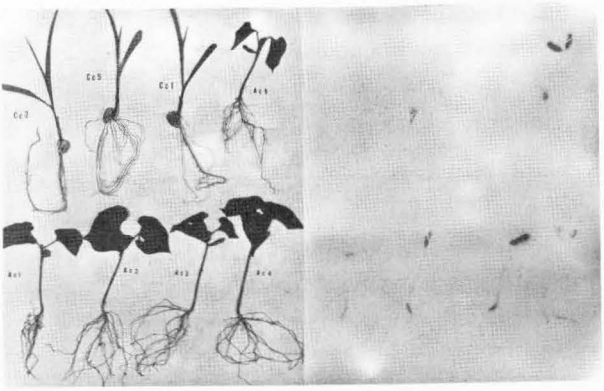
Seeds of corn treated with ^{14}C EPTC. Plant mounts and radioautographs in Figure 5 (Cb1, Cb2, Cb3, Cb4 and Cb5) show the distribution of ^{14}C EPTC in corn seedlings following 1, 2, 4, 8 and 20 hour seed treatment. In seedlings from 1 and 2 hour treated seeds (Cb1 and Cb2), the label gave only a faint image on the radioautogram. With more time (4, 8 and 20 hour treatments), the distribution and the accumulation of radioactivity in seedlings became more evident. Following the longest exposure (20 hour, Cb5), the accumulation of the label seemed to be in the leaf tips.

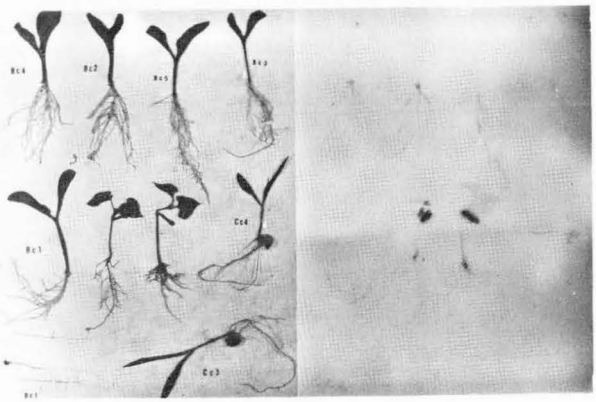
Seeds of onion treated with ^{14}C EPTC. Mounted plants and radioautographs in Figure 2 (Db1, Db2, Db3 and Db4) and in Figure 6 (Db5) show the distribution of ^{14}C EPTC in onion seedlings following 1, 2, 4, 8 and 20 hour seed treatment. In the 1 hour treatment (Db1) the isotope was distributed throughout the plant and gave a light image on the radioautogram. With more time, 2, 4 8 and 20 hour treatments (Db2, Db3, Db4 and Db5), the image of the activity in the seedlings was somewhat darker than that Db1, and the accumulation of the isotope in the roots was evident.

Seeds of bean treated with ^{14}C 2,4-D. Plant mounts and radioautographs in Figure 7 (Ac1, Ac2, Ac3, Ac4 and Ac5) show the distribution of ^{14}C labeled 2,4-D in bean seedlings following 1, 2, 4, 8 and 20 hour seed treatment. In seedlings from 1 hour treated seed the isotope in the hypocotyl gave a clear image on the radioautogram. With more time (2, 4, 8 and 20

Figure 7. Distribution of ^{14}C 2,4-D from treated seeds of bean (Ac. .) and corn (Cc. .). Treatment time for bean, 1, 2, 4, 8 and 20 hour (Ac1, Ac2, Ac3, Ac4 and Ac5) and for corn, 1, 2, and 20 hour (Cc1, Cc2 and Cc5). Radioautographs are at right, mounted plants left.

Figure 8. Distribution of ^{14}C 2,4-D from treated seeds of squash (Bc. .), corn (Cc. .) and onion (Dc. .). Treatment time for squash, 1, 2, 4, 8 and 20 hour (Bc1, Bc2, Bc3, Bc4 and Bc5), for corn, 4 and 8 hour (Cc3 and Cc4) and for onion, 1 hour (Dc1). Radioautographs are at right, mounted plants left.





hour), the accumulation of radioactivity in the hypocotyls became evident. The label in the roots and leaves gave only a faint image on the radioautogram.

