Tracer Studies with Alligator Weed Using 2,4-D-C^{14}

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Alligator weed (*Alternanthera philoxeroides* (Mart.) Griseb.) is one of the prolific weeds found in the waterways of the Gulf coastal area. The use of 2,4-D in the control of a similar pest, the water hyacinth, has been reported by Hitchcock, Zimmerman, Kirkpatrick, and Earle (*1*). Since 2,4-D was apparently less effective on alligator weed, a series of studies was begun to investigate the action of the herbicide on this plant, with particular reference to absorption and translocation rates, using 2,4-D-C^{14}.

In spite of the higher cost, 2,4-D-C^{14} was chosen for the investigations rather than 2,4-D-5I^{131}, because of the suspected change in absorption characteristics introduced by such a large increase in molecular weight. Linder (*2*) reports that there is a small but decided variance in the growth-inhibiting properties of 2,4-D and 2,4-D-5I.

The counting equipment used was a Tracerlab SC-2A Scaler, with associated sample holder, tube mount, and electrical stop clock. The Geiger-Muller tubes had a mica window of thickness less than 2 mg/cm^2 to accommodate the low energies of the β-particles emitted by the C^{14}. The isotope has a maximum β-energy of 150 kev. The sample holder and tube mount were assembled in a wooden box with a close-fitting door. The wide temperature and humidity variations experienced in New Orleans had a rather deleterious effect on the counter tubes, which was eliminated by performing the counting in an air-conditioned laboratory with controlled humidity.

The 2,4-D-C^{14} was obtained from Tracerlab, Inc., and had a specific activity of 1 mc/mM. This implied 3.48 mg/mc. The greater part (98.44%) of the 2,4-D used was unlabeled. Ethyl alcohol and methyl cellosolve were tested as solvents. Both had good evaporation characteristics, but the cellosolve dried and withered the leaves within 24 hr, whereas the ethyl alcohol had no appreciable effect. The 2,4-D-C^{14} was used in 95% ethyl alcohol solution, 98% alcohol by weight. In some of the experiments a spreading agent, Tween 20, a product of the Hercules Powder Company, was added to the solvent.

Samples of alligator weed were grown in a greenhouse under simulated field conditions. The radioactive material was placed on the upper surfaces of one pair of leaves of each plant by means of a tuberculin syringe and hypodermic needle. To prevent surface flowing a lanolin dam, in the form of a chevron, was placed just above the petiole attachment with the apex of the chevron toward the stem. Cut sections of the stem of the treated plant were weighed, placed in ¼-oz ointment tins, and macerated manually. Small quantities of ethyl alcohol were added as the grinding proceeded. A warm plate was used to evaporate the residual alcohol. The tins were placed in the sample holder for counting.

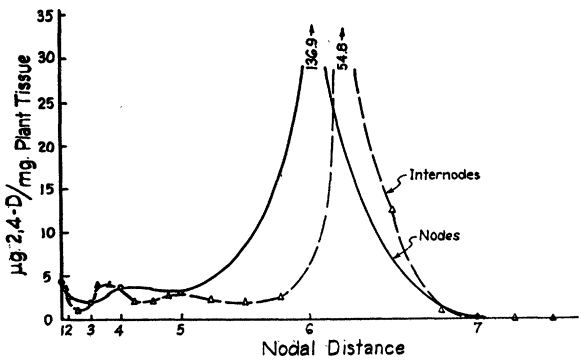

FIG. 1. Distribution plot (12-hr exposure).

For identification, the nodes of each plant were numbered from the top—that is, No. 1 was the node for the top pair of leaves, No. 2, the node of the second pair, etc. The node-to-node distances were measured. Distribution plots of 2,4-D-C^{14}/mg of tissue versus the node-to-node distances were prepared (Fig. 1).

The counting apparatus was checked daily against a standard uranium acetate sample. The geometrical efficiency factor of the counter was calculated to be 1.14%, indicating that the counter will detect 1.14% of the total radiation emitted by the sample.

Eighty-two plants were studied. The plants were exposed to the radioactive 2-4-D for 1–9 days. The results of the runs showed no marked differences in absorption in plants exposed for short intervals as compared with those exposed for longer periods. The entire absorption pattern apparently reached its steady state within 24 hr. A second set of tests showed that the 2,4-D-C^{14} reached its maximum distribution in about 8 hr. A typical plant profile is shown in Fig. 2. Those plants on which the solvent containing

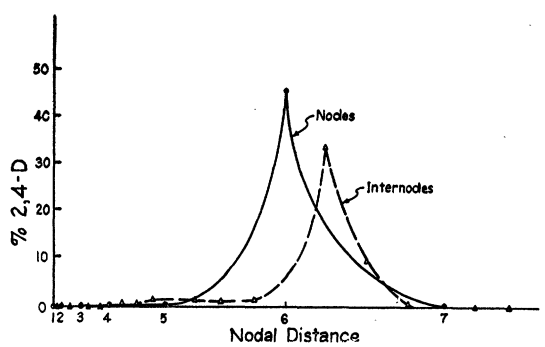

FIG. 2. Typical plant profile (12-hr exposure).

