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Effects of Endothall and Other Aquatic Herbicides on Chlorophyll Fluorescence, Respiration and Cellular Integrity[1]

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ABSTRACT

Part of the mode of action of several aquatic herbicides is cellular disruption which can be caused by the generation of oxygen radicals or loss of adenosine triphosphate (ATP) needed to maintain cellular integrity. In an attempt to distinguish between the varying mechanisms by which certain compounds cause cellular disruption, ion leakage (light and dark regimes), chlorophyll fluorescence, and oxygen consumption (a normal consequence of respiration) were monitored over time from leaf tissue exposed to endothall, simazine, dinoseb, diquat and gramicidin. All compounds, except simazine, caused high ion leakage in both light and dark. Diquat caused more rapid leakage in light, while endothall and gramicidin caused more rapid leakage in the dark. Diquat, dinoseb and simazine increased chlorophyll fluorescence, but endothall and gramicidin did not. Oxygen consumption was stimulated by gramicidin and diquat but inhibited by endothall and dinoseb. Comparing the effects of compounds with known mechanisms-of-actions on ion leakage, chlorophyll fluorescence and oxygen consumption suggest that endothall acts to inhibit respiration.

Key words: dinoseb, diquat, simazine, gramicidin, conductivity, oxygen radical, mode-of-action, cucumber.

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INTRODUCTION

Endothall (7-oxabicyclo[2,2,1]heptane-2,3-dicarboxylic acid) has been used in aquatic plant management since the early 1960's (7) and provides good control of many submersed species (19,25). In addition, endothall is or has been used as a defoliant in cotton (17) and other crops (18,26), as a potato-vine dessicant (5), and for selective weed control in sugar beets (27) and turf (24). Endothall is classified as a phthallic acid herbicide (1) and is a derivative of cantharidin (23) which is a natural compound produced by the blister beetle (*Epicauta* spp.) that causes burning and blistering of the skin (3).

Several formulations of endothall (mainly salts) have been or are presently registered for various weed management uses. The halflife of the potassium and sodium salt formulations in the aquatic environment is 2 to 3 days under normal conditions, while the alkylamine salts are more persistent (14 to 21 days). Microbial degradation is the major mechanism of dissipation (15,16). Although accumulation of endothall is limited due to its short halflife, this compound in concentrated form is highly toxic: LD_{50} (rat) for technical endothall acid is 38 to 51 mg/kg, 182 to 198 mg/kg for Na and K salts, and 206 mg/kg for the amine salt formulation (9).

When applied to the soil, endothall is taken up by plant roots and translocated via the transpiration stream. Endothall is not phloem mobile (10). In contrast, movement in aquatic plants is limited to the symplast (20) and uptake in hydrilla [*Hydrilla verticillata* (L.f.) Royle] is enhanced by high temperatures and low light levels (6). Endothall affects several plant processes including lipid (11) and protein synthesis (12) and dipeptidase and proteinase activities (21). Furthermore, Penner and Ashton (14) found that endothall decreased proteolytic activity and was similar to that caused by actinomycin D. From this they postulated that endothall interferred with mRNA metabolism.

Endothall is considered to be a membrane-active compound, causing cellular disruption of plant tissue within 2 to 5 days (10). Herbicides that cause rapid cellular disruption are not mobile in plants because cellular integrity is essential for translocation. This may help to explain the limited phloem mobility of endothall. Herbicides that interfere with protein, lipid, or amino acid synthesis often require 2 to 4 wk to cause plant death after initial application. The symptomology of plants treated with these types of herbicides includes discoloration and stunting. However, endothall produces necrotic lesions and an overall browning of the tissue, characteristic of an oxygen radical generating compound. Therefore, the reported mechanisms-of-action do not appear to adequately explain the symptoms of plants treated with endothall. The objective of this research was to determine a more plausible mechanism-of-action for endothall. This was accomplished by utilizing compounds with known mechanisms of action and comparing their effects on chlorophyll fluorescence, oxygen consumption and cellular integrity to those effects caused by endothall.

