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Short Communication

Action Spectrum of the Activity of Acifluorfen-methyl, a Diphenyl Ether Herbicide, in *Chlamydomonas eugametos*[1]

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ABSTRACT

Light is required for the herbicide activity of diphenyl ether herbicides. An action spectrum of acifluorfen-methyl activity with *Chlamydomonas eugametos* (Moewus) determined that cell death occurred at two peaks of light; 450 and 670 nanometers. These data indicate both chlorophyll and carotenoids, but not riboflavin, are involved in herbicide toxicity.

DPE[3] herbicides, which disrupt membranes, require light for herbicide activity (4, 9), although the specific wavelengths of light required for herbicide toxicity are uncertain. In buckwheat (*Fagopyrum esculentum* (Moench 'Tokyo') leaf discs, light between 565 and 615 nm (yellow to orange) or between 515 and 565 nm (green to yellow) was reported to be necessary for activity of the DPE herbicide oxyfluorfen (2-chloro-1-[3-ethoxy-4-nitrophenoxy]-4-[trifluoromethyl]benzene) (14). Wavelengths of light between 465 and 515 nm (blue green to green) gave some herbicide damage, whereas minimal herbicide toxicity occurred in plants exposed to the blue spectrum (415–465 nm). No oxyfluorfen activity occurred in the red wavelengths (14). In *Scenedesmus acutus* (Turp.), oxyfluorfen was active with red light greater than 610 nm, as measured by ethane evolution (12).

To better define the light requirement, an action spectrum of AFM toxicity in the unicellular green alga *Chlamydomonas eugametos* (Moewus) was determined. This action spectrum is more detailed than previous action spectra measured for other DPE herbicides.

MATERIALS AND METHODS

All action spectrum studies were conducted using the unicellular green alga *Chlamydomonas eugametos* (Moewus) (Indiana University Culture Collection number 9). *C. eugametos* cells were maintained on agar slants and liquid cultures were initiated as previously described (3, 8). To initiate an action spectrum study, the cells of *C. eugametos* in liquid culture were counted with a hemacytometer (8) and the cell population was adjusted to 0.75×10^6 cells ml^{-1}. Then 1.5 ml of the *C. eugametos* cells were added to a 6-ml scintillation vial (14 mm diameter by 45 mm in height). The herbicides AFM, MC15608 (methyl 5-[2-chloro-4-(trifluoromethyl) phenoxy]-2-chlorobenzoate), or oxyfluorfen were added in the dark. Nontreated control cells of *C. eugametos* contained ethanol (0.1%, v/v). Vials containing the algae were placed in a dark compartment of a VIS-UV Chromatogram Analyzer (Farrand Optical Co., Inc., Valhalla, NY) and the wavelengths of light inducing cell death were recorded between 310 and 690 nm by selecting 20-nm wavelength intervals of light. The reported wavelengths of light are in the middle of the 20-nm band width of light; for example, the recorded 450 nm of light encompassed 440 to 460 nm of light. The number of $\mu E\ m^{-2}\ s^{-1}$ of each 20-nm wavelength interval between 310 and 690 nm was determined by a spectroradiometer (Li-Cor Li-1800). The spectrometer scanned at 1 nm intervals and readings for each 20-nm wavelength interval are the summation of intensity readings across the 20-nm wavelength of light. The light source was from a 150-w d.c. Xenon arc lamp where emission was mainly the wavelengths between 200 and 1400 nm of light. The algae were gently agitated by a magnetic stirrer in the light for 12 h (31°C). Cell death was recorded by the stain FDA (3, 15). Tests of each 20 nm wavelength band of light were repeated three times.

RESULTS AND DISCUSSION

The DPE herbicide AFM induced cell death in the presence of two different wavelength bands of light (Fig. 1). Most AFM herbicide activity in *C. eugametos* occurred at 450 nm, where about 24% cell death resulted from treatment with 1 nM AFM. AFM herbicide damage was also induced by the red wavelength bands (650–670 nm), where 10 to 13.5% cell death resulted from these two wavelength bands of light. Little or no AFM herbicide activity was observed between 310 and 400 nm, between 490 and 630 nm, or at 690 nm (Fig. 1). The DPE herbicides MC15608 and oxyflurofen also had herbicidal activity at 450 and 670 nm (Table I).