Tween 20 was used showed greater absorption than the controls. There is no dependence of travel rate in the plant on the solvent, or on the quantity of 2,4-D-C^{14} absorbed by the plant. The average rate of upward travel was found to be about 4.3 cm/hr, and the downward rate 4.2 cm/hr. The radioactive material traveled to the top of each plant, but downward only one or two internodes.

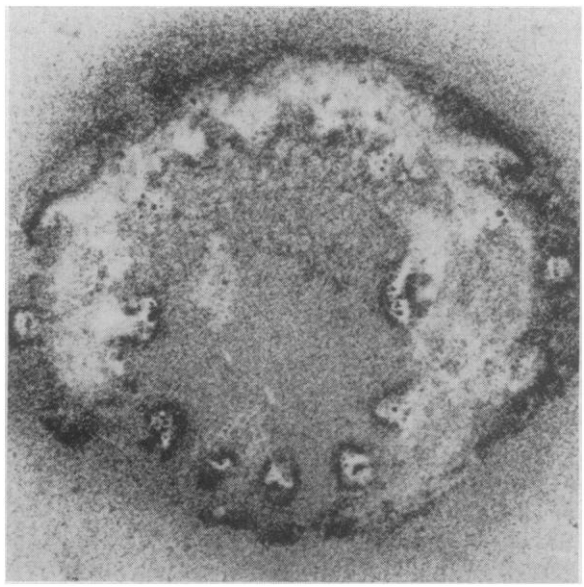

FIG. 3. Radioautograph of section of alligator weed at a treated node. (Dark spots indicate accumulation of 2,4-D-C^{14}.)

Radioautographs were made of sections in various positions of the plant and with various types of film. Eastman contrast lantern slide plates and Ansco stripping film were too slow and required long exposures. Ansco nonscreen x-ray film worked well, with exposure flux of 6 million β-particles/cm^2. This is six times the flux reported by Calvin (*3*). The films were developed in Kodak D-19. Individual nodal or internodal sections did not give sufficient activity to secure a suitable radioautograph when the 2,4-D-C^{14} was applied at only one point on the plant. If adjacent pairs of leaves were treated and a section taken between, good pictures were obtained.

The normal methods of preparing histological sections by dehydration and paraffin embedding would remove most of the 2,4-D-C^{14} in the section. Since the energy of the emitted β-particle is low, thick sections cut manually with a razor blade could be used, for the only effective radiation will be from the tissue layer nearest the film. The radiation from the more distant portions of tissues would be absorbed. In the preliminary experiments the stem sections dried out readily and distorted markedly, not producing satisfactory radioautographs. Interposing thin films between the x-ray film and the section did not prove satisfactory. The problem of distortion was solved by making the radioautographs in a plastic box with a piece of filter paper lining the bottom of the box and a small film support inside. If the filter paper was kept moist, the tissue sections were kept from dehydrating and distorting. Lead foil strips were placed between the films to cut down background fogging from adjacent sections. The plastic box was made light-tight by sealing with masking tape and, as an additional precaution, was placed inside a sealed box. A typical radioautograph is shown in Fig. 3.

Additional radioautographs must be taken before a complete interpretation may be made. The botanical significance of these results will be reported later.

References

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Purification of the Anticoagulant Principle Obtained from the Indian Cattle Leech, *Hirudinaria*

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Lal and Chowdhury (*1*) pointed out that the anticoagulant substance obtained from the Indian cattle leech, *Hirudinaria,* was not only nontoxic but quite effective *in vitro* and also *in vivo* in white mice, both intravenously and orally. In their experiments a crude extract of the anticoagulant substance was obtained by simple maceration of the leech heads in distilled water, and separation of proteins was effected by coagulation at 80°–85° C in a water bath for 20–30 min. The protein content of the anticoagulant substance at this stage was found to be 600 mg%. In our attempts to obtain the active anticoagulant principle in purer form several methods were tried.

To the crude extract obtained after maceration of the leech heads in distilled water was added trichloracetic acid in sufficient quantity to make a 4% solution of the acid for the precipitation of the proteins. The mixture was kept overnight and was subjected to filtration and centrifuging at 2,500 rpm the next day. The supernatant fluid was buffered with phosphate buffer and pH adjusted to 7.2, but a large quantity of phosphate buffer had to be used in order to adjust the pH. The fluid was then evaporated to dryness on a water bath and left in a desiccator overnight. The residue when dissolved in isotonic saline was found to exhibit the anticoagulant activity. The protein content at this stage was estimated and found to be 224 mg%. As the quantity of phosphate required to adjust the pH is very large, the method does not seem satisfactory for obtaining the active substance in pure form.

In another method the macerated extract of leech heads in distilled water was kept at 80°–85° C for 20–30 min and filtered. Absolute alcohol in sufficient quantity was added to the filtrate to precipitate the proteins, and the mixture was left overnight. Next morning it was filtered and then centrifuged to obtain a clear supernatant fluid, which was evaporated to dryness on a water bath to remove the alcohol. The residue was once more dissolved in distilled water, and the solution so obtained was again evaporated to dryness on a water bath to remove the remaining traces