# SOME FACTORS AFFECTING THE TRANSLOCATION OF RADIOACTIVE PARAQUAT IN CYPERUS SPECIES

by G. H. WOOD and J. M. GOSNELL

## Introduction

Paraquat (1, 1'-dimethyl-4, 4'- bipyridilium dichloride or dimethyl sulphate) has been used successfully on an extensive scale for the post-emergent control of *Cyperus rotundus L.* and *Cyperus esculentus L.* in sugarcane. It is primarily a contact herbicide and has no residual effect in the soil. Regrowth of *Cyperus spp.* (watergrass) after scorching of the foliage always occurs, the rate and extent depending on the stage of growth and conditions under which paraquat is applied. Experiments in trays have indicated that best control of watergrass, together with maximum reduction of tubers and roots can be achieved by spraying with paraquat about 3 weeks after emergence (S.A.S.A. 1964, 1965). This coincides with the beginning of the flowering stage.

Since regrowth originates from plant parts which escape contact with the paraquat spray, namely the tubers, rhizomes, basal bulbs and portions of the shoots still below ground level, spraying under conditions favouring maximum translocation of paraquat to these parts would be of advantage. Research with this objective was undertaken using radioactive paraquat.

An investigation into the starch reserves of tubers at different stages of growth was also undertaken, as it is was thought that this might have a bearing on recovering from the effects of paraquat. (Thakur and Negi 1954).

## Experimental details

### General

Watergrass tubers were planted singly in 5 x 5 inch earthenware or plastic pots filled with a Clansthal sand. The pots were kept in the open, watered daily, and the plants were subsequently treated with radioactive paraquat.

Radioactive paraquat labelled with Carbon-14 in the methyl radical was received in two consignments with specific activities 2.02 and 10.1 millicuries per millimole. Preliminary work had shown that translocation of paraquat could be traced satisfactorily when a watergrass plant was treated with a 0.01 ml. droplet of aqueous paraquat solution containing 0.5 microcuries of carbon-14. Hence, stock solutions were prepared from the low and high specific activity consignments containing 6330 and 1272 p.p.m. respectively. The former was used for all except experiment D.

The standard method of treatment was to apply the 0.01 ml. droplet to the primary tiller in each pot, one third of this being spread over the upper surface of the middle inch of each of three adjacent mature leaves. To confine the solution to the treatment site, a band of lanolin was placed across the leaf above and below it. No surfactant was required, as *Cyperus* leaves are able to retain and spread aqueous solutions efficiently (Ennis *et al.* 1952).

After treatment, the plants were either removed directly into the open or greenhouse, or kept in the dark for a 24-hour period prior to this. They were harvested intact about a week after treatment by carefully removing the soil from the roots. The treated tiller and a few adjacent tillers and any attached tubers were selected and kept intact for autoradiography. These were carefully inserted between sheets of blotting paper and dried in a plywood-hardware cloth press at 80° C for approximately 24 hours. After mounting the dried plants on 10 x 12 in. sheets of white paper and covering them with Melinex film of 6 microns thickness, they were autoradiographed following the method developed by Yamaguchi and Crafts (1958). Ilford Industrial G film was used, as previous work had shown this to give entirely satisfactory and unambiguous autoradiographs. The exposure time varied from 3 to 6 weeks.

Three replicates were used for each treatment together with a control to check for pseudoautoradiography. The treated plants were selected for uniformity from a large number of pots.

