J. Aquat. Plant Manage. 40: 68-71

Activity of Endothall on Hydrilla[1]

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ABSTRACT

Endothall was compared to compounds with known mechanisms-of-action in an attempt to elucidate its mode-of-action on hydrilla (*Hydrilla verticillata* [L.f.] Royle). In measurements of ion leakage over time, endothall caused 30% greater cellular damage in darkness than in light after 30 hours of exposure. Endothall did not affect chlorophyll fluorescence but inhibited photosynthetic oxygen evolution of hydrilla shoots after 2 hours. Endothall reduced respiration at 100 µM but stimulated respiration at 1000 µM. In comparative studies of phytotoxicity, the effects of endothall most closely resembled those of the ionophore, gramicidin or the uncoupler, dinoseb. This suggests the activity of endothall in hydrilla is membrane associated, possibly through the inhibition of ATP production in photosynthesis and respiration. The cessation of ATP production would cause a loss of cell membrane integrity, which more accurately explains the rapid, contact-type symptomology associated with endothall activity on hydrilla.

Key words: Hydrilla verticillata, herbicides, dinoseb, simazine, diuron, gramicidin, diquat, mode-of-action, conductivity, photosynthesis inhibition, uncoupler, ionophore.

INTRODUCTION

Endothall (7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid) has been used as an aquatic herbicide for nearly 40 years. It effectively controls several submersed aquatic species including hydrilla, Illinois pondweed *(Potamogeton illinoensis* Morong), and coontail (*Ceratophyllum demersum* L.) (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 alfalfa (*Medicago sativa* L.) and clover *(Trifolium* spp.) (Anonymous 2001).

Endothall is classified as a phthallic acid herbicide by Anderson (1993), although it has been regarded as unclassified due to its unique mono-oxygen bridge structure. Endothall is a derivative of cantharidin, a skin irritant produced by blister beetles (*Epicauta* spp.), and was first synthesized in 1929 (vonBruchhausen and Bersch 1929). It was found to possess herbicidal properties in 1948 and was registered for use as a plant growth regulator in 1951. In 1960, Pennsalt Chemical Company registered endothall for use as an aquatic herbicide.

Endothall is utilized for hydrilla management, and provides excellent, albeit short-term, control (Westerdahl and Getsinger 1988). Endothall is fairly short-lived in the aquatic environment; the half-life of the dipotassium salt formulation is 3 days while the half-life of the alkylamine salt is 14 to 21 days (Simsimann and Chesters 1975). Endothall movement within submersed aquatic plants is symplastic (Thomas and Seaman 1968) and uptake in hydrilla is enhanced by low light levels and high temperatures (Haller and Sutton 1973).

There have been a number of published reports on the herbicidal modes of action of endothall. Mann et al. (1965) reported that endothall severely inhibited protein synthesis in hemp sesbania (*Sesbania exaltata* [Raf.] Rybd.) but later reported that endothall inhibited lipid synthesis (Mann and Pu 1968). Penner and Ashton (1968) and Tsay and Ashton (1971) found that endothall decreased proteolytic activity and dipeptidase activity, respectively, and concluded that endothall inhibited mRNA synthesis. Turgeon et al. (1972) found that endothall rapidly inhibited photosynthesis in annual bluegrass (*Poa annua* L.). Abeles and Abeles (1972) also found that endothall inhibited photosynthesis and stimulated respiration in excised bean leaves. Collectively, these studies showed that endothall can affect diverse biochemical reactions in plants but failed to address the generally devastating and rapidly lethal symptoms observed in plants treated with endothall.

The activity of endothall on hydrilla appears to be contact in nature, with severe cellular disruption occurring within 72 hours of treatment (Keckemet 1968, Keckemet and Nelson 1968). This mode of action on hydrilla is similar to the activity on terrestrial plants, where wilting and leaf dessication is the commonly observed symptom (Haderlie 1989). Keckemet (1968) referred to endothall as a membrane-active herbicide, noting its phytotoxic symptoms to be a breakdown of the cellular osmotic system and abnormal permeability. More recent research by MacDonald et al. (1993) suggested that endothall was a respiratory poison, but does not completely account for the dramatic effects exhibited by this compound, especially as it relates to submersed aquatic species.

