ABSTRACT

Endothall has been used as an aquatic herbicide for more than 40 years and provides very effective weed control of many weeds. Early research regarding the mechanism-of-action of endothall contradicts the symptomology normally associated with the product. Recent studies suggest endothall is a respiratory toxin but the mechanism-of-action remains unknown. To further elucidate the activity of endothall, several endothall formulations were evaluated for their effects on ion leakage, oxygen consumption and photosynthetic oxygen evolution from hydrilla shoot tips. The influence of pH, buffering and divalent cations was also evaluated. Based on ion leakage, the LD$_{50}$ of the alkylamine formulation was substantially lower under both light and dark conditions than the acid or dipotassium salt formulations. Lowering the pH increased ion leakage, and therefore activity, of the dipotassium salt, while buffering had the opposite effect on the acid formulation. Neither pH nor buffer concentration had an effect on the alkylamine salt, however addition of divalent cations increased ion leakage by all formulations. The rate of oxygen consumption was initially increased followed by a sharp decrease for both the dipotassium and alkylamine salt formulations. All formulations also caused a marked reduction in photosynthetic oxygen evolution within 60 minutes of treatment. Based on these data, endothall appears to inhibit respiration and photosynthesis, possibly due to a similar mechanism. There also appears to be fundamental differences between the alkylamine salt and the other formulations that may help to explain the mechanism-of-action of endothall in hydrilla and other plants.

Key words: mode-of-action, conductivity, photosynthesis inhibition, oxygen consumption, uncoupler, ionophore.

INTRODUCTION

Endothall (7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid) has been widely used for effective control of several submerged aquatic species including hydrilla (Hydrilla verticillata [L.f.]Royle), Illinois pondweed (Potamogeton illinoensis Morong), and southern naiad (Najas guadalupensis [Spreng.] Magnus) (Hiltibran 1963, Thayer et al. 2001). Endothall is also registered for use as a preharvest defoliant for cotton (Gossypium hirsutum L.), as a potato vine (Solanum tuberosum L.) desiccant and harvest aid for legume forages (Anonymous 2002).

Endothall was first discovered in 1929 by vonBruchhausen and Bersch as a derivative of cantharidin, a natural product of the blister beetle (Epicauta spp. - Davidson and Lyon 1979) and was reported to possess herbicidal activity in 1948 by Niagara Chemical Company. This compound was registered for use as a plant growth regulator by the Sharples Chemicals Company in 1951 (Tischler et al. 1950). Endothall was first registered for aquatic weed control in 1960 by Pennsalt Chemical Company (Hiltibran 1963).

Several formulations of endothall have been developed and marketed and include the sodium salt which is no longer registered for use, the dipotassium salt, and mono- and dimethylalkylamine salt formulations. The sodium and dipotassium formulations form salts with the parent acid at the carboxylic acid moieties. This is similar to the alkylamine, which either forms salts at one carboxylic acid group (monoalkylamine) or both (di-methylalkylamine). The alkyl groups are a mixture of C$_{10}$ to C$_{12}$ straight chains, although chain length varies from 8 to 14 carbons (Anonymous, 1976).

Both dipotassium (Aquathol® K) and alkylamine (Hydrothol® 191) salts of endothall are formulated as liquid or granule and are registered for aquatic use. The dipotassium salt is recommended for hydrilla control at 0.5 to 3.0 ppm and the alkylamine from 0.05 to 0.5 ppm. While the alkylamine salt formulation is more effective, its use is limited in several areas due to fish toxicity issues (Thayer et al. 2001). The oral LD$_{50}$ (rat) for endothall is also variable with respect to formulation. The LD$_{50}$ for the parent acid is 38 to 51 mg/kg while the dipotassium and alkylamine salts are 198 and 206 mg/kg, respectively (Ahrens 1994). This discrepancy between lower LD$_{50}$ yet higher fish toxicity for the alkylamine salt is not well understood.

Endothall dissipates very rapidly after application. The half-life of the dipotassium salt is often as short as 2 to 3 days in water while alkylamine salts may persist for 14 to 21 days.
Microbial breakdown is the primary means of dissipation in the aquatic environment (Sikka and Saxena 1973). Endothall has limited soil activity in terrestrial systems and is taken up by the roots and translocated apoplastically (Turgeon et al. 1972). There is no appreciable phloem movement and post-emergence selectivity among terrestrial plant species appears to be differential absorption, uptake, or by both processes. In contrast, movement within aquatic plants is symplastic (Thomas and Seaman 1968).