MATERIALS AND METHODS

Plant material for all experiments was obtained from approximately 10-day-old cucumber (*Cucumis sativa* L.) seedlings ('Poinsett 76') which were grown in potting soil (Metro-mix 200) in a growth chamber under the following conditions: 14 hr light/10 hr dark photoperiod with an average light intensity of 350 $\mu mol\ m^{-2}\ sec^{-1}$ at 25C. Leaf disks (0.9 cm^2) were excised from the cotyledons and utilized similarily in all experiments.

Ion leakage. Four leaf disks were placed in distilled water (control) or 4 ml of solutions containing one of the following compounds: herbicides; endothall, simazine (6-chloro-N,N'-diethyl-1,3,5-triazine-2,4-diamine), dinoseb[3] (2-sec butyl-4,6-dinitrophenol), diquat (6,7-dihydrodipyrido[1,2-α:2',1'-c]pyrazinediium ion); respiratory poisons; gramicidin S[3]. Treated tissue was maintained under continuous light (400 $\mu mol\ m^{-2}\ sec^{-1}$) or in continuous dark conditions at 25C. Conductivity (μmhos/cm) was measured utilizing a conductivity bridge[4] at 6, 12, 18, 24, 36, and 48 hr after initial exposure. Dark-adapted tissue was assayed at the same times as those under light conditions, with an additional 72-hr measurement. Initial measurements of conductivity were taken on the solutions alone. Following completion of the experiment, the tissue was frozen and thawed twice to release all ions, providing a measurement of total conductivity. Treatment concentrations of compounds in all studies were: 10 mM endothall, 0.1 mM dinoseb, 0.1 mM diquat, 0.1 mM gramicidin, and 0.1 mM simazine. These concentrations were the approximate I_{50} concentrations based on preliminary studies (data not shown). However, for simazine, an accurate I_{50} value could not be determined due to limited activity under the described experimental conditions. 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.

Fluorescence. Leaf disks were exposed to the same compounds (with the exception of gramicidin) and I_{50} concentrations used in the ion leakage study, but were placed under continuous light (400 $\mu mol\ m^{-2}\ sec^{-1}$) for 2, 4, and 6 hr

[3]Sigma Chemical Company, St. Louis, MO 63178.

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

after initial exposure. Before determining chlorophyll *a* fluorescence treated tissue was equilibrated for 10 min in complete darkness. Induction curves were recorded and data analyzed using initial (after 5 ms), peak (after 1 sec) and steady-state (after 50 sec) time intervals. Data are presented as the ratio of peak/terminal fluorescence as follows: (peak-initial)/(terminal-initial).

Oxygen consumption. Twenty-five leaf disks were exposed in the dark to treatment solutions with the exception of simazine which was omitted. Leaf disks were vacuum infiltrated with the treatment solution for 20 min to eliminate possible diffusion differences between the compounds. Since vacuum infiltration removes dissolved oxygen, leaf disks were transferred to fresh solutions. The oxygen probe was then placed in the solutions and containers were sealed to prevent exposure to outside air. Oxygen concentrations (mg/l) were monitored 0.0 (initial), 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 hr after initial exposure using an oxygen probe[5]. Data are presented as oxygen consumption, derived from the formula: Oxygen consumption (mg/l) = (oxygen initial - oxygen measured), where oxygen measured represents levels at time of measurement.

Data were subjected to analysis of variance to determine if the effects from herbicides were significant. Because the responses as a function of time and herbicides were consistent for both experiments ($P < 0.05$) data are presented averaged over experiments. However, time of evaluation and herbicides differentially influenced the responses ($P < 0.05$) and data are presented accordingly. Standard errors of the mean (four replications) are present for ion leakage and fluorescence whereas oxygen values were regressed to obtain a best-fit model ($P < 0.05$).

RESULTS

Ion leakage. Following exposure, simazine caused less than a 20% increase in conductivity in the light (Figure 1), while gramicidin caused increases of 30 and 55% after 6 and 48 hr, respectively. Diquat and dinoseb also caused dramatic increases in conductivity with diquat causing a slightly larger increase than dinoseb. However, by 48 hr both compounds produced nearly a 100% increase in conductivity. The effect from endothall was more gradual, with a 50% increase after 24 hr. However, by 48 hr endothall also caused nearly a 100% increase in conductivity.