Therefore, studies were conducted to assay the activity of endothall herbicide on the submersed aquatic weed hydrilla and compare the activity to compounds with known mechanisms-of-action. More specifically, this research was conducted to: 1) determine if the activity of endothall is similar between aquatic and terrestrial plants and 2) provide further evidence as to the mode of action of endothall.

MATERIALS AND METHODS

Preparation of hydrilla shoots. Plant material for all experiments consisted of 2 cm apical shoots 2 cm in length collected from hydrilla cultured under greenhouse conditions. Each shoot tip had 20 to 30 leaves, and each individual leaf

[1]Published with the approval of the Florida Agricultural Experiment Station as J. Series No. R-08885.

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was approximately 2 to 3 mm wide by 7 to 10 mm long. The mean fresh weight of a sample (blotted dry) was 200 ± 30 mg. Shoot tips were excised with dissecting scissors, washed with tap water and stored in sterile water for at least 1.0 hour prior to use in experiments.

Chemicals. Technical grade endothall acid was supplied by Cerexagri, Inc.—North America. Gramicidin, diuron (N'[3,4-dichlorophenyl]-N,N-dimethylurea) and dinoseb (2-sec-butyl-4,6-dinitrophenol) were purchased from Sigma Chemicals (St. Louis, MO.). Technical simazine (6-chloro-N,N'-diethyl-1,3,5-triazine-2,4-diamine) was provided by Syngenta Crop Protection, Inc. and recrystalized diquat (6,7-dihydropyrido [1,2-a:2',1'-c]pyrazinediium) dibromide was provided by Dr. Thai Van, USDA-ARS, Ft. Lauderdale.

Ion leakage. Hydrilla shoot tips (one tip per vial) were incubated in 5 ml of treatment solution and placed under continuous light (300 $\mu mol \cdot m^{-2} \cdot s^{-1}$) at 21C or continuous dark conditions at 26C. Conductivity ($\mu mhos/cm^2$) was measured utilizing a conductivity bridge[6] at 4, 8, 12, 24, 30 and 48 (dark regime only) hours after initial exposure. 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: % conductivity = ((measured - initial)/(total - initial))*100; where measured equaled the amount of conductivity at each time of measurement. Treatments for both light and dark experiments included 100 µM endothall technical acid, 100 µM simazine, 10 µM gramicidin, 10 µM diquat, and 10 µM dinoseb. The concentrations tested were approximate I_{50} concentrations based on previous research (MacDonald et al. 1993). However, an accurate I_{50} value for simazine could not be obtained under the described experimental conditions. The entire study was conducted twice with four replications per treatment were conducted at least twice with four replications.

Fluorescence. Individual leaflets from hydrilla shoot tips were exposed to 100 µM endothall acid, or 10 µM diquat, dinoseb or simazine. Leaflets were placed under continuous light (300 $\mu mol \cdot m^{-2} \cdot s^{-1}$) at 21C for 90 minutes. Treated leaflets were dark-equilibrated for 10 minutes prior to chlorophyll *a* fluorescence determination.[7] Initial, peak and terminal fluorescence measurements were taken. Terminal fluorescence was measured after 50 seconds with a 1.0-second gain between initial and peak measurements. Data are presented as the ratio of peak to terminal fluorescence derived from the following equation: (peak - initial)/(terminal - initial). Results are the means of six replications.

Oxygen uptake. Hydrilla shoot tips were placed in treatment solutions containing 0 (distilled water control), 100, 1000 or 10,000 µM of endothall acid. Dark respiration was monitored by first acclimating shoot tips in complete darkness for 1.0 hour then placing them in 15 ml of treatment solution in a glass vial, maintaining complete darkness. A dissolved oxygen probe[8] 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. 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 endothall acid, 100 µM gramicidin, 1 µM diquat or 1 µM diuron. Shoot tips were placed in a glass vial containing the treatment solution and 5 mM sodium bicarbonate ($NaHCO_3$). The vial was clamped in a wrist-action shaker and exposed to 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light. Tips were positioned so that light could be focused on the abaxial and adaxial surfaces of the leaves. The oxygen meter probe[8] was wrapped with teflon tape and placed in the vial so that it sealed just as head-space was eliminated. Measurements were recorded for 1.0 to 5.0 minutes, depending on the rate, hourly for 2 hours because faster rates caused rapid oxygen-saturation of the treatment solution, leading to oxygen-inhibition of the reaction. Between measurements the samples were stored in an illuminated water bath shaker. Results are the means of six replications.