Photon flux density (measured in $\mu E\ m^{-2}\ s^{-1}$) of the light source remained fairly constant between 450 and 700 nm, but decreased gradually in the blue and UV region of the spectrum (Fig. 2). There was adequate light intensity for DPE herbicide toxicity at all wavelengths of pigment absorbance. Pigments present in *C. eugametos*, are the same pigments common to higher plants (1, 7). Chl *a* (in methanol) has absorbance peaks at 430 and 662 nm, whereas Chl *b* (in methanol) has absorbance peaks at 470 and 643 nm (5). β-Carotene (in hexane) and lutein (in ethanol) both have absorbance peaks between 440 and 455 nm and between 470 and 490 nm (5, 17), whereas absorbance peaks of riboflavin (*in vivo*) are at 380 and 460 nm (6, 10, 13). At 380 nm, a riboflavin absorbance peak, there was only 3% cell death (Fig. 1). The light intensity at 380 nm was 6 $\mu E\ m^{-2}\ s^{-1}$, which was less than the average light intensity of 8.5 $\mu E\ m^{-2}\ s^{-1}$

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[3] Abbreviations: DPE, diphenyl ether; AFM, acifluorfen-methyl (methyl 5-[2-chloro-4-(trifluoromethyl)phenoxyl]2-nitrobenzoate); FDA, fluorescein diacetate.

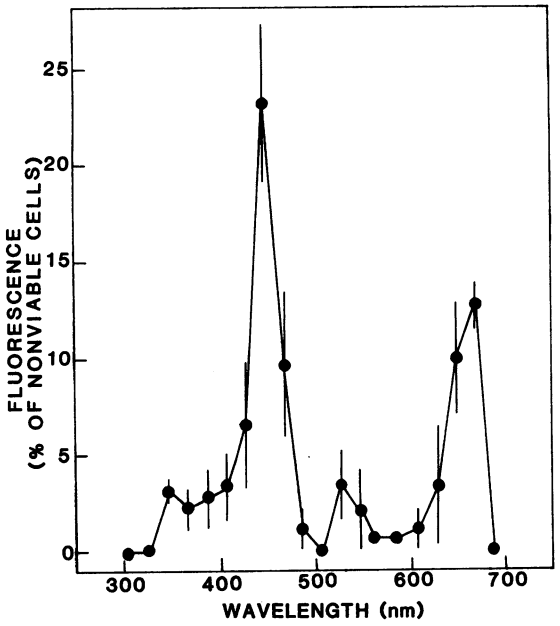

FIG. 1. Action spectrum of the DPE herbicide AFM. *C. eugametos* cells were exposed to 20 nm bandwidths of light between 310 and 690 nm for 12 h with 1 nM AFM or ethanol (0.1%, v/v). Cell death was recorded by the stain FDA. Ethanol controls did not cause cell death. Vertical bars represent ± SE.

Table I. *Light Requirement of MC15608, Oxyfluorfen, and AFM*

C. eugametos cells were exposed to either 450 or 670 nm of light for 12 h (31°C). The concentrations of the DPE herbicides were determined from the I_{50} values in *C. eugametos* (2). Cell death was recorded by the stain FDA. Values are mean ± SE of three experiments.

Treatment	Dead Cells
	%
MC15608, 50 nM	
450 nm	22 ± 1.61
670 nm	18 ± 1.88
Oxyfluorfen, 5 nM	
450 nm	31 ± 3.91
670 nm	26 ± 3.03
AFM, 1 nM	
450 nm	24 ± 4.12
670 nm	13 ± 1.28

present between 450 and 700 nm. However, 6 μE m^{-2} s^{-1} was sufficient light intensity to initiate AFM toxicity when *C. eugametos* was exposed to this level of light intensity at 450 or 670 nm (data not shown). If riboflavin is involved in AFM toxicity, there should have been significant cell death at 380 nm.

