Uptake Of Bivert-Applied Diquat By Hydrilla

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ABSTRACT

The fate of 6,7-dihydrodipyrido[1,2-a:2,1-c]pyrazinidium ion (diquat) applied to hydrilla (Hydrilla verticillata Royle) in the form of an invert emulsion termed "bivert" was studied with 14C-ethylene bridge labeled herbicide. The distribution of the isotope in the plant and surrounding water as well as radioautography of the plant suggests that diquat is released into the aqueous phase and subsequently taken up uniformly by the plant.

INTRODUCTION

The bipyridylidum herbicides, of which diquat and 1,1-dimethyl-4,4-bipyridinium ion (paraquat) are the best known, have been used extensively in chemical weed control because of their relatively low toxicity and rapid inactivation in soil (2). In aquatic weed management, diquat is applied as the soluble dibromide which is very effective in the control of a wide array of free-floating, emergent, and submerged aquatic weeds (2). Generally the effective range is 0.25 to 1.0 ppm in static or slow moving waters, although control with as little as 0.2 ppm has been reported (1).

The marked water solubility of diquat, while desirable in certain circumstances, may be a disadvantage in the treatment of vascular aquatic weeds if only a small amount of herbicide actually reaches the plant surface. Furthermore, inactivation by hydrosoil and light is apt to be extensive. Application by the bivert system might obviate some of these difficulties as well as allow the use of less herbicide thereby reducing costs and ecological perturbations.

In the bivert system, which was originally developed to reduce drift in aerial application to terrestrial weeds, the water soluble herbicide is wrapped in a non-aqueous substance and weighted to reduce drift in the meniscus between the applicator and the plant (4). In the case of delivery to aquatic weeds, a typical field application formulation (per surface acre basis) is diquat, 1 gal (3.785 l); xylene, 5 gal (19.925 l); ammonium sulfamate, 95% (“Ammate x”) (as weighting agent), 50 lb (22.68 kg); dilon application adjuvant (61% fatty amine salts, 35% petroleum distillates - “DPN”), 1 gal (3.785 l) and Cu (7%) complexed as triethanolamine (Catrine), 3 gal (11.355 l). The latter is a desirable adjunct to diquat since they act synergistically (7). The oil and water phases pass through a mixing valve and leave the spray nozzle as droplets of 200 μm.

Although we have observed the toxic effect of biverted diquat on hydrilla under field conditions (8), it is not known whether the herbicide acts directly upon the plant via the adherent bivert droplet, is slowly released into the water to be taken up by the plant, or by a combination of both. The object of this investigation was to follow the fate of diquat(14C) labeled in the ethylene bridge when applied via the bivert system to hydrilla under defined laboratory conditions.

METHODS AND MATERIALS

Plant Materials

Hydrilla was collected from nearby lakes or river areas which were not subjected to any known chemical treatment, washed thoroughly with tap water in the laboratory to remove epiphytic algae and detritus and only obviously healthy tissue was used to plant aquaria. Apical portions varying from 12 to 20 cm were placed in 10% Hoagland’s solution supplemented with iron EDTA (527 ppm Fe) and NaHCO₃ (10 ppm) in 10 g and 37.8 l glass aquaria for at least 1 week prior to use to minimize tissue variability. Aquaria were wrapped on the sides with black mylar plastic and covered with a double thickness of 18 by 14 mesh plastic screen to reduce illumination to the point where algal growth was minimal. Plant nutrient was recirculated by a Metalprime pump at 7.56 l/min through fine cotton filter floss. Aquaria were located in a controlled 23°C environmental room with a 12 hr light-dark cycle giving maximum illumination at mid-day of 660 μw/cm².

Chemicals

The commercial formulation of diquat was purified by a combination of recrystallization from ethanol and paper chromatography which yielded a crystalline product with the spectral characteristics of authentic pure diquat. The specific activity of the diquat (14C bridge labeled) was 60.2 μCi mg⁻¹. With the exception of xylene and diquat, the remaining substances used for bivert preparations were commercial grade.