Statistics. Data were subjected to analysis of variance (ANOVA) to determine the significance of treatment effects ($P < 0.05$). Data for ion leakage were pooled across experiments Means for photosynthetic oxygen evolution were separated using Waller-Duncan *k*-ratio *t* test at the 0.05 level. Results for other studies are presented with standard errors of the mean (minimum of four replications).

RESULTS AND DISCUSSION

Ion leakage. Endothall caused nearly 60% conductivity under light conditions after 30 hours, which was similar to that observed from diquat and dinoseb (Figure 1). Simazine caused a minimal increase in conductivity (<25%) under light conditions, with only a slight increase in conductivity over the treatment period. Gramicidin caused the most rapid increase in conductivity, producing over 60% total conductivity from hydrilla shoot tips after 6 hours. However, the effect of this compound did not increase over the treatment period. Percent conductivity caused by endothall, diquat and dinoseb increased over time with diquat inducing nearly 90% conductivity after 30 hours under continuous light conditions. The ion leakage of dinoseb and endothall was similar after 30 hours (approximately 60% conductivity), although the leakage from endothall was more rapid than dinoseb in the light.

The effect from endothall and dinoseb was much more rapid under dark conditions compared to those observed in the light regime and resulted in higher percent conductivity values (>80%) after 30 hours of treatment (Figure 2). Simazine caused similar leakage under dark conditions, with less than 15% conductivity observed over the treatment period. The leakage caused by gramicidin was also similar, with slightly higher percent conductivity observed. Diquat caused less conductivity under dark conditions, with approximately 45% leakage observed after 48 hours.

Endothall caused rapid ion leakage and cellular disruption from hydrilla shoot tips under light and dark regimes with greater and more rapid leakage in the dark, indicating

[6]Model 31, Yellow Springs Instrument Co., Inc., Yellow Springs, OH 45387.

[7]Plant Productivity Fluorometer, Model SF20, Richard Brancker Research, Ottawa, Ont., Canada.

[8]Model 820, Orion Research, Boston, MA.

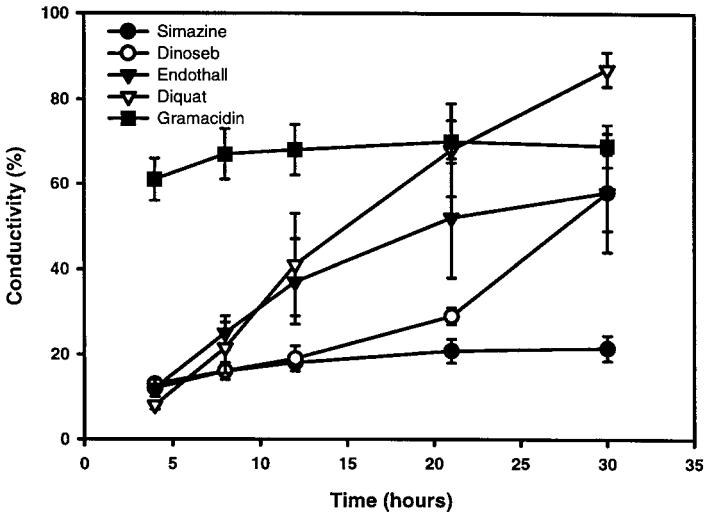

Figure 1. The effect of endothall acid, gramicidin, dinoseb, diquat, and simazine on ion leakage from hydrilla shoot tips in the light.