### Treatments

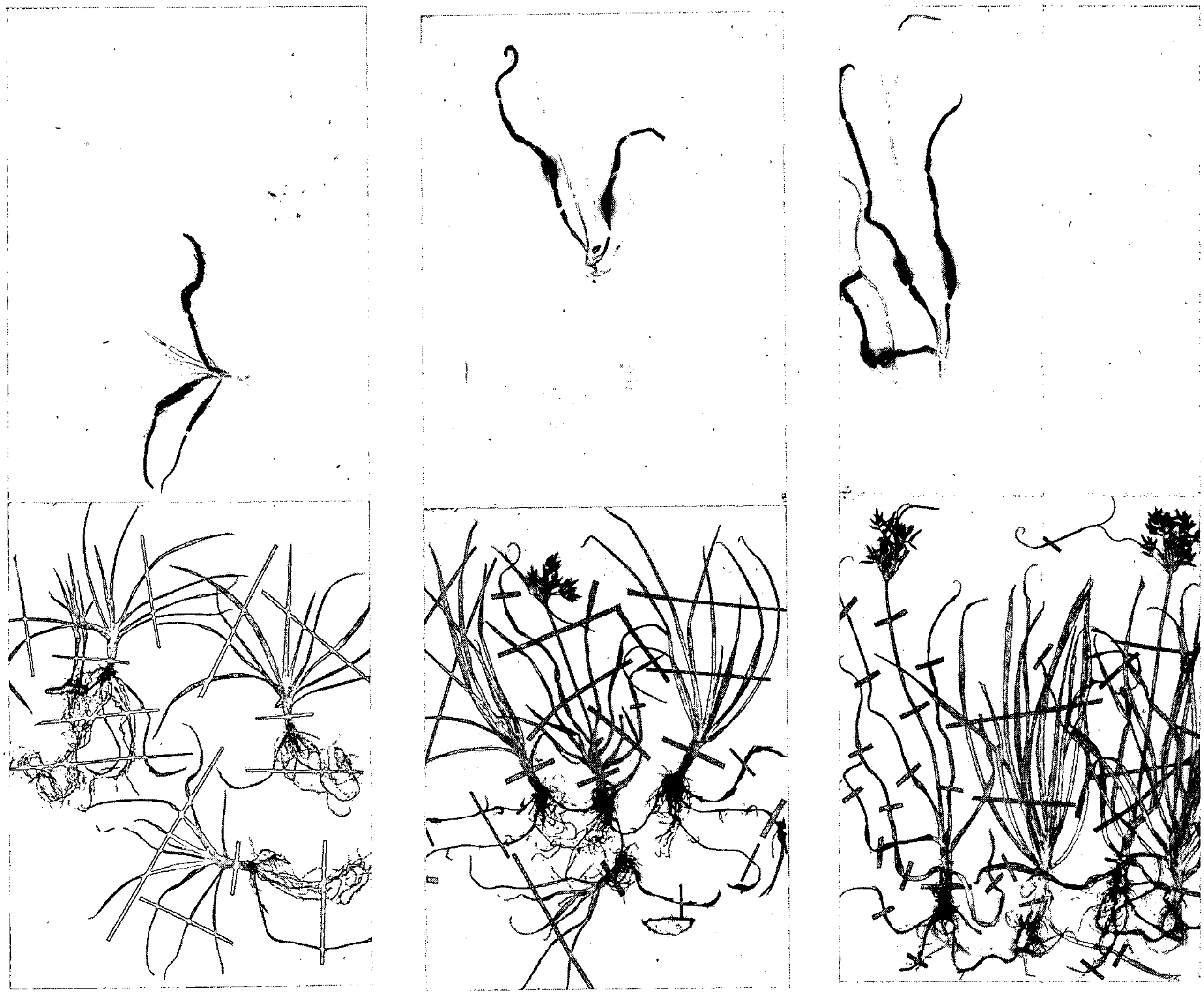
*Experiment A—Application of paraquat to* C. rotundus *at different stages of growth*

Uniform emergence had occurred by 25 days after planting and this was taken to be the reference date. Radioactive paraquat solution was applied at 1, 2, 3, 4, 6 and 8 weeks after the reference date of emergence. The pots were left in the dark for 24 hours after treatment and then placed in the open for 5 days before harvesting.

*Experiment B—Application of paraquat to* C. rotundus *at various times of the day*

Three weeks after emergence, paraquat was applied at the following times of the day: 6.00 a.m., 10.0 a.m., 2.00 p.m. and 6.00 p.m. Following treatment, the plants were placed in the open. In addition, a "standard" treatment (as used in most of the other experiments) was included; paraquat was applied at 2.00 p.m. and the plants left in the dark for 24 hours before being placed in the open. Harvesting took place a week after treatment.

*Experiment C—Combined application to* C. esculentus *of paraquat and bromacil* (5-*bromo*-6-*methyl*-3-(1-*methylpropyl*) *uracil*)



A B C

PLATE 1

Comparison of translocation of paraquat in *C. rotundus* at A:1 week, B:4 weeks and C:6 weeks after emergence. (Autoradiographs above, plants below).