Gramicidin caused more rapid ion leakage in the dark than in the light and resulted in almost 90% ion leakage after 72 hr (Figure 2). The effect from diquat closely mirrored that of gramicidin in the dark, but was slighty less until 72 hr, when

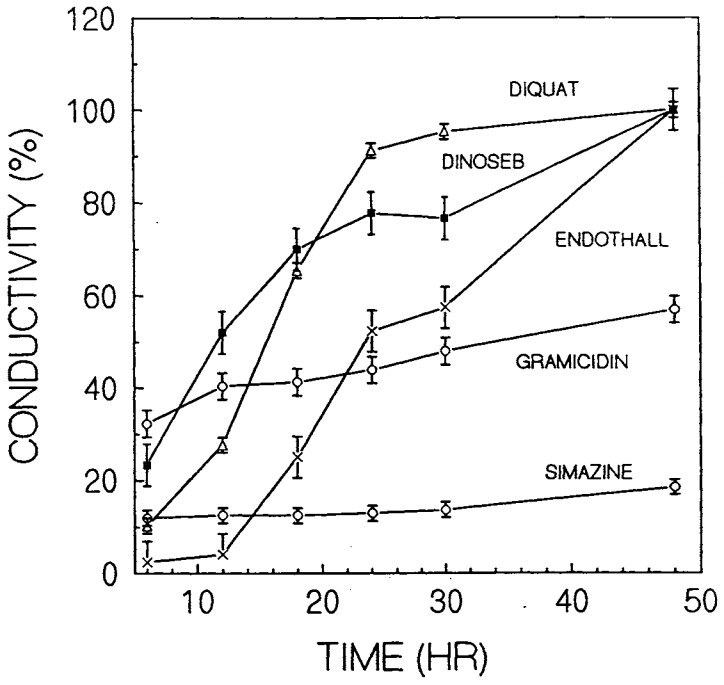

Figure 1. The effect of simazine, gramicidin, dinoseb, diquat and endothall on ion leakage from cucumber leaf disks in the light.

it also caused 90% leakage. The effect of diquat in the dark was markedly reduced from that produced in the light. Dinoseb produced similar results in the dark compared to the light, while the effect from endothall in the dark was greatly enhanced. Endothall caused a greater than 30% increase in conductivity after 6 hr and nearly 90% after 24 hr in the dark. The effect caused by simazine in the dark was neglible.

Fluorescence. Dinoseb, simazine, and diquat lowered the peak/terminal ratio, indicating increased chlorophyll *a* fluorescence relative to the control 2, 4, and 6 hr after treatment (Figure 3). Endothall did not affect fluorescence until 6 hr after treatment when fluorescence decreased below that of the control, probably due to indirect effects caused by membrane disruption. However, endothall caused minimal effects on chlorophyll fluorescence compared to the other compounds tested. Gramicidin did not affect fluorescence regardless of time (data not shown).

Oxygen consumption. Gramicidin and diquat caused a rapid increase in oxygen consumption (Figure 4) in the first 2 hr of exposure. After 2 hr, the rate of oxygen consumption caused by diquat slowed but gramicidin-treated tissue continued to consume oxygen at a rapid rate. Dinoseb and endothall reduced the ability of the tissue to use oxygen, with the effect from dinoseb being slightly greater than that of endothall.

[5] Monitor II, Beckman Instruments, Inc., Irvine, CA 92713.