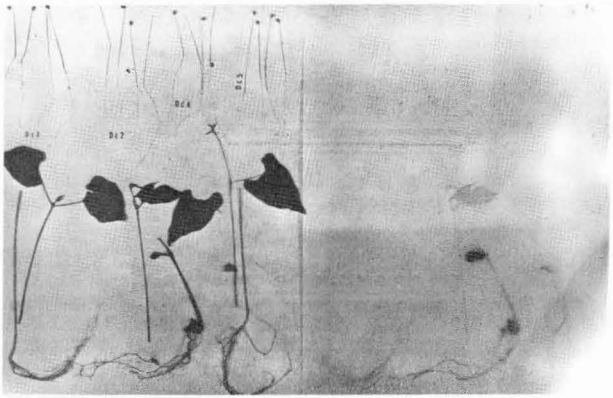
Seeds of squash treated with ^{14}C 2,4-D. Plant mounts and radioautographs in Figure 8 (Bc1, Bc2, Bc3, Bc4 and Bc5) show the distribution of ^{14}C 2,4-D in squash seedlings following 1, 2, 4, 8 and 20 hour seed treatment. In the 1 hour (Bc1) treatment, the label in the roots gave a very clear image on the radioautogram. With more time (2, 4, 8 and 20 hour), the accumulation of ^{14}C in the roots became strongly evident. The isotope in the shoots gave only a faint image on the radioautogram.

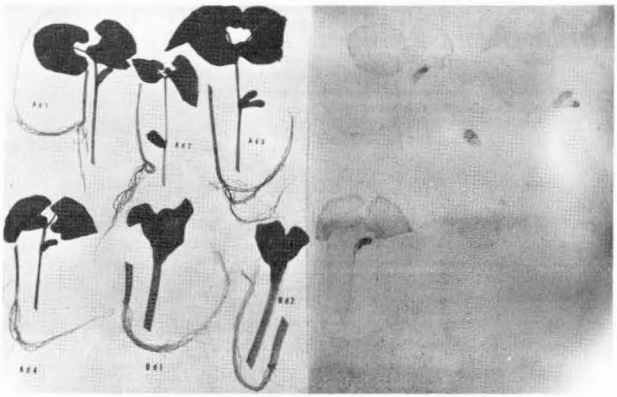
Seeds of corn treated with ^{14}C 2,4-D. Mounted plant and radioautogram in Figure 7 (Cc5) shows the distribution of ^{14}C 2,4-D in corn seedling following 20 hour seed treatment. No image of the plants following 1, 2, 4 and 8 hour seed treatment was shown on the radioautogram. In seedling from 20 hour treated seed, only isotope in the seed gave an image on the radioautogram. No evidence of translocation of 2,4-D was found.

Seeds of onion treated with ^{14}C 2,4-D. Plant mounts and radioautograms in Figure 9 (Dc3, Dc4 and Dc5) show the distribution of ^{14}C 2,4-D in onion seedling following 4, 8 and 20 hour seed treatment. In the 1 hour seed treatment (Figure 8, Dc1) and 2 hour seed treatment (Figure 9, Dc2), no image of the plants was seen on the radioautogram. In seedlings from 4, 8 and 20 hour treatments (Dc3, Dc4 and Dc5), the accumulation of the activity in the hypocotyls became strongly evident.

Figure 9. Distribution of ^{14}C 2,4-D from treated seeds of onion (Dc. .). Treatment time, 2, 4, 8 and 20 hour (Dc2, Dc3, Dc4 and Dc5). Radioautographs are at right, mounted plants left.

Figure 10. Distribution of ^{14}C pyrazon from treated seeds of bean (Ad. .) and squash (Bd. .). Treatment for bean, 1, 2, 4, and 8 hour (Ad1, Ad2, Ad3 and Ad4) and for squash, 1 and 2 hour (Bd1 and Bd2). Radioautographs are at right, mounted plants left.





Seeds of bean treated with ^{14}C DCPA. Plant mounts and radioautograms in Figure 14 (Ae1, Ae2 and Ae3) and in Figure 12 (Ae4 and Ae5) show the distribution of ^{14}C DCPA in bean seedlings following 1, 2, 4, 8 and 20 hour, respectively. In seedlings from 2, 4, 8 and 20 hour seed treatment, the label in the roots and hypocotyls gave only a faint image on the radioautogram. The label in the cotyledon following 1 hour treatment (Ae1) can be seen on the radioautogram.

Seeds of squash treated with ^{14}C DCPA. Plant mounts and radioautograms in Figure 12 (Be1, Be2, Be3, Be4 and Be5) show the distribution of ^{14}C DCPA in squash seedlings following 1, 2, 4, 8 and 20 hour seed treatment. The label in the roots and cotyledons of the seedlings (Be1, Be2, Be3, Be4 and Be5) gave only a faint image on the radioautogram. However, there was some evidence of accumulation of herbicide in the roots (Be3, Be4 and Be5).

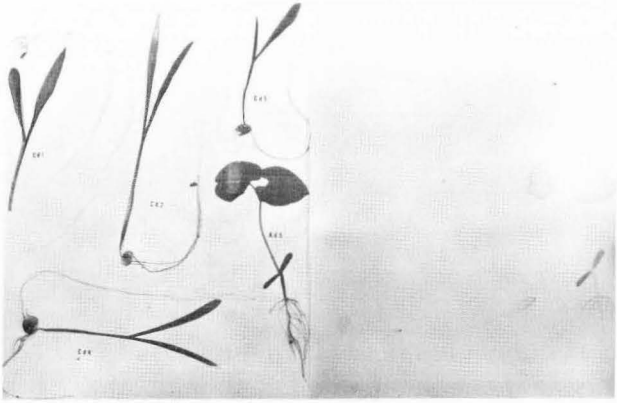
Seeds of corn treated with ^{14}C DCPA. Mounted plants and radioautograms in Figure 16 (Ce1, Ce2, Ce3, Ce4 and Ce5) show no evidence of the distribution of ^{14}C DCPA in the seedlings of corn following 1, 2, 4, 8 and 20 hour seed treatment. This indicates that no DCPA entered the seed.