The following treatments were applied 3 weeks after emergence:

(i) Paraquat only

(ii) Paraquat + bromacil in the proportion 1:2

(iii) Paraquat + bromacil in the proportion 1:6

Bromacil in suspension was added to the paraquat solution as required. The plants were placed in the open immediately after treatment and harvested after 6 days.

*Experiment D—Application of paraquat to* C. rotundus *growing under various soil moisture regimes*

Six weeks after emergence, the plants were removed to a greenhouse, where the following water treatments were applied:

(i) High — sufficient water for optimum growth.

(ii) Medium — sufficient water to maintain a reduced rate of growth, with relatively slight wilting.

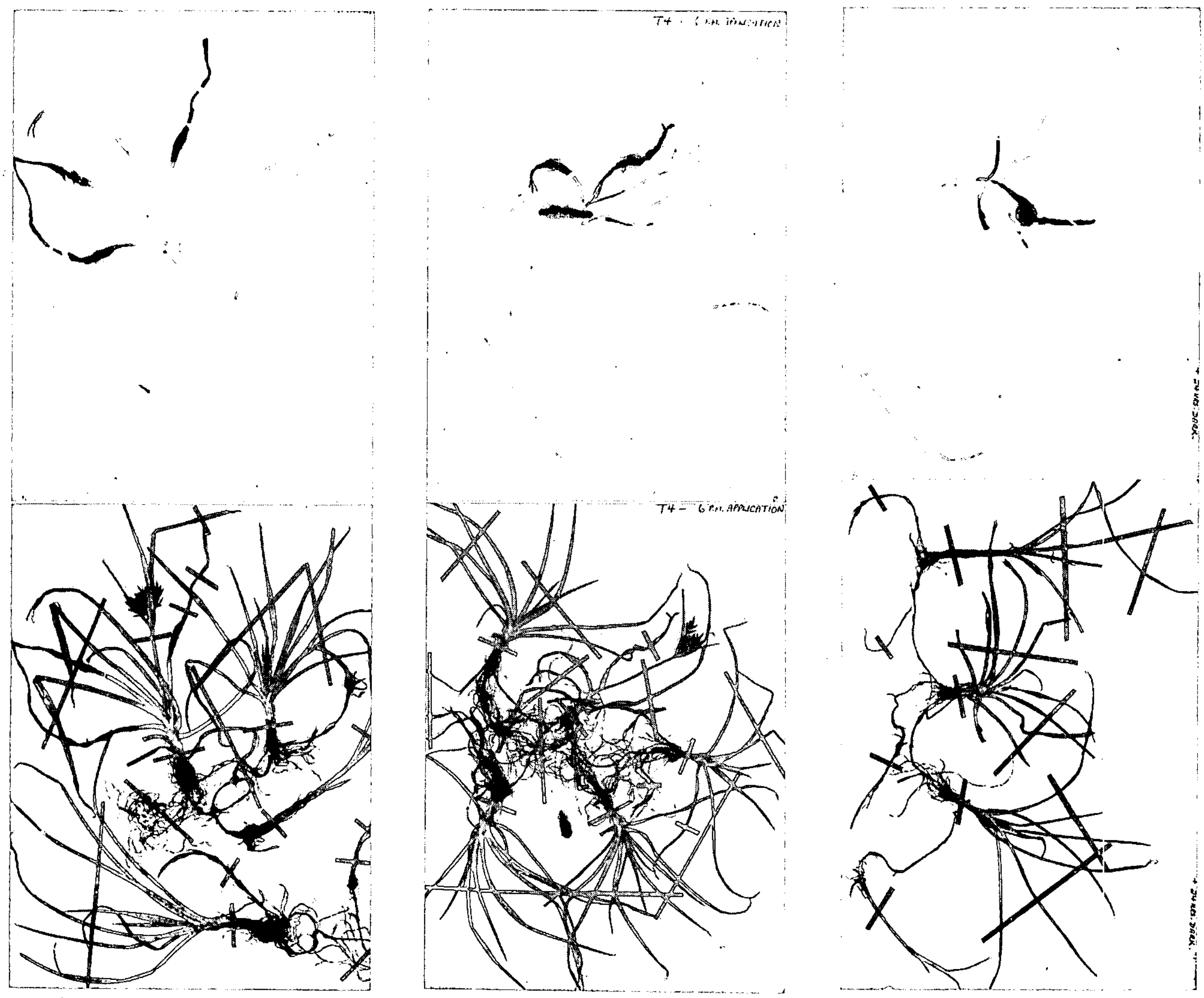
(iii) Low — just sufficient water to keep the plants alive.