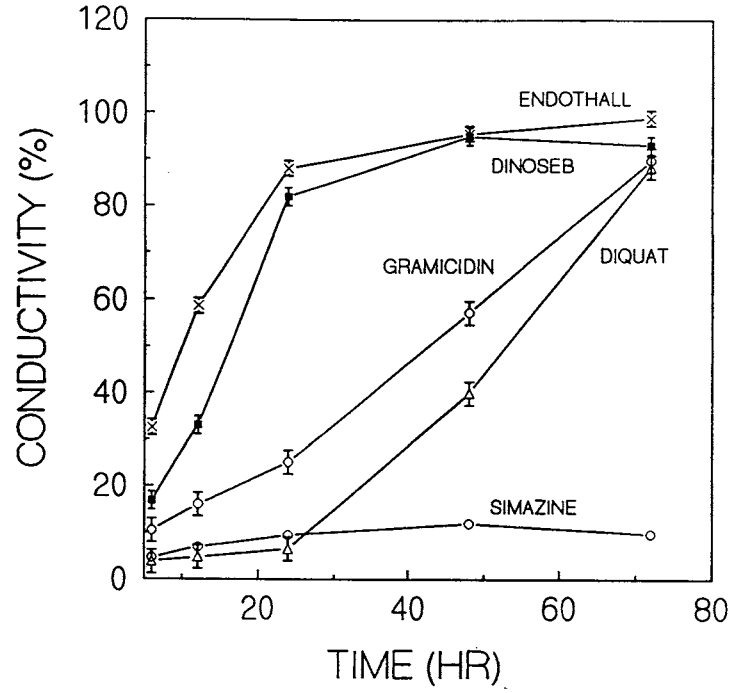

Figure 2. The effect of simazine, gramicidin, dinoseb, diquat and endothall on ion leakage from cucumber leaf disks in the dark.

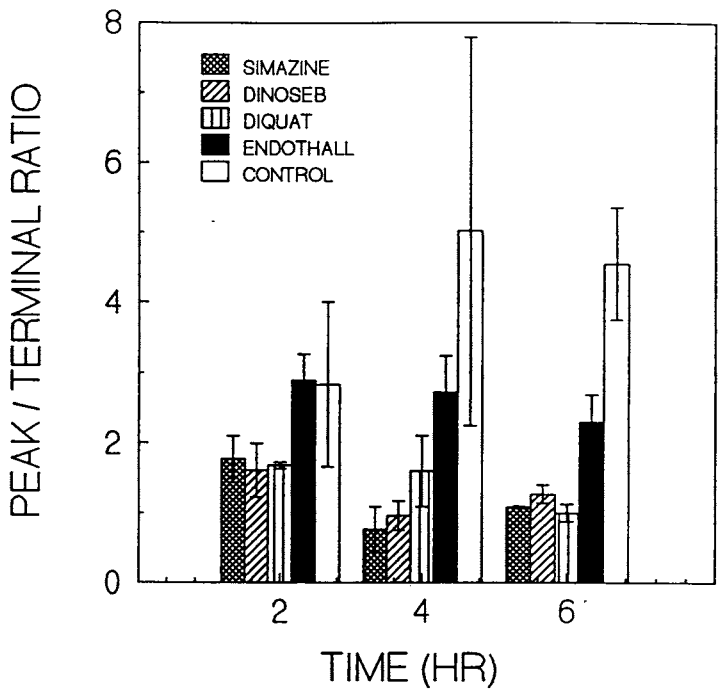

Figure 3. The effect of simazine, dinoseb, diquat and endothall on chlorophyll *a* fluorescence in cucumber leaf disks.

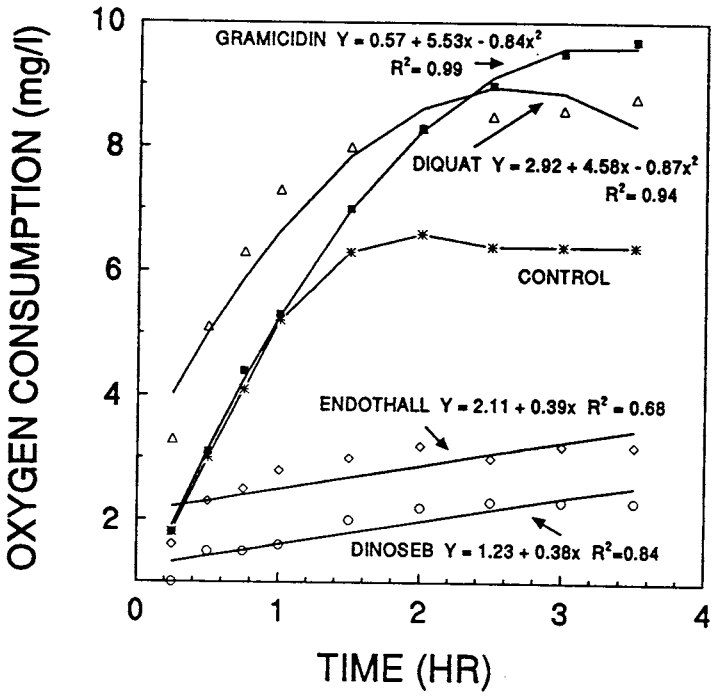

Figure 4. The effect of gramicidin, dinoseb, diquat and endothall on oxygen consumption (mg/l) in cucumber leaf disks.