Seeds of onion treated with ^{14}C DCPA. Plant mounts and radioautograms in Figure 14 (De1, De2, De3, De4 and De5) show the distribution of ^{14}C DCPA in onion seedlings following 1, 2, 4, 8 and 20 hour seed treatment. The label in the seedlings gave only a faint image on the radioautograms. The accumulation of ^{14}C was primarily in the roots.

Seeds of oat treated with ^{14}C DCPA. Mounted plants and radioautograms in Figure 15 (Ee5 and Ee6) show the distribution of ^{14}DCPA in oat

Figure 11. Distribution of ^{14}C pyrazon from treated seeds of corn (Cd..) and bean (Ad..). Treatment time for corn, 1, 2, 8 and 20 hour (Cd1, Cd2, Cd4 and Cd5) and for bean, 20 hour (Ad5). Radioautographs are at right, mounted plants left.

Figure 12. Distribution of ^{14}C DCPA from treated seeds of squash (Be..) and bean (Ae..). Treatment time for squash, 1, 2, 4, 8 and 20 hour (Be1, Be2, Be3 and Be5) and for bean, 8 and 20 hour (Ae4 and Ae5). Radioautographs are at right, mounted plants left.



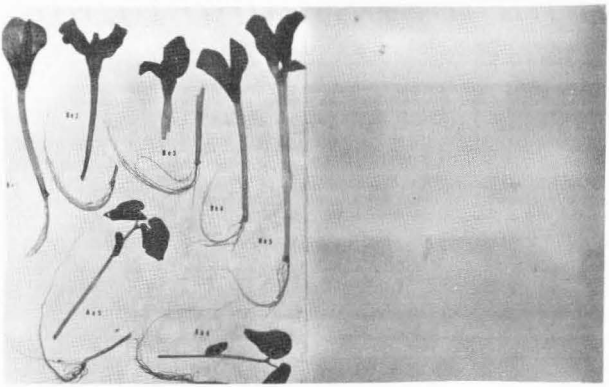
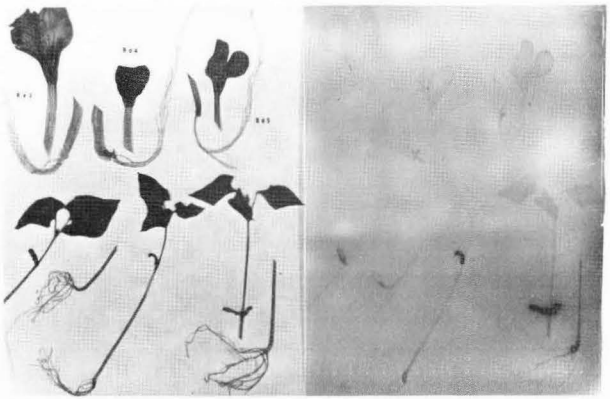


Figure 13. Distribution of ^{14}C pyrazon from treated seeds of squash (Bd. .). Treatment time, 4, 8 and 20 hour (Bd3, Bd4 and Bd5). Radioautographs are at right, plant mounts left.

Figure 14. Distribution of ^{14}C pyrazon from treated seeds of onion (Dd. .) and corn (Cd. .); distribution of ^{14}C DCPA from treated seeds of bean (Ae. .). Treatment time for onion, 1, 2, 4, 8 and 20 hour (Dd1, Dd2, Dd3, Dd4 and Dd5), for corn, 4 hour (Cd3) and for bean, 1, 2 and 4 hour (Ae1, Ae2 and Ae3). Radioautographs are at right, mounted plants left.