Treatments were controlled by daily weighing and addition of water to constant weight. Plastic pots were used to avoid errors inherent with the use of earthenware pots. After ten days the application of radioactive paraquat was made. The plants were left in the dark for 24 hours and then returned to the greenhouse, where the same watering treatments were carried out as before, until they were harvested a week later.

*Experiment E — Starch in* C. rotundus *tubers at different stages of growth*

A bulk sample of tubers was divided into 7 subsamples each comprising 24 tubers. One subsample was prepared for subsequent starch analysis by slicing the tubers, drying them at 80°C for 24 hours and grinding, while the remaining six were planted out into trays. Harvesting was carried out at weekly intervals from 1 to 6 weeks after planting, and the tubers were prepared as before.

To extract the starch, 1 gram of the ground sample was boiled with 40 ml. calcium chloride solution (S.G. = 1.3). The subsequent procedure was identical to that used for cane juice. (Wood 1962).



A B C

PLATE 2

Comparison of translocation of paraquat in *C. rotundus* when applied A: at 10.00 a.m., B: at 6.00 p.m. and C: at 2.00 p.m. (standard treatment). (Autoradiographs above, plants below).

**Results and Discussion**

The autoradiographs of both species of *Cyperus* showed massive translocation of paraquat to the tip of the treated leaf with a smaller amount moving downwards. With the exception of the treated tiller the difference in growth between the control and treated plants was not large. This was due to the short treatment time (1 week) and to the fact that only a relatively small amount of paraquat moved out of the treated tiller to other tillers and underground parts, even when optimum translocation occurred. This small quantity was unlikely to retard new growth seriously. It must be borne in mind, however, that in these experiments only a small proportion of the total leaf area of a single tiller was treated. It is reasonable to assume that the amount absorbed and translocated by the plant as a whole would increase with the area of leaf covered, and, hence, in the field, where all the foliage is sprayed, the quantity of paraquat going into certain underground parts might well be lethal.