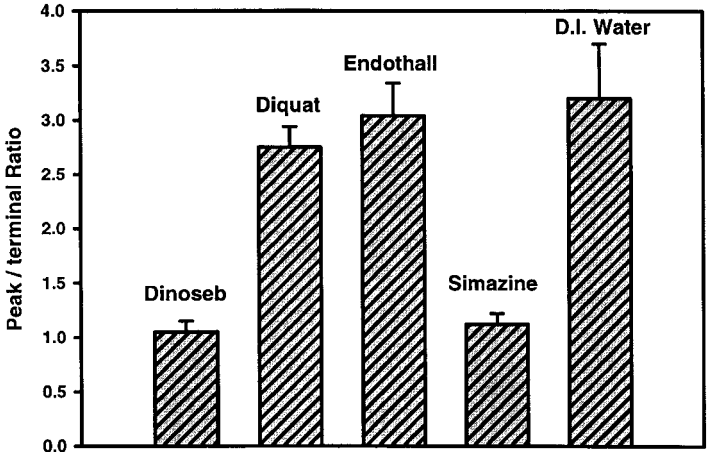

Figure 3. The effect of endothall acid, gramicidin, dinoseb, diquat, and simazine on chlorophyll *a* fluorescence in hydrilla leaflets.

that the herbicidal activity of endothall is not totally light-dependent. This pattern of ion leakage was also similar to leakage caused by the known membrane uncoupler dinoseb (Moreland 1988).

Fluorescence. Endothall and diquat produced no increase in fluorescence as indicated by peak/terminal ratios around 3, similar to those observed for distilled water. Dinoseb and simazine lowered the peak/terminal ratio after 90 minutes, indicating increased chlorophyll a fluorescence relative to the distilled water control (Figure 3).

Chlorophyll fluorescence is a direct measure of light reaction efficiency where chlorophyll molecules re-radiate 'excess' absorbed light energy as fluorescence (Lawlor 1987). Chlorophyll fluorescence is typically measured through the ratio of peak to terminal fluorescence. Higher values indicate normal light reaction efficiency, while ratios near 1 signify an inhibition of electron flow. Dinoseb and simazine are known to block electron flow in photosystem II and produced characteristically low ratios (Devine et al. 1993) while endothall had little to no effect when compared to the distilled water control. This indicates that the activity of endothall on hydrilla is not directly associated with an inhibition of the light reactions of photosynthesis.

Oxygen uptake. Endothall, depending on concentration, caused suppression or stimulation of oxygen consumption compared to the distilled water control over the 3-hour treatment period (Figure 4). Endothall at 100 µM suppressed respiration while the higher rates of 1000 and 10,000 µM stimulated respiration. Both higher rates caused a similar amount of oxygen consumption over the treatment period.

Stimulation of respiration is a common response for most plants when exposed to a membrane uncoupler (Moreland 1988) and previous research by MacDonald et al. (1993) showed that the uncoupler gramicidin caused a marked in-

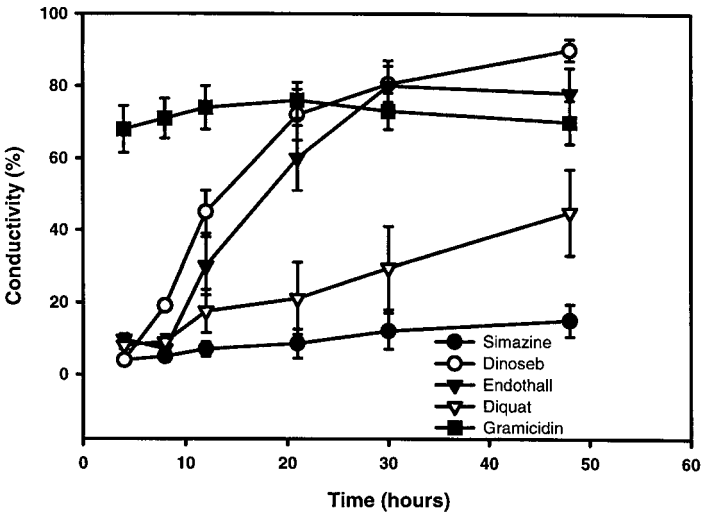

Figure 2. The effect of endothall acid, gramicidin, dinoseb, diquat, and simazine on ion leakage from hydrilla shoot tips in the dark.