Endothall appears to be contact in activity, but research on its mode-of-action indicates otherwise. Mann and Pu (1968) showed that endothall inhibited lipid synthesis and caused a decrease in protein synthesis (Mann et al. 1965). Tsay and Ashton (1971) showed a reduction in dipeptidase and proteinase activity, while Penner and Ashton (1968) showed this decrease was similar to actinomycin D and therefore postulated that endothall interfered with mRNA metabolism.

Collectively, the modes-of-action proposed in the preceding paragraph describe a slow-acting herbicide, quite in contrast to the rapid, contact-type activity of endothall noted in field studies. Research by MacDonald et al. (1993, 1999) has reported that endothall may have similar activity in plants and animals, and indicated respiratory toxicity. In an attempt to further elucidate the mechanism-of-action of endothall, several studies were conducted to evaluate the influence of formulation, buffering, pH, and divalent cations on endothall activity. Hydrilla was used as an assay system due to its susceptibility to endothall and the importance of this weedy aquatic species (Langeland 1990).

MATERIALS AND METHODS

Preparation of hydrilla shoots. Plant material for all experiments consisted of apical shoots 2 cm in length collected from dioecious hydrilla cultured under greenhouse conditions. Each shoot tip contained 20 to 30 leaves, with each individual leaf approximately 2 to 3 mm wide by 7 to 10 mm long. The mean fresh weight of a blotted dry sample was 200 ± 30 mg (standard deviation). Shoot tips were excised with dissecting scissors, washed with tap water and stored in sterile water for at least one hour prior to use in experiments. Based on preliminary experiments with deionized water and microscopic observations (data not shown), this treatment maximized cell turgor and allowed the formation of callose at the excision site. Technical grade endothall formulations of the alkylamine salt, dipotassium salt, and acid monohydrate were used for all studies. All herbicide formulations were supplied by Cerexagri, Inc.

Ion Leakage Studies. Hydrilla shoot tips (one tip per 5 dram vial) were incubated in 5 ml of treatment solution, similar to previous studies (MacDonald et al. 1993). Identical treatments were either in continuous light or in constant darkness for the duration of studies. Treatments maintained in continuous light (300 µmol·m⁻²·s⁻¹) were held in clear 5 dram vials in a water bath shaker at 21 C. Dark treatments were maintained in vials on a rotary shaker in a photographic darkroom at 26 C. Conductivity (µmhos/cm⁻¹) was measured utilizing a conductivity bridge with the probe modified to contain the entire 5 ml of treatment solution. Initial conductivity was measured on the solutions alone and total conductivity was obtained by freezing and thawing the samples twice to release all ions. Data are presented as percent conductivity derived from the following equation:

\[
\text{% conductivity} = \left( \frac{(\text{measured} - \text{initial})}{(\text{total} - \text{initial})} \right) \times 100; \text{ where measured equaled the amount of conductivity at each time of measurement.}
\]

For initial comparisons of formulation, ion leakage from hydrilla shoot tips was monitored in continuous light or complete darkness at 4, 8, 12, 24, 48 and 72 hours after initial treatment. A concentration of 100 µM was used for all formulations with deionized water as a control. A dose response over a 24-hour period was obtained for all formulations and this was used to calculate the IC₅₀ (the concentration required to cause 50% ion leakage) for continuous light and constant dark treatments. To evaluate the effect of pH, 50 µM alkylamine and 2 mM dipotassium salt formulations were placed in unbuffered solutions at pH 3, 5, or 7. Hydrochloric acid (HCl) or potassium hydroxide (KOH) was used to achieve desired pH. The effect of buffering and ammonium chloride (2 mM) was also tested on the acid and alkylamine formulations of endothall. In addition, 2 mM concentrations of monovalent and divalent cations were tested for their effects with all endothall formulations. Ion leakage was monitored for 24 hours for pH, buffering and cation studies. Percent ion leakage data was adjusted for the amount of leakage that occurred in control treatments. All experiments were conducted at least twice with a minimum of four replications.