DISCUSSION

Rapid ion leakage and cellular disruption caused by endothall occurred under both light and dark conditions. However, the effect from this compound was greater in the dark, indicating the mechanism-of-action of endothall is not light dependent. Since all energy under dark conditions is produced via respiration, these data implicate respiratory inhibition by endothall.

Dinoseb and diquat are known to affect both photosynthesis and respiration, injuring the cell through two separate mechanisms (2,13). Therefore, in the light these compounds cause injury through a disruption of photosynthesis (as evidenced by change in fluorescence) and respiration, whereas in the dark they only affect respiration. This is evident from elevated ion leakage in the light, whereas both these herbicides caused slower rates of leakage in the dark. Therefore, the activity of these compounds in the light was additive. These compounds are known respiratory poisons, acting directly on the cell's ability to produce adenosine triphosphate (ATP)(4). The loss of ATP causes the cell to 'leak' because its ability to maintain electro-chemical gradients is diminished. Although some oxidative stress is induced by inhibition or diversion of oxidative electron flow, the major cause of

membrane disruption is the collapse of the membrane gradient due to a lack of energy. Endothall appears to be acting similarly to the respiratory poisons in the dark; however, the increase in activity under dark conditions correlates with a compound that only inhibits respiration. This is because under light conditions, photosynthesis would provide some energy for respiration, thus diminishing the activity of endothall.

Gramicidin directly affects respiration but has little influence on photosynthesis (22). This compound also caused greater ion leakage in the dark. The similarity of endothall to gramicidin provides additional evidence that endothall affects respiration.

Chlorophyll fluorescence is often measured to determine the effect of various compounds on the light reactions of photosynthesis (15). In normal light reactions, light is absorbed by chlorophylls and other pigments and transmitted to reaction centers where light energy is converted to chemical energy through the donation of electrons. However, not all of the energy absorbed by chlorophyll molecules can be utilized and some is re-radiated as fluorescence (2). Normal fluorescence values for the ratio of peak to terminal for our study ranged from 3 to 5. This ratio indicates the ability of the plant to utilize light energy with higher ratios corresponding to more efficient light use, while low ratios (near 1.00) indicate that most of the energy is being lost to fluorescence.

Both simazine and dinoseb produced very low peak to terminal fluorescence ratios, which is characteristic of their known mechanisms-of-action. These compounds block electron flow at photosystem II, causing a feed-back effect (2). Chlorophyll molecules continue to absorb light energy, and must re-radiate most of this energy as fluorescence to avoid photo-oxidation. Diquat also produced low ratios, but this was probably due to the degradation of the photosynthetic apparatus by oxygen radicals. This is characteristic of diquat's mechanism-of-action. Endothall had virtually no effect on chlorophyll *a* fluorescence but lower peak/terminal ratios occurred after 6 hr in conjunction with significant ion leakage. Once ion leakage increases, the disruption of the photosynthetic apparatus can occur and the use of chlorophyll fluorescence to determine the mechanism of action is diminished.

In this study, endothall and dinoseb severely reduced the ability of cucumber tissue to utilize oxygen for respiration, while diquat and gramicidin increased oxygen consumption. Gramicidin acts directly on the mitochondrial membrane by dissipating the pH and charge gradient that allows ATP production (22). The cell increases its respiration rate and consumes more oxygen. Diquat, on the other hand, acts as an alternative reductant, diverting electrons away from the electron transport chain and utimately to oxygen. This also decreases the gradient, increasing respiratory rate, and creates a greater demand for oxygen.

Dinoseb inhibits respiration at site IV in the oxidative electron transport chain, blocking the flow of electrons to oxygen (13). Oxygen consumption can also be inhibited by compounds that block phosphorylation, causing an increase of the gradient and feedback inhibition of respiration, and resulting in a decrease in oxygen consumption. Endothall may be acting at either of these two sites to inhibit respiration.

In conclusion, higher ion leakage in the dark, minimal effects on fluorescence, and a reduction in oxygen consumption collectively indicate that endothall is a respiratory poison. In addition, endothall has been suggested to affect the mitochondria of animals (8) and the mode-of-action in plants may be similar.

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