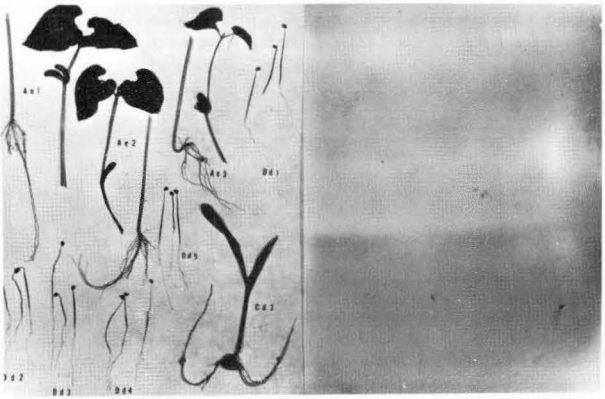
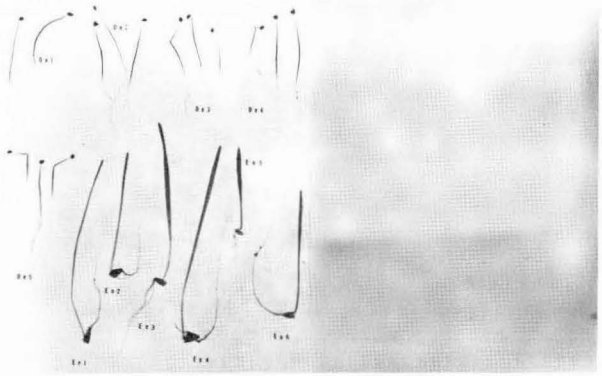
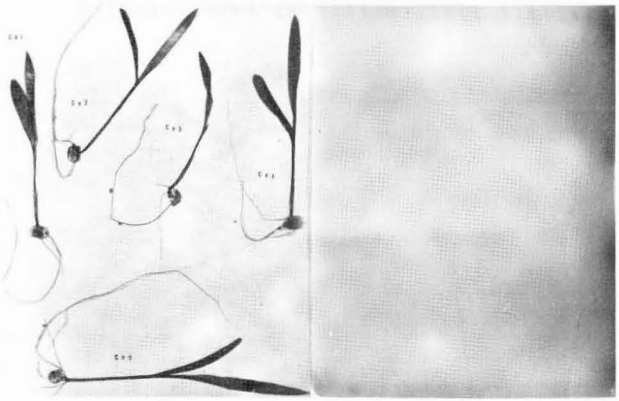

Ae1
Ae2
Ae3
Dd1
Dd5
Dd3
Dd2
Dd3
Dd4

Figure 15. Distribution of ^{14}C DCPA from treated seeds of onion (De..) and oat (Ee..). Treatment time for onion, 1, 2, 4, 8 and 20 hour (De1, De2, De3, De4 and De5) and for oat, 1, 2, 4, 8, 20 and 48 hour (Ee1, Ee2, Ee3, Ee4, Ee5 and Ee6). Radioautographs are at right, mounted plants left.

Figure 16. Distribution of ^{14}C DCPA from treated seeds of corn (Ce..). Treatment time, 1, 2, 4, 8 and 20 hour (Ce1, Ce2, Ce3, C34 and Ce5). Radioautographs are at right, plant mounts left.





seedlings following 20 and 48 hour seed treatment, respectively. The label in the seedlings gave only a faint image on the radioautograms and show a uniform distribution throughout the plants.

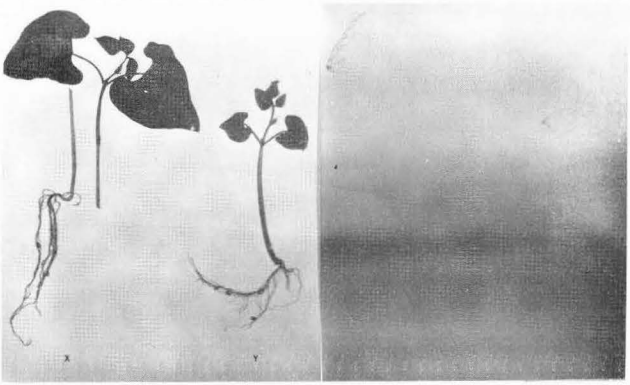
Seeds of bean treated with ^{14}C pyrazon. Plant mounts and radioautograms in Figure 10 (Ad1, Ad2, Ad3 and Ad4) and in Figure 11 (Ad5) show the distribution of ^{14}C pyrazon in bean seedlings following 1, 2, 4, 8 and 20 hour seed treatment, respectively. The label in the aerial portions gave a very clear image on the radioautograms. The density increased greatly from the 1, 2, 4 and 8 hour treatments (Ad1, Ad2, Ad3 and Ad4) through the 20 hour treatment (Ad5). This indicated that with longer treatment time more pyrazon entered the seed or developing seedling. The accumulation in the leaf margins became strongly evident.

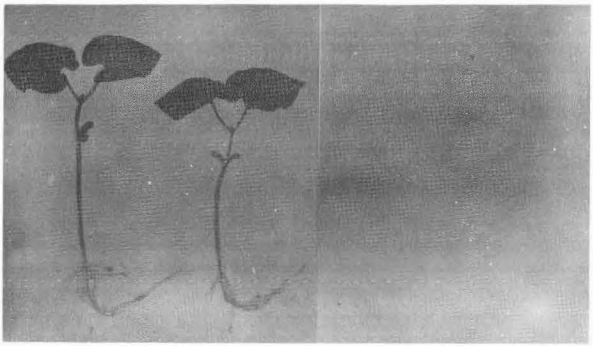
The plant mount and radioautogram in Figure 17 (Y) shows the distribution of ^{14}C pyrazon in a 25-day-old bean plant following 8 hours of seed treatment. The label in the aerial portion gave a very clear image on the radioautogram. The distribution of ^{14}C in the trifoliate leaves became evident, and there was evidence of herbicide accumulation in the leaf margins.