Oxygen Uptake. Hydrilla shoot tips were placed in treatment solutions containing 10 or 100 µM of alkylamine salt or dipotassium salt formulation of endothall. Distilled, deionized water was used as a control. Dark respiration was monitored by first acclimating shoot tips in complete darkness for 1 hour then placing them in 15 ml of treatment solution in a 5 dram glass vial, maintaining complete darkness. A dissolved oxygen probe was wrapped with teflon tape so that it was sealed in the mouth of the vial just as head-space (air) was eliminated. Agitation was provided by clamping the vial in a wrist-action shaker. Oxygen uptake as a measure of respiration was measured over 10-minute periods at hourly intervals for 4 hours. Reported results are the means of eight replications.

Photosynthetic Oxygen Evolution. Photosynthesis was assayed as light-induced oxygen evolution from hydrilla shoot tips in 15 ml of treatment solution. Treatments included distilled water control, 2,000 µM acetic monohydrate, 2000 µM dipotassium salt or 100 µM alkylamine salt of endothall. Shoot tips were placed in a 5 dram glass vial containing the treatment solution and 5 mM sodium bicarbonate (NaHCO₃). The vial was clamped in a wrist-action shaker and exposed to 300 µmol·m⁻²·s⁻¹ light. Tips were positioned so that light could be focused on the abaxial and adaxial surfaces of the leaves. The oxygen meter probe was wrapped with teflon tape and placed in the vial so that it sealed just as head-space was eliminated. Measurements were recorded for 1 to 5 minutes, depending on the rate, hourly for 2 hours. Between measurements the samples were stored in an illuminated water bath shaker. The reported results are the means of 6 replications.

Statistics. Data were subjected to analysis of variance (ANOVA) to determine the significance of treatment effects.
and interactions (P < 0.05). Data for ion leakage were pooled across experiments and means separated using Fisher’s least significant difference procedure at the 0.05 level. Means on the effect of formulation on oxygen consumption and photosynthetic oxygen evolution were separated using Fisher’s least significant procedure at the 0.05 level. Results for other studies are presented with standard errors of the mean with a minimum of four replications.

RESULTS

Ion Leakage. Initial conductivity experiments showed that the alkylamine formulation of endothall caused greater damage in both light and dark than either of the other formulations. The alkylamine salt caused over 60% conductivity within 4 hours of treatment and almost complete ion leakage after 72 hours in the light (Figure 1). There was clearly a greater effect of this formulation on ion leakage in darkness, where > 95% conductivity was measured after 4 hours and complete ion leakage had occurred with 24 hours of treatment (Figure 2). The dipotassium salt and acid formulations showed < 25% conductivity in the light with little difference between formulation over the treatment period (Figure 2). In the dark, endothall acid caused more rapid and greater ion leakage as compared to the dipotassium salt formulation (Figure 2). Based on \( I_{50} \) values, the alkylamine formulation was nearly 100 times more toxic than either the acid or dipotassium salt formulations (Table 1). There was no difference in \( I_{50} \) between the acid and dipotassium salt in the dark.

Changes in pH had no effect on the activity of the alkylamine salt (Table 2). Acidification of the medium greatly enhanced the activity of dipotassium endothall and this effect was observed under both light and dark conditions. There was no impact of buffering, the addition of ammonium chloride or the combination of the two on ion leakage from the alkylamine formulation in the light (Table 3). Alkylamine activity was enhanced by buffering alone in the dark and the activity of buffering was greater with ammonium chloride.

Conversely, buffering the treatment medium greatly decreased the activity of technical endothall acid under both light and dark conditions. The addition of ammonium chloride to buffer ameliorated this decrease in activity in the light but had little impact under dark conditions. The addition of ammonium sulfate and the divalent cations iron, calcium or magnesium caused a dramatic increase in activity across all formulations of endothall (Table 4). The dipotassium salt formulation showed slightly less activity compared to the other formulations and there was little difference between treatments under light or dark conditions.

Oxygen Uptake. Both dipotassium and alkylamine salts of endothall, regardless of concentration tested, caused an increase in oxygen consumption immediately after treatment (Table 5). There was no effect of the acid formulation after 10 minutes, but the dipotassium and alkylamine formulations of endothall inhibited photosynthesis within 10 minutes by 72 and 82%, respectively. Endothall alkylamine caused complete inhibition after 1 hour.