1Purchased from Amersham Searle, Arlington Heights, Illinois.
2DPN (Dow Chemical Co., San Antonio, Texas); Ammate X (Dupont Chemical Co., Wilmington, Delaware); Catrine (Applied Biochemists Inc., Milwaukee, Wisconsin).
Preparation and Application of Bivert

Since it was quite likely that the composition of the bivert might influence the release of diquat from the droplet, three types of biverts were employed (Table 1). A laboratory equivalent of the bivert was compounded by preparing the oil phase (xylene and DPN) and water phase (diquat, Cutrine, Ammate x and water) separately followed by mixing in a vortex mixer. Although the drop size from the pipette was much larger than that obtained in field spraying, there was no means available for generating radioactive microdrops in a confined system in which excessive amounts of isotope would be avoided.

The bivert material thus prepared was first used in model systems without the plant in order to gain some knowledge of the rate of release of \(^{14}\)C-herbicide under simple conditions, (a) bivert in water under static conditions (b) bivert in water under mild agitation and (c) bivert in water which was flowing through the container at 25 ml/hr. Samples of the water were removed for counting at appropriate intervals.

In the laboratory model system containing the plant, apical segments of hydrilla 12 cm long were weighted with split lead shot and placed in 22 by 150 mm test tubes containing 50 ml of the modified 1% Hoagland's solution. A small aluminum foil collar was placed below the third node and sealed to the stem with melted eicosane in order to prevent any bivert which might be dislodged during handling from spreading to the remainder of the system (Figure 1). One hundred \(\mu\)l of bivert diquat-\(^{14}\)C (357,000 cpm) was carefully pipetted onto the apex.

Preparation of Materials for Counting

One hundred \(\mu\)l samples were withdrawn with a micropipette and transferred to 2.1-cm discs of Whatman glass fiber pads, type GF/A. The pads were air dried at room temperature, placed in a scintillation vial and covered with 10 ml of a scintillation fluid.

Plants were carefully removed from the tube so that the bivert did not become dislodged, and the plant was cut at the collar. The apical section was rubbed gently under flowing water to remove the bivert. The sinker and point of attachment tissue were discarded and the lower section was cut into two pieces.

![Figure 1. Experimental set-up for application of bivert droplet to apical meristem of hydrilla. Bivert droplet is at arrow.](image)

Table 1. Formulation of Three Biverted Mixtures Employed in Model Systems in Which Diquat Release Was Studied.

<table>
<thead>
<tr>
<th>Component</th>
<th>Minus Cutrine</th>
<th>Minus Ammate x</th>
<th>Complete</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylene (ml)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>DPN (ml)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Diquat (mg)</td>
<td>58.1</td>
<td>47.7</td>
<td>67.1</td>
</tr>
<tr>
<td>Diquat-(^{14})C ((\mu)l)</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Cutrine (ml)</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Water (ml)</td>
<td>0.6</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Ammate x (g)</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Total Volume (ml)</td>
<td>1.3</td>
<td>1.0</td>
<td>1.5</td>
</tr>
</tbody>
</table>

\(^{1}\)Oil phase consisted of xylene and DPN while the remaining compounds were in the water phase. Activity of the \(^{14}\)C diquat was 25 \(\mu\)C/ml.

When the tissue was to be used for radioautography, it was fixed on Scotch tape and dried overnight at 67 C. The tape containing the dried plant tissue was adhered to Kodak Industrial X-ray film, Type M, and placed in the dark for 3 weeks, after which the taped plant was removed and the film developed with Kodak Microdol-X.

Plant tissue which was to be assayed for radioactivity by scintillation counting was dried in a tared weighing dish, cut into small pieces and 2 mg of random samples were burned in a specially fabricated 50 ml Erlenmeyer fitted with a scintillation vial side arm by a modification of the method of Gupta (5). The scintillation vial contained a scinttered glass pad impregnated with 0.1 ml phenethylamine to absorb the \(^{14}\)CO\(_2\) formed during the 2-hr equilibration period after oxidation.

All counting was done in a Packard Tricarb Scintillation Counter, Model 3375, using a scintillation fluid of the following composition: 2.5-diphenyloxazole (PPO), 5.5 g; 1,4-bis-[2-(5-phenyloxazolyl)]-benzene (POPOP), 0.1 g; toluene, 667 ml; Triton X-100, 333 ml.