Seeds of squash treated with ^{14}C pyrazon. Plant mounts and autoradiograms in Figure 10 (Bd1 and Bd2) and in Figure 13 (Bd3, Bd4 and Bd5) show the distribution of ^{14}C pyrazon in squash seedlings following 1, 2, 4, 8 and 20 hour seed treatment. In the 1 and 2 hour (Bd1 and Bd2) treatments, the label in the seedlings gave moderately clear images on the radioautogram

Figure 17. Bean plant (X) showing the absorption of ^{14}C pyrazon 8 hours after treated leaf. Bean plant (Y) showing the distribution of ^{14}C pyrazon in the unifoliage and trifoliage leaves following 8 hour treated seed. Radioautographs are at right, mounted plants left.

Figure 18. Distribution of ^{14}C pyrazon 48 hours after treatment to the leaf. The left picture is the plant; the right, the radioautographs. (Z), plant treatment. (C), control.


X
Y



and show a uniform distribution throughout the plants. In seedlings from 4, 8 and 20 hour treated seeds, a greater accumulation of radioactivity was observed judging from the image density. The ^{14}C tended to accumulate in the cotyledon margins and in the upper portions of the roots (Bd3, Bd4 and Bd5).

Seeds of corn treated with ^{14}C pyrazon. Mounted plants and radioautograms in Figure 11 (Cd1, Cd2, Cd4 and Cd5) and in Figure 14 (Cd3) show the distribution of ^{14}C pyrazon in corn seedlings following 1, 2, 8, 20 and 4 hour seed treatment, respectively. The accumulation of the isotope in the coleoptile tips of all seedlings is very evident on the radioautograms. In seedlings from 2, 4 and 8 hour treatments (Cd2, Cd3 and Cd4), the radioactivity was distributed throughout the plants and gave a light image on the radioautograms. An accumulation of label was also found in the foliage leaf tips.

Seeds of onion treated with ^{14}C pyrazon. Plant mounts and radioautograms in Figure 14 (Dd1, Dd2, Dd3, Dd4 and Dd5) show the distribution of ^{14}C pyrazon in onion seedlings following 1, 2, 4, 8 and 20 hour seed treatment, respectively. In seedlings from the 8 hour treatment, the image of seedlings shows the label was distributed uniformily throughout the plant. In the 1, 2, and 4 hour treated seeds (Dd1, Dd2 and Dd3), the label gave only a faint image on the radioautograms.

Bean leaf treated with ^{14}C pyrazon. The radioautograph of bean plants following 8 hour leaf treatments (Figure 17, (X)) shows the absorption

of ^{14}C pyrazon into the leaf. No evidence of translocation of herbicide was observed.

The radioautograph of a bean plant following 48 hour leaf treatment (Figure 18, (Z)) shows the movement of ^{14}C pyrazon from the treated spot towards the tip of the treated leaf (apoplastic movement) without any noticeable basipetal movement.

Microradioautography

The distribution of the radioactivity within the tissues of seedlings treated with various labeled herbicides is shown in the Figures 19-25.

Figure 19 is a photomicrograph of the cross section of a bean leaf from a plant treated with ^{14}C simazine. The section shown in this figure is covered by the developed and fixed emulsion. The isotope label is located in distinct patches of vascular tissue as well as in the adjacent tissues.

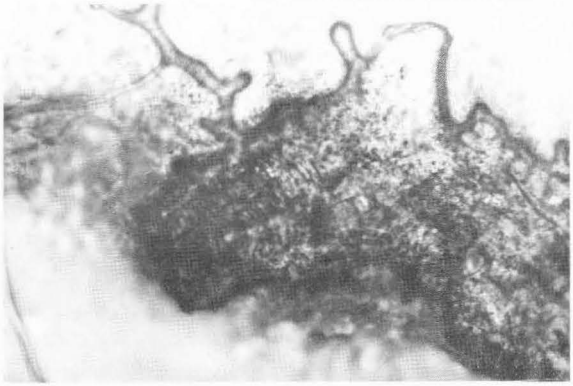
Figure 20 shows a squash root section with superimposed radioautograph from a plant treated with ^{14}C EPTC 10 days after treatment. Only traces of radioactivity were found in the cells of the cortex tissue.

Figure 21 shows a portion of the cross-section of bean leaf from a plant treated with ^{14}C EPTC 12 days after treatment. The radioactivity was generally found surrounding the vascular bundle.

Figure 22 is a cross-section of onion hypotoctyl from a seedling treated with ^{14}C EPTC. Radioactivity is concentrated in the vascular tissue with considerable amounts in the adjacent areas.