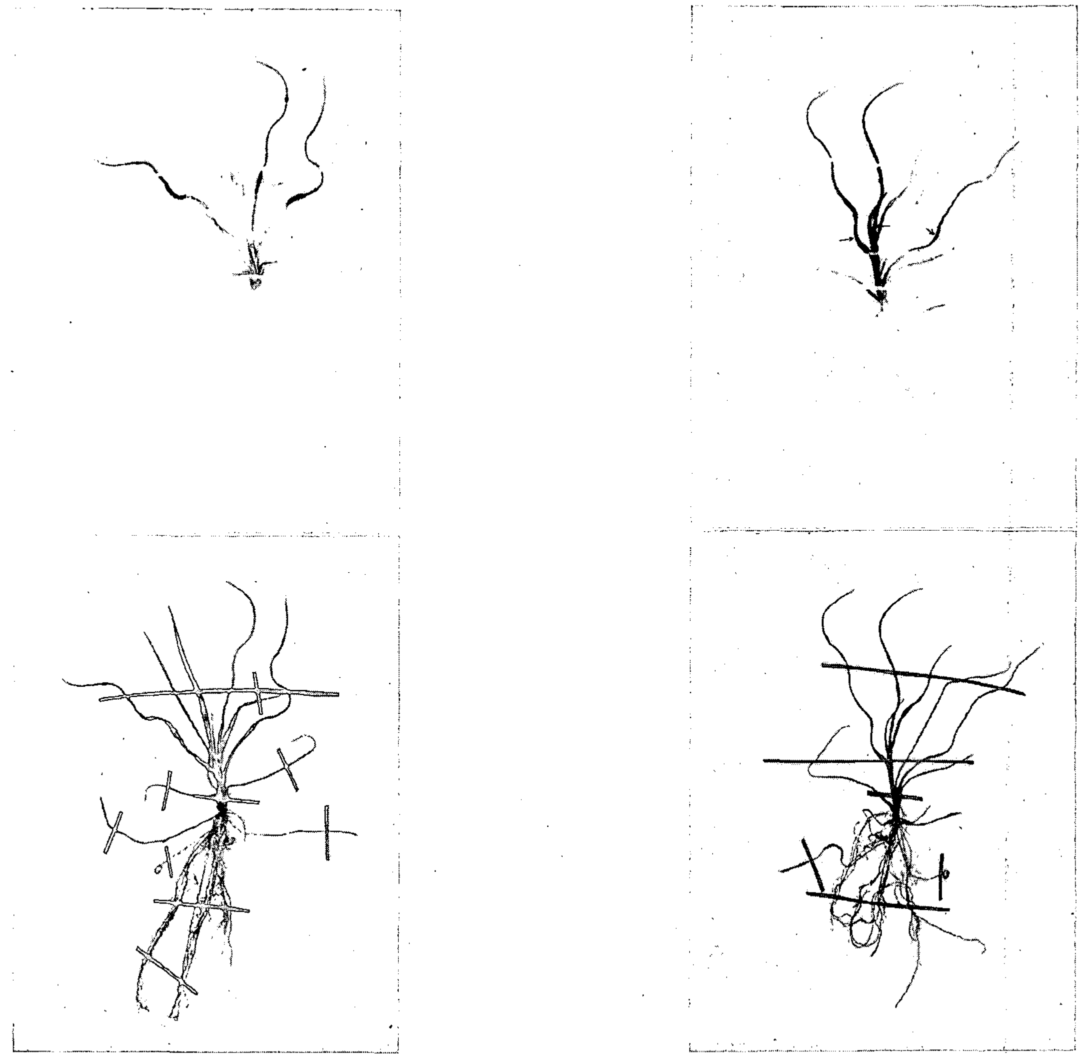
Accumulation of paraquat was very seldom observed in the tubers of either species. This confirms field observations that tubers from treated and untreated plants are equally viable.

*The effect of increasing age on paraquat movement in* C. rotundus

Little or no difference was found in the extent of translocation occurring from treatment 1 to 4 weeks after the date of emergence.

During this period, the greatest amount of translocation, apart from that to the tips and base of the treated leaves, was in general either to the younger leaves or to the inflorescence of the treated tiller, depending on the stage of growth. The older leaves of the treated tiller, the foliage of connected tillers and underground parts excluding the tuber accumulated paraquat to a lesser extent.

At 6 to 8 weeks, the extent of translocation was much reduced with little or no movement into attached tillers and roots. However, some accumulation was observed in the few new shoots that were present. Maximum translocation into the inflorescence occurred from 4 to 6 weeks. This corresponds to the



A PLATE 3 B

The effect of bromacil on paraquat translocation in *C. esculentus* A: paraquat only, B: paraquat + bromacil (1:6). (Autoradiographs above, plants below).

period immediately preceding maturity of the seeds.

Representative plants and corresponding autoradiographs are shown in Plate 1.

Although the quantity of paraquat translocated per unit area of leaf remained approximately the same from 1 to 4 weeks after emergence, the number and size of tillers rapidly increased and total ground cover was achieved at about 3-4 weeks. Thus the total amount translocated must increase rapidly during this period. This, together with the fall-off in translocation, after the fourth week, is probably the main reason for optimum control at this stage.