The action spectrum reported here for AFM toxicity differs from that of oxyfluorfen reported by Vanstone and Stobbe (14). In their studies with buckwheat leaf discs, most of the light required for activity of oxyfluorfen was between 515 and 615 nm (green to orange), with little or no light requirement in the blue or red wavelengths. Vanstone and Stobbe (14) suggested carotenoids were the pigments absorbing light for DPE herbicide toxicity and concluded a xanthophyll-protein complex in buckwheat accounted for the measured toxicity between 515 and 615 nm. In the study reported here, the light requirement peaks (450 and 670 nm) correspond to known peaks of carotenoid and Chl absorption. Sandmann and Böger (12) reported red light greater than 610 nm causes oxyfluorfen toxicity in *S. acutus* which

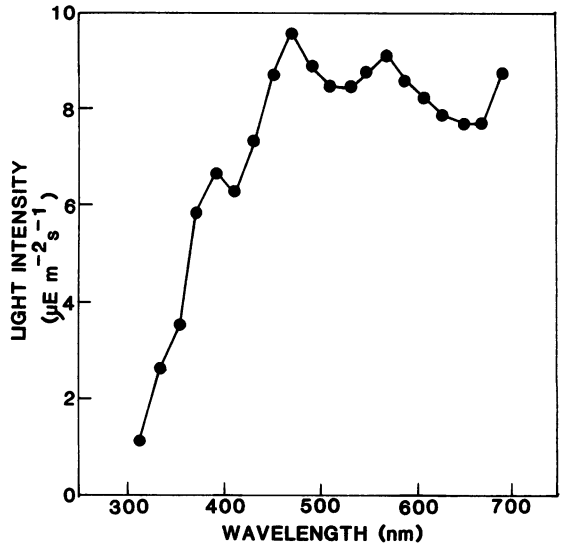

FIG. 2. Light intensity (μE m^{-2} s^{-1}) of light from a VIS-UV Chromatogram Analyzer, Farrand Optical Company. Light source was from a 150-w d.c. Xenon arc lamp. The reported wavelengths of light were in the middle of a 20 nm wavelength band of light. The number of μE m^{-2} s^{-1} of each 20 nm wavelength interval between 310 and 690 nm was determined by a spectroradiometer (LI-Cor LI-1800).

suggests Chl is the light-absorbing pigment in DPE herbicide toxicity.

Because no herbicide activity was observed in the UV (380 nm), where riboflavin absorbs light (6, 10, 13), riboflavin appears not to be required for DPE herbicide toxicity. These data show Chl is somehow involved in DPE herbicide toxicity (Fig. 1). The peaks in the blue do not discriminate between Chl and carotenoid absorption. Both carotenoids and Chl are probably responsible for DPE herbicide activity since carotenoids are required for DPE herbicide activity in plants without Chl (4, 9, 11). In yellow rice (*Oryza sativa* L.) mutants, Matsunaka (9) found that xanthophylls were important in absorbing light to achieve toxicity with the DPE nitrofen (2,4-dichlorophenyl *p*-nitrophenyl ether). Fadayomi and Warren (4), using mutant plants that did not contain Chl, and Orr and Hess (11), using etiolated cucumber cotyledons or cotyledons treated with the carotenoid inhibitor fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)-phenyl]-4(1*H*)-pyridinone), thus lacking carotenoids, conclude that carotenoids are important in DPE herbicide activity.

The toxicity occurring in the red region (650–670 nm) is difficult to explain. Recent evidence (2) concludes noncyclic photosynthetic electron transport is not involved in DPE toxicity. Chl could be indirectly involved by passing light energy to carotenoids (15). If DPE treatment causes carotenoids to function improperly, energy directly from light or from Chl could be involved in the lipid peroxidation process. However, when noncyclic electron transport is inhibited, toxicity should increase when energy transfer from Chl to carotenoids is increased as a result of an inability of transfer from Chl to photosynthetic electron transport. This does not occur (2). Perhaps cyclic photosynthetic electron transport or Chl itself is involved in initiation of lipid peroxidation after DPE treatment. Initial studies using DBMIB (2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone) to block cyclic electron transport in whole cucumber cotyledons suggest this reaction sequence may not be involved in DPE toxicity (11).

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