Figure 19. Radioautograph of 16 μ transverse section of bean leaf showing evidence of translocation of ^{14}C simazine from treated seed. The heaviest concentration of radioactivity is in the vascular bundle. Exposure 2 weeks. Magnification X 1000.

Figure 20. Radioautograph of 16 μ transverse section of squash root showing evidence of translocation of ^{14}C EPTC from treated seed. Traces of radioactivity in cortex are apparent. Exposure 2 weeks. Magnification X 1000.



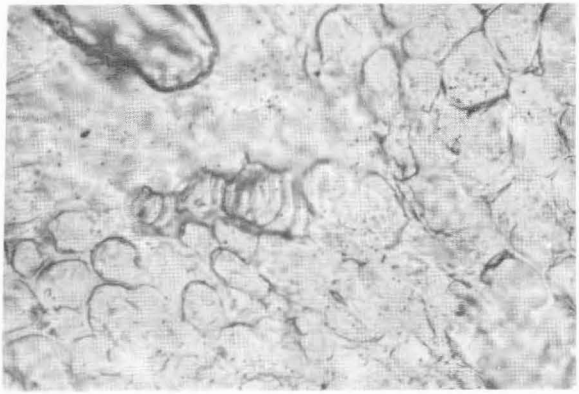
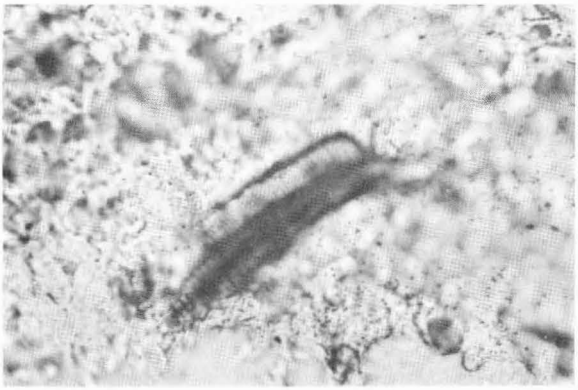
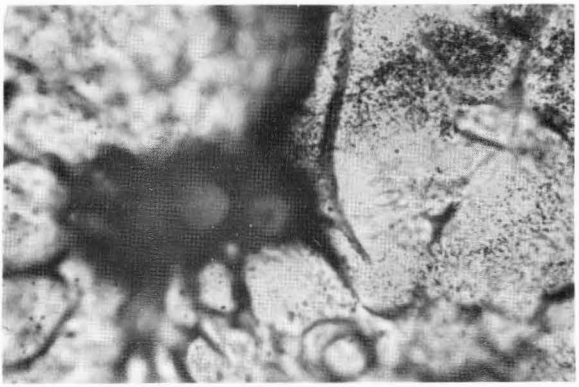


Figure 21. Radioautograph of 16 μ transverse section of bean leaf showing evidence of translocation of ^{14}C EPTC from treated seed. Radioactivity is widely distributed in the surrounding vascular tissue. Exposure 2 weeks. Magnification X 1000.

Figure 22. Radioautograph of 16 μ transverse section of onion hypocotyl showing evidence of translocation of ^{14}C EPTC from treated seed. Radioactivity is concentrated mainly in the vascular bundle and adjacent vascular bundle. Exposure 2 weeks. Magnification X 1000.





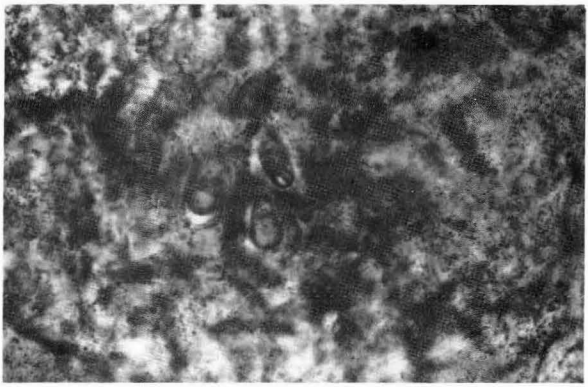
The radioautograph in Figure 23 is a cross-section of a bean leaf from a plant treated with ^{14}C 2,4-D. Radioactivity is concentrated in the vascular tissue with lesser amounts distributed in the surrounding tissues.

The onion hypocotyl cross-section with superimposed radioautograph from a plant treated with ^{14}C 2,4-D (Figure 24) shows isotope label distribution in the vascular bundle and tissue adjacent to the vascular bundle.

Figure 25 is a cross-section of bean cotyledon from a plant treated with ^{14}C pyrazon. It is evident that a small amount of activity is located in the cotyledon cells.

Figure 23. Radioautograph of 16 μ transverse section of squash leaf showing evidence of translocation of ^{14}C 2,4-D from treated seed. Radioactivity is concentrated mainly in the vascular bundle and adjacent tissues. Exposure 2 weeks. Magnification X 1000.