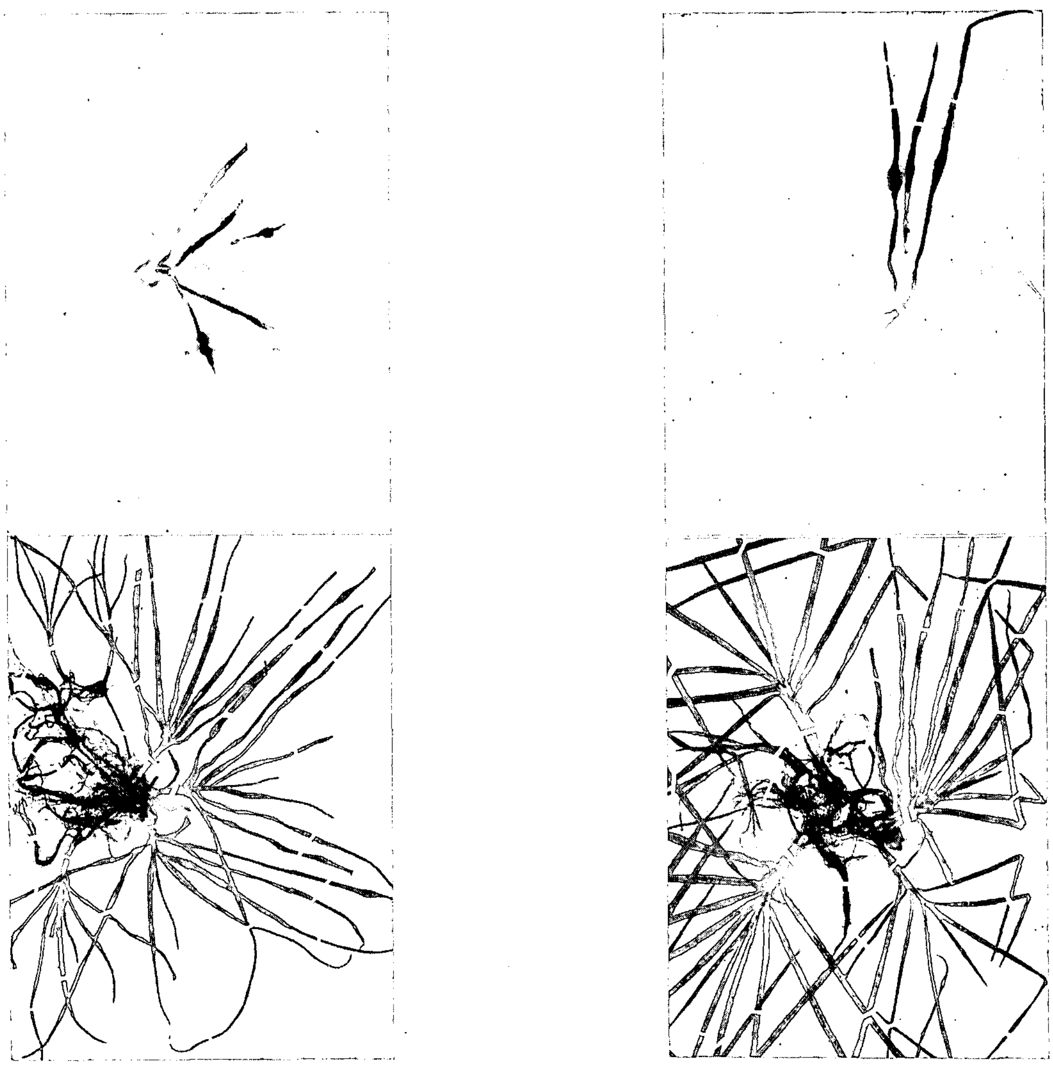
Increased translocation to the inflorescence from 4 to 6 weeks could have practical significance in reducing seed production, as it is possible that paraquat spray falling directly onto the seed may simply scorch the seed coat without affecting its viability.

*The effect of application of paraquat to* C. rotundus *at different times of the day*

The autoradiographs showed that most extensive translocation and uniform distribution in all plant parts including the inflorescence, occurred in the "standard" treatment where application of paraquat was immediately followed by a period of darkness. The extent of translocation was very similar when paraquat was applied at 6.00 p.m. but was considerably smaller in the case of the earlier applications where no paraquat accumulated in the roots and new shoots. Paraquat was deposited preferentially in the older leaves when the plants were treated in the morning or early afternoon, while uniform distribution in the foliage together with good accumulation in the inflorescence took place when paraquat was applied shortly before dark.