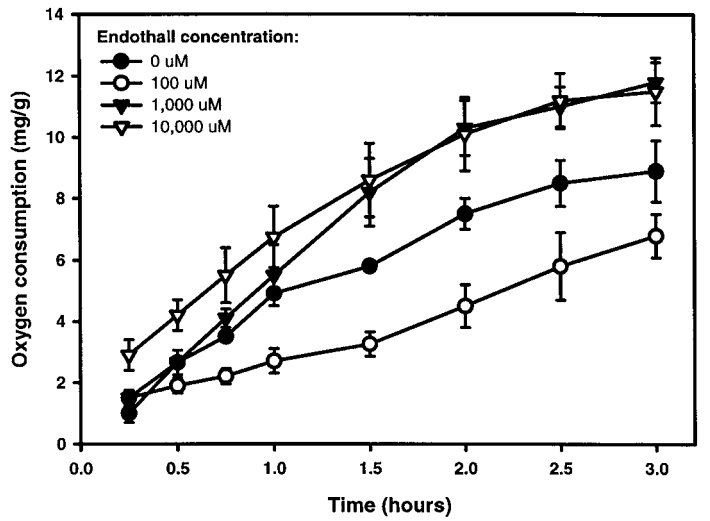

Figure 4. The effect of endothall acid at 0.0, 100, 1000 and 10,000 µM on oxygen consumption by hydrilla shoot tips.

crease in oxygen consumption in cucumber (*Cucumis sativa* L.). However, in the current study, endothall at a lower concentration of 100 µM suppressed respiration. Herbicides that affect mitochondrial respiration, such as dinoseb, have been shown to stimulate or inhibit oxygen uptake as a function of concentration (Moreland 1988).

Photosynthetic oxygen evolution. Gramicidin, diquat and diuron caused a significant decrease in photosynthetic oxygen evolution after 10 minutes (Table 1). Hydrilla shoot tips treated with 2,000 µM endothall acid showed no effect after 10 minutes and showed highly dissimilar rates of inhibition after 1 hour. However, complete inhibition was observed for endothall after 2 hours. Complete inhibition was also observed for diquat and diuron after 2 hours.

Collectively these data suggest that endothall elicits rapid, membrane active effects in hydrilla. Previous research (MacDonald 1993) suggested that the activity of endothall on cucumber leaf discs was a respiratory inhibition due to the lack of chlorophyll fluorescence, greater or more rapid ion leakage in the dark and suppressed respiration rates. The data reported here also show similar results with endothall having a profound effect on respiration. However, endothall was also shown to reduce oxygen evolution via photosynthesis, which not occur if respiratory inhibition was the sole mechanism of action. Although Turgeon et al. (1972) reported endothall to affect photosynthesis and respiration in turfgrass, specific activity was not determined. The activity of endothall observed in this study was similar to gramicidin, which is a membrane active compound that causes proton leakage from both chloroplast and mitochondrial membranes. Endothall caused similar effects as gramicidin and would partially explain the results observed and symptoms associated with its use.

TABLE 1. THE EFFECT OF ENDOTHALL AND OTHER HERBICIDES ON PHOTOSYNTHETIC OXYGEN EVOLUTION FROM HYDRILLA SHOOT TIPS.

		Oxygen evolution ($nmol \cdot g^{-1} \cdot min^{-1}$) minutes after initial exposure		
Treatment	Concentration	10	60	120
Distilled water	—	619 a[1]	569 a	612 a
Endothall acid	2,000 µM	527 ab	232 ab	0 b
Gramicidin	100 µM	197 cd	134 b	56 b
Diquat	1 µM	344 bc	316 ab	0 b
Diuron	1 µM	35 d	0 b	0 b

[1]Means in a column followed by the same letter do not significantly differ (∝ = 0.05, Waller-Duncan *k*-ratio *t* test).

ACKNOWLEDGMENTS

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

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