Figure 24. Radioautograph of 16 μ transverse section of onion hypocotyl showing evidence of translocation of ^{14}C 2,4-D from treated seed. Radioactivity is over-exposed but radioactivity is concentrated mainly in the vascular bundle. Exposure 2 months. Magnification X 1000.



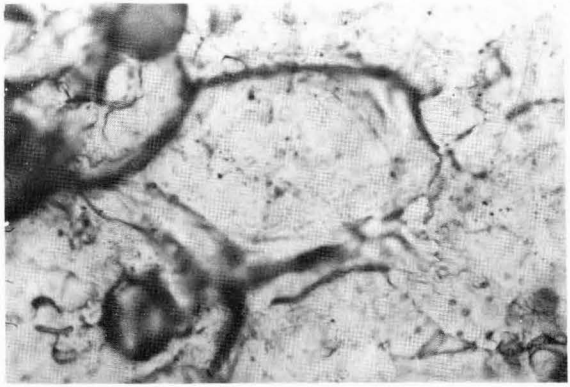


Figure 25. Radioautograph of 16 μ transverse section of bean cotyledon showing evidence of translocation of ^{14}C pyrazon from treated seed. Traces of radioactivity in the cells are apparent. Exposure 2 weeks. Magnification X 1000.

DISCUSSION

The uptake of the herbicides by emerged plants is closely associated with the ingress of water required for transpiration and development (Davis, Gramlish and Funderburk, 1965). During the earlier stages of seed germination (turnip seed) the initial accumulation of herbicide (atrazine) can be independent of water uptake, and take place into the seed coat (Hocombe, 1968). A physical accumulation process, independent of water uptake, has been reported for CIPC on maize, soya and castor seeds (Ashton and Helfgott, 1966). The results of seed or seedling uptake of simazine, EPTC, 2,4-D, DCPA and pyrazon from a time series of treatments correlate well with these observations. The results show further that the initial accumulation of these herbicides appeared to depend on the duration of exposure. The radioautographs of each plant species (bean, squash, corn and onion), each time treatment (1, 2, 4, 8 and 20 hour) and each chemical treatment (simazine, EPTC, 2,4-D and pyrazon), Figures 1-16, clearly show high accumulation of herbicides in these seedlings with long exposure period (except plants treated with DCPA). It seems likely that such a relationship exists because there has been some direct accumulation of these herbicides by the expanding seedling.

Radioautography

No information was available to indicate the distribution of these herbicides (simazine, EPTC, 2,4-D and pyrazon) in the plants following their

imbibition by seeds. However, some of the results of this study are in agreement with those were mentioned in the review of literature, i.e., the translocation and distribution of these herbicides in the plants.

^{14}C simazine treatments of bean, squash, corn and onion. Experimental results clearly show that translocated ^{14}C simazine was distributed throughout treated beans (Figure 1) and accumulated in the leaf margins, typical of xylem transport. Simazine absorption from treated bean seed or developing seed would seemingly involve the same principles as absorption from root application. This result indicates that the simazine was absorbed by the radicle or developing root and moved through the xylem in the transpiration stream. The results of treating seeds of squash, corn and onion should also be the same as that of the bean in that simazine entered through seed coat. In the case of corn it seems likely that the results could be explained by the work of Haskell and Rogers (1960), in which the simazine was found in the radicle, spicotyl, endosperm or cotyledon.

^{14}C EPTC treatments of bean, squash, corn and onion. When ^{14}C EPTC was applied to the seeds or developing seeds, it was absorbed and translocated throughout the plants (bean, squash, corn and onion). This indicates that EPTC was absorbed by the seeds or developing seeds (some part of the embryo) and was translocated or redistributed. EPTC appears to be freely mobile in the plants and can be translocated upward and downward (Appleby, Furtick and Fang, 1965). However, the results of this study showed that EPTC appears predominantly to move upward to the aerial portions of the plants. This can be clearly seen in the case of squash and corn.

^{14}C 2,4-D treatments of bean, squash, corn and onion. Following the treatment of dry bean seeds little activity was found in the leaves. It appears that this labeled compound moved to the leaves in the xylem. However, a very little movement under such cases occurs in the xylem. The translocation of 2,4-D from a treated seed shows a characteristic fixation along the path of the translocation. The accumulation occurs in the region around the location of application, i.e., the seed or developing seed. This also was found in squash and onion plants. In corn seedlings the translocation of 2,4-D is even more limited. The activity was mainly centered in the vicinity of the treated seeds.

^{14}C pyrazon treatments of bean, squash, corn and onion. Radioautographs of plants indicate the upward movement of pyrazon and accumulation of the isotope in the leaf margins of bean, squash and corn. The upward movement and the distribution pattern indicate that pyrazon was translocated mainly through the xylem following absorption by the developing seed. This conclusion explains the accumulation in the leaf margins. Redistribution was found when pyrazon moved up to the trifoliate leaves of the bean and accumulated in the leaf margins. This result is in agreement with the results of Rodebush (1968). It is difficult to explain the translocation and distribution pattern of pyrazon in onion seedlings on the basis of xylem transport, since the label was distributed uniformly throughout the plant. It seems likely that xylem transport should also occur in this case, however.