These observations agree with the results of field experiments which showed that the extent of scorching of watergrass increased the closer to evening the plants were sprayed (Gosnell 1965). Baldwin (1963) also found that more efficient translocation of dipyridilium compounds was obtained when a period of darkness followed spraying. The reduction of paraquat to an active radical only occurs when photosynthesis is actively proceeding (Boon 1964). Either absence of light or the presence of a photosynthetic inhibitor therefore delays the scorching and allows greater translocation to occur.

Representative plants and corresponding autoradiographs are shown in Plate 2.



A B

PLATE 4

The effect of moisture stress on paraquat translocation in *C. rotundus*. A: low watering treatment, B: high watering treatment. (Autoradiographs above, plants below).

A further interesting observation from the autoradiographs is the patchy distribution of translocated paraquat that occurred in all cases, where the plants were exposed to light immediately after treatment. The paraquat appears to have a tendency under these circumstances to accumulate in isolated cells or groups of cells. The same phenomenon was frequently observed when paraquat was translocated to the older less turgid leaves, irrespective of the light intensity following treatment. Absence of light after treatment usually resulted in more even distribution of paraquat in the turgid or actively growing tissue into which it moved.

The results of this experiment indicate that optimum control of watergrass should be obtained with evening spraying. However, no significant differences were obtained in a field experiment comparing these times of spraying (Gosnell 1965), and it is probable that the increased control of watergrass was countered by increased damage to the cane. In addition, practical considerations, especially the effect of wind, favour morning spraying.

*The effect of bromacil on the translocation of paraquat in* C. esculentus

Bromacil, being a photosynthetic inhibitor, might be expected to improve the translocation of paraquat by delaying the scorch. This has in fact been observed in field experiments, but did not occur in the work with radioactive paraquat. The lower level of bromacil had no noticeable effect on the translocation of the paraquat applied with it. Slightly improved translocation did, however, occur with the higher level.

The following plant parts are listed in the order of decreasing paraquat accumulation in this experiment. Tip of applied leaf >> root tips > older leaves of treated tiller > younger leaves of treated tiller = roots of treated tiller > roots and older leaves of connected tillers > rhizomes and new shoots > younger leaves of connected tillers; this was the overall picture for all treatments.

Representative plants and corresponding autoradiographs are shown in Plate 3.

From the autoradiographic results it can be concluded that in this experiment bromacil had only a slight effect on the translocation of paraquat, even at the highest rate of application.

*The effect of water stress on paraquat translocation in* C. rotundus

The plants which were subjected to severe water stress showed patchy accumulation of translocated paraquat, mainly in the older leaves, with some movement into the basal bulb. In addition, small isolated concentrations of paraquat occurred throughout the above ground and underground portions of the plant system.

With adequate watering, the paraquat tended to be distributed evenly in the leaves and other plant parts into which it was transported. Accumulation took place mainly in the younger leaves of the treated tiller, with a somewhat lower amount going into the older leaves and basal bulb of the treated tiller and the younger leaves of attached tillers. A small amount was deposited in the roots and rhizomes and in one replication appreciable accumulation occurred in the tuber. This was the only instance noted of any deposition of paraquat in tubers.

The translocation pattern in plants which received the medium watering treatment fell somewhere between the two extremes described above.

Representative plants and corresponding autoradiographs are shown in Plate 4.

*The starch content of tubers at different times after planting*

The starch contents of the samples of tubers taken at different times after planting are listed in Table I.

TABLE I.

**Percent Starch in Tubers dug at intervals after planting.**

| *Time after Planting (weeks)* | *Per cent starch (dry wt. basis)* |
|---|---|
| 0 | 40.1 |
| 1 | 32.8 |
| 2 | 34.4 |
| 3 | 34.8 |
| 4 | 27.7 |
| 5 | 29.4 |
| 6 | 30.1 |

The results show that initiation of growth appreciably depleted the starch reserves in the tubers, as demonstrated by Thakur and Negi (1954). The lowest content was found at 4 to 5 weeks after planting, which coincides with the period of maximum control of watergrass obtained at 3 weeks after emergence (S.A.S.A. 1964, 1965). It is therefore possible that recovery from paraquat application is related to the starch reserves in the tuber.

## Conclusions

The foregoing results and discussion indicate that translocation of paraquat is most extensive and effective when watergrass is sprayed approximately 3 weeks after emergence and in the evening. These findings agree well with the results of field and greenhouse trials.