**RESULTS AND DISCUSSION**

A somewhat surprising preliminary observation was the relative ease with which radioactivity entered the water phase from the bivert droplet, especially in the complete
system (Figure 2). In the complete system, 70% of the counts were in the water after 80 hr, whereas only 4% was released in the minus cutrine system. In other experiments not reported here, we noted that other alterations in the composition of the bivert also changed droplet stability, there being a tendency for droplets without Ammato x to fall apart quickly and render kinetic experiments impossible to interpret. Although the flowing system may resemble the situation in the field, it was not greatly different from the stationary one for the first 40 hr.

It was of interest to determine whether the plant tissue became increasingly radioactive with time for this might reveal something of the kinetics of diquat uptake by the plant. Counts of samples of tissue oxidized to CO₂ showed that apical portions were initially more radioactive than basal, but that this difference rapidly lessened with time (Table 2). The phenomenon was not noticeably influenced by light and temperature over the range of conditions tested.

One cannot determine from this type of experiment whether the increase in radioactivity of the tissue distal to the point of application was the result of passage through the plant tissue, release into the water followed by uptake, or both. Therefore, the radioactivity of tissue as a function of time was monitored by radioautography (Figure 3). The relatively even distribution, yet increasing

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Expt 1 (26 C, light)</th>
<th>Expt 2 (26 C, dark)</th>
<th>Expt 3 (20 C, light)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Apical Basal</td>
<td>Apical Basal</td>
<td>Apical Basal</td>
</tr>
<tr>
<td>0.25</td>
<td>225 18</td>
<td>— 1</td>
<td>— 1</td>
</tr>
<tr>
<td>5.0</td>
<td>534 122</td>
<td>581 281</td>
<td>319 14</td>
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<tr>
<td>15</td>
<td>830 804</td>
<td>1183 695</td>
<td>— 574</td>
</tr>
<tr>
<td>24</td>
<td>— —</td>
<td>707 788</td>
<td>986 707</td>
</tr>
</tbody>
</table>

A dash indicates that no determination was made.

amount of radioactivity with time, suggests that the origin of counts noted above in the combusted samples was from free diquat previously released into the water from the bivert droplet. It is known that diquat as the usual soluble salt is rapidly taken up reversibly by elodea and that both absorption and metabolic accumulation are involved (3). The results reported here, which are in agreement with this, suggest that the phytotoxic effects of bivert applied diquat are due to the extensive release of diquat into the water surrounding the plant and subsequent uptake. Furthermore, the xylene probably disrupts the plant surface to facilitate uptake.

It is possible that the radioactivity was due to the

Figure 2. Release of biverted ¹⁴C-diquat from the model systems under varying conditions of agitation. Upper: complete system; lower: minus cutrine Left, static; center, agitated; right, flow through.
uptake of substances other than diquat in view of the known light sensitivity of bipyridylum herbicides. This was unlikely since spectrophotometric monitoring of the water phase demonstrated the gradual liberation of substance absorbing at 310 nm and a curve very much like that of diquat.

These findings have considerable implications regarding the application of diquat in the field by the bivert technique. The rate of release from the pipetted droplets must represent minimal values for the release in the field from microdrops 200 µm in diameter would be much faster due to the vast difference in surface area between the two. Rapid release into the environment adjacent to the plant is in accord with our field observations of the rapid appearance of phytotoxicity after spraying. Although release followed by rapid uptake is desirable from the viewpoint of control of the plant, the basic assumption previously made that the herbicide passes through the water to the plant without accumulating in the water phase may be questioned. Whether degradation products are also in the water cannot be answered at this time.

The method employed in these studies should be readily applicable to studying the fate of other herbicides which can be obtained or prepared with appropriate isotopic labels. One herbicide which would be desirable to study is 1,2-dicarboxy-3,6-endocyclohexane (endothall) which has been successfully applied in the bivert form in the field.

ACKNOWLEDGEMENTS

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LITERATURE CITED