The results from the bean leaves treated with ^{14}C pyrazon indicate that pyrazon was foliarly absorbed and translocated. Basipetal movement

of the isotope was limited. The radioautogram of bean leaves treated with ^{14}C pyrazon did not exhibit phloem translocation, but there was some distribution in the expanding leaf. Acropetal movements appeared to occur readily. The distribution pattern following acropetal translocation indicated that the labeled compound involved appeared to move within xylem tissues.

^{14}C DCPA treatments of bean, squash, corn, onion and oat. The radioautograms of bean, squash, onion and oat plants showed the distribution pattern of ^{14}C DCPA. However, there was very little evidence of accumulation in the plant or within the seeds themselves. It should be noticed that ^{14}C DCPA was being translocated, however. Of the distribution ^{14}C more accumulated in the root system than in the aerial portions, except in the case of oat in which more activity accumulated in the shoot than in the root system. These studies indicate that penetration of DCPA into the seed or developing seedling may occur via the seed coat.

Microradioautography

The interpretation of the tissue radioautograms is complicated by the following factors. (a) It is often difficult to distinquish between the background and the silver grains from the labeled compounds. (b) In some of the photomicrographs the resolution was inadequate to determine the presence of some in different plant tissues. However, my interpretation is that the tissues producing densest radioautographs were the tissues involved in distribution and translocation or accumulation.

Simazine appears to be translocated in the vascular tissue (Figure 19). The opaqueness of the vascular bundles should be interpreted as a high concentration of developed silver grains. It is evident that a large amount of activity above the general background is present in the xylem. Some simazine diffused into the adjacent tissues as indicated by a small amount of activity present in cells surrounding the vascular tissue of the bean leaf.

The resolution of Figure 20 was inadequate to determine in which root tissue EPTC accumulated. However, there were small amounts of ^{14}C label widely distributed in the cortex. Vertical translocation may take place in cortex. Since living cells are connected by plasmodesmata producing what Arisz (1956) has termed symplast, there should be longitudinal as well as radial translocation through the cortex of the squash root.

EPTC appears to be widely distributed surrounding the vascular bundle tissues of the bean leaf (Figure 21). This seems likely that it occurs in the mesophyll. Similar results were found in onion hypocotyl (Figure 22) where EPTC is widely distributed in the cells surrounding the vascular bundle.

The squash vascular bundle (Figure 23) contains a large amount of radioactive 2,4-D. Some isotope is also found in the adjacent living cells. Similar results were found in onion hypocotyl (Figure 24) where 2,4-D is widely distributed in the cells surrounding the vascular bundle.

In the experiment in which bean seed was soaked in the radioactive pyrazon, pyrazon was found in the cotyledonary cells (Figure 25).

The resolution was inadequate to determine whether pyrazon accumulated in the cell, between the cells, or both. However, in other radioautograms of sections having a relatively high ^{14}C content (unsuitable for photography), the accumulation of ^{14}C was primarily between the cells.

SUMMARY AND CONCLUSIONS

The effect of stage of seedling development on absorption of five soil applied herbicides by bean, squash, corn, onion and oat was investigated. By radioautography and microradioautography herbicide translocation, distribution and accumulation were studied. Seeds were treated with labeled ^{14}C herbicides for varying periods of time.

^{14}C simazine, ^{14}C EPTC, ^{14}C 2,4-D and ^{14}C pyrazon were absorbed by seeds of bean, squash, corn and onion, the amount increasing with time. Very little ^{14}C DCPA was absorbed. Distribution and translocation of ^{14}C simazine, ^{14}C EPTC and ^{14}C pyrazon were observed in young of bean, squash, corn and onion plants. The accumulation of simazine and pyrazon was found to occur in the leaf margins of bean. EPTC and pyrazon were accumulated in the cotyledon margins of squash. Pyrazon also accumulated in the coleoptile tips and leaf tips of corn. The translocation of 2,4-D in bean, onion and squash showed a characteristic fixation along the path of translocation. Very little translocation or distribution of ^{14}C DCPA were found in bean, squash, onion and oat.

Radioautograms indicated that ^{14}C pyrazon applied to the first true leaf of bean was absorbed and moved towards the tip of treated leaf; however, basipetal movement of pyrazon was limited.

Microradioautography indicated that simazine and EPTC were translocated in the vascular tissues of bean leaf. Limited vertical translocation

may also take place in the cortex of squash root. EPTC was also found in the vascular bundles of onion hypocotyl, 2,4-D was found in the vascular tissues localized in the cells surrounding the vascular bundle of the squash cotyledon and onion hypocotyl. Pyrazon was found in the cells and between the cells of bean cotyledon.

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