By far the greatest movement of paraquat took place in the xylem towards the tip of the treated leaf. However, there was generally appreciable translocation downwards to the basal bulb, and movement also occurred to a smaller extent to the young shoots, rhizomes and root tips. This type of movement is not associated with xylem transport, but rather with phloem movement, and the evidence from these experiments thus tends to confirm that of Thrower *et al.* (1965) who found that diffusive movement occurred out of the xylem. van Oorschot (1964) has also found accumulation of paraquat in root tips, believed to be due to phloem movement. This symplastic movement probably accounts for the small residual effect which is obtained with paraquat on *Cyperus spp.*

Although bromacil in combination with paraquat is undoubtedly useful in extending control of watergrass for considerably longer periods than is possible with paraquat alone, it only increased the translocation of paraquat slightly in these experiments.

Adequate soil moisture appears to favour even distribution of translocated paraquat and deposition in younger rather than older leaves. The phenomenon of patchy deposition associated with plants growing under severe moisture stress and those which were exposed to light after treatment, has also been frequently observed when paraquat was deposited in older, less turgid leaves. A possible explanation may be the collapse of large numbers of cells when the leaves become old or start to wilt as a result of moisture stress, while paraquat continues to be transported through the conducting tissues to groups of functional cells where accumulation can still take place.

From these studies no firm conclusion can be drawn regarding the possibility that low starch reserves may influence paraquat translocation, although this appears possible.

## Summary

In a series of pot experiments to study the translocation of paraquat in *Cyperus spp.*, this herbicide labelled with radioactive carbon was applied at different stages of growth, various times of the day, alone or mixed with the photosynthetic inhibitor, bromacil, and under various soil moisture regimes. Autoradiographic methods were used to determine the translocation pattern.

The autoradiographs showed that optimum paraquat translocation occurred when the plants were sprayed 1 to 4 weeks after emergence. When the

development of ground cover was taken into consideration, the quantity of paraquat transported to underground parts was estimated to be greatest at approximately 3-4 weeks, agreeing with the results of field and greenhouse trials.

The extent of translocation following evening application was similar to that of the "standard" treatment, where 24 hours of darkness followed application, and was much greater than when treatments were applied at 6.00 a.m., 10.00 a.m., or 2.00 p.m.

Bromacil increased the translocation of paraquat only slightly in these experiments.

Patchy deposition of paraquat was associated with plants growing under moisture stress, whereas adequate moisture favoured even distribution of the translocated herbicide. In general, plants growing vigorously tended to accumulated paraquat preferentially in new growth. In only one case was accumulation in the tubers noted.

Investigation of the starch reserves of the tubers at the different stages of growth indicated the possibility that recovery from paraquat application could be related to the starch reserves of the tuber.

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**Mr. Gilfillan:** Has Mr. Wood mixed bromacil with paraquat? In my experience paraquat will dominate any mixture of herbicides.

**Mr. Wood:** The bromacil was applied as a suspension in the paraquat solution. Field tests to determine the effect of bromacil on paraquat carried out by the Agronomy Section indicated a delay in scorch and a greater residual effect. I am not sure whether they were applied mixed together or separately.

**Dr. Thompson:** In one instance they were applied together in a ratoon crop and we did get a delayed reaction which might have contributed to the translocation of paraquat but in subsequent experiments we have gained no advantage by mixing these two herbicides.

**Mr. Glover:** Your summary would make it reasonable to associate movement of paraquat with the metabolised products in the phloem.

Cyperus seems to accummulate starch in leaves during the day and may translocate it as sugar at night so that the paraquat may be flowing in linkage with the sugar down the transportation stream towards the sink. The meristematic tissues will have a high demand and therefore you will get a very fast shift particularly to active cells which you have clearly described. I would like to see if you are collecting more starch during the day than you are discharging sugar to find out if the light inhibiting and dark promoting reactions are associated with the carbohydrates.

**Mr. Alexander:** Did Mr. Wood experience any difficulty owing to radiation due to the presence of potassium in the plants in establishing where translocation of the paraquat had occurred?

**Mr. Wood:** Preliminary work showed that the amount of potassium in the leaves is not sufficient to produce any image on the X-ray film.