Photosynthetic Oxygen Evolution. All formulations of endothall inhibited photosynthetic oxygen evolution at the doses tested, and caused complete inhibition within 2 hours (Table 6). There was no effect of the acid formulation after 10 minutes, but the dipotassium and alkylamine formulations of endothall inhibited photosynthesis within 10 minutes by 72 and 82%, respectively. Endothall alkylamine caused complete inhibition after 1 hour.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>( I_{50} ) in light</th>
<th>( I_{50} ) in dark</th>
</tr>
</thead>
<tbody>
<tr>
<td>acid monohydrate</td>
<td>2.68 mM</td>
<td>2.71 mM</td>
</tr>
<tr>
<td>dipotassium salt</td>
<td>3.91 mM</td>
<td>2.83 mM</td>
</tr>
<tr>
<td>alkylamine salt</td>
<td>0.09 mM</td>
<td>0.04 mM</td>
</tr>
</tbody>
</table>

Figure 1. The effect of 100 µM endothall technical acid, dipotassium salt, and alkylamine salt on ion leakage from hydrilla shoot tips in the light.

Figure 2. The effect of 100 µM endothall technical acid, dipotassium salt, and alkylamine salt on ion leakage from hydrilla shoot tips in the dark.
The alkylamine formulation of endothall was found to be much more active than either the acid or dipotassium (K\(^+\)) salt, causing greater and more rapid ion leakage in hydrilla. This effect was similar under light or dark conditions, with greater ion leakage under darkness. MacDonald et al. 1993 also found greater leakage under dark conditions for the parent acid, similar to the results observed in this study. However, the most striking observation was the concentration difference between the alkylamine and the other formulations. The alkylamine exhibited activity at rates 97% and 98% lower concentrations than the acid and K\(^+\) salt, respectively. Walker (1963) also reported major differences between the disodium salt and the alkylamine salt of endothall with respect to herbicidal activity. He reported a nearly 100-fold greater activity in fish mortality of fish exposed to the alkylamine salt compared to the disodium salt which is similar to the nearly 100-fold difference in activities reported here. This effect was similar under light or dark conditions, with decreasing pH (Briggs et al. 1987, Devine et al. 1987). The alkylamine salt appears to react differently, suggesting that this formulation does not dissociate from the parent acid, at least in the conditions present in this study. It also suggests that the alkylamine formulation is more lipophilic than either the acid or K\(^+\) salt and may be forming a micelle (Benjamin Horenstein, pers. comm.). Another possibility could be that the alkylamine salt dissociates from the parent acid and reacts directly with the membrane to increase permeability for the parent acid to be absorbed.

Buffering dramatically reduced the activity of endothall acid under both light and dark but had little effect on the alkylamine. Buffering the solution at pH 7.0 maintains endothall acid in the dissociated form, limiting uptake. The addition of a proton donor such as ammonium chloride enhanced activity, allowing more of the endothall acid to be undissociated.

**DISCUSSION**

### Table 2. The Effect of pH on Ion Leakage (% Conductivity) from Hydrilla Shoot Tips in the Light and the Dark for Two Salt Formulations of Endothall.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Light</th>
<th>Dark</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Dipotassium (2 mM)</td>
<td>80 ± 6</td>
<td>43 ± 2</td>
</tr>
<tr>
<td>Alkylamine (50 µM)</td>
<td>36 ± 2</td>
<td>49 ± 2</td>
</tr>
</tbody>
</table>

\(^1\)Means within formulation and light regime followed by a different letter are significantly different (\(\alpha = 0.05\), Fisher’s least significant difference procedure).

\(^2\)Means followed by standard error.

### Table 3. The Effect of Buffering and Ammonium Chloride on Ion Leakage (% Conductivity) from Hydrilla Shoot Tips in the Light and the Dark for Two Formulations of Endothall.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Light</th>
<th>Dark</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amine</td>
<td>Acid</td>
</tr>
<tr>
<td>None</td>
<td>75 ± 8</td>
<td>53 ± 4</td>
</tr>
<tr>
<td>Buffer(^1)</td>
<td>82 ± 9</td>
<td>25 ± 2</td>
</tr>
<tr>
<td>NH(_4)Cl</td>
<td>79 ± 12</td>
<td>79 ± 3</td>
</tr>
<tr>
<td>Buffer + NH(_4)Cl</td>
<td>88 ± 8</td>
<td>59 ± 3</td>
</tr>
<tr>
<td>LSD(\text{so}_\text{a})</td>
<td>NS</td>
<td>19</td>
</tr>
</tbody>
</table>

\(^1\)Concentrations for amine and acid formulations are 50 µM and 2 mM, respectively.

\(^2\)Means followed by standard error.

\(^3\)Mean comparison within a column (\(\alpha = 0.05\), Fisher’s least significant difference procedure).

### Table 4. The Effect of Ammonium and Divalent Cations on Ion Leakage (% Conductivity) from Hydrilla Shoot Tips in the Light and the Dark for Three Formulations of Endothall.

<table>
<thead>
<tr>
<th>Treatment(^1)</th>
<th>Light</th>
<th>Dark</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amine</td>
<td>Acid</td>
</tr>
<tr>
<td>None (D.I. Water)</td>
<td>28 ± 4</td>
<td>27 ± 3</td>
</tr>
<tr>
<td>NH(_4)(SO(_4))(_2)</td>
<td>75 ± 5</td>
<td>—</td>
</tr>
<tr>
<td>FeSO(_4)</td>
<td>80 ± 2</td>
<td>76 ± 3</td>
</tr>
<tr>
<td>MgCl(_2)</td>
<td>93 ± 4</td>
<td>74 ± 4</td>
</tr>
<tr>
<td>CaCl(_2)</td>
<td>93 ± 6</td>
<td>75 ± 2</td>
</tr>
<tr>
<td>LSD(\text{so}_\text{a})</td>
<td>9</td>
<td>6</td>
</tr>
</tbody>
</table>

\(^1\)Concentrations for all treatments was 2 mM. Treatments included ammonium sulfate [(NH\(_4\))(SO\(_4\))]\(_2\), ferrous sulfate [FeSO\(_4\)], magnesium chloride [MgCl\(_2\)], and calcium chloride [CaCl\(_2\)].

\(^2\)Mean comparison within a column (\(\alpha = 0.05\), Fisher’s least significant difference procedure).

\(^3\)Means followed by standard error.

\(^4\)Mean comparison within a column (\(\alpha = 0.05\), Fisher’s least significant difference procedure).

---

Divalent cations greatly enhanced the activity of all formulations, with slightly less enhancement observed for the K+ salt. This could be due to competition between the cations and the K+ itself. The cations could also be reacting directly to form a different salt (molecule). Chelation between the mono-oxygen bridge of endothall and divalent cations such as Mg have been reported (Matsuzawa et al. 1987). This chelation could act to stabilize the molecule, making it more lipophilic and consequently increasing diffusion through membranes. Acidification of the media could also have occurred by hydrilla absorbing the cations, exchanging protons for cation uptake.

The effect of the alkylamine and K+ salt formulations on dark respiration was similar, with both causing an initial stimulation of respiration followed by complete cessation of oxygen consumption within 3 hours. However, the alkylamine salt caused a longer stimulation of respiration compared to the K+ salt. This stimulation effect was previously reported with the acid formulation by MacDonald et al. (1993). Moreover, there were striking differences between the alkylamine formulation and the acid and K+ salts. The most obvious was the rate response, but also the differential impact of buffering and pH between formulations. Other interesting observations were the differences observed between the formulations and respiration and photosynthetic oxygen evolution. The alkylamine salt appeared to have greater impact on photosynthesis and less of an impact on respiration compared to the K+ salt.

This study does not define the primary mechanism-of-action of endothall and suggests that endothall has multiple phytotoxic modes-of-action and that the mechanism-of-action might be associated at the membrane level. The differences between formulations raise further questions regarding activity of endothall on hydrilla, suggesting that formulation, especially in the case of the alkylamine salt, may have a direct bearing on the mechanism of action of this herbicide.

Collectively, these studies further support the hypothesis that the activity of endothall is similar to inhibitory uncouplers such as dinoseb (2-sec-butyl-4,6-dinitrophenol) (MacDonald et al. 1993). Moreover, there were striking differences between the alkylamine formulation and the acid and K+ salts. The most obvious was the rate response, but also the differential impact of buffering and pH between formulations. Other interesting observations were the differences observed between the formulations and respiration and photosynthetic oxygen evolution. The alkylamine salt appeared to have greater impact on photosynthesis and less of an impact on respiration compared to the K+ salt.

These studies were supported by the Center for Aquatic and Invasive Plants and the Agronomy Department at the University of Florida. Cerexagri graciously donated supplies to support this research. The advise of Drs. William Haller and Benjamin Horenstein was greatly appreciated.

ACKNOWLEDGEMENTS

These studies were supported by the Center for Aquatic and Invasive Plants and the Agronomy Department at the University of Florida. Cerexagri graciously donated supplies to support this research. The advise of Drs. William Haller and Benjamin Horenstein was greatly appreciated.

LITERATURE CITED


