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Photo-enhancement of Hydrogen Peroxide Toxicity to Submersed Vascular Plants and Algae[1]

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ABSTRACT

Fanwort (*Cabomba caroliniana* Gray), egeria (*Egeria densa* Planch.), hydrilla [*Hydrilla verticillata* (L.f. Royle], eelgrass (*Vallisneria americana* Michx.), and Eurasian watermilfoil (*Myriophyllum spicatum* L.) were injured in direct proportion to quantum flux density (QFD) after 1 h exposure to 1 or 2 mM H_2O_2. Chlorophyll decreased linearly with increasing QFD for *Anabaena, Raphidiopsis,* and *Ankistrodesmus* continuously exposed for 24 h to 0.2 or 0.5 mM H_2O_2. *Ankistrodesmus* and *Raphidiopsis* were completely bleached by H_2O_2 at all QFD after 48 h. H_2O_2 partially bleached *Anabaena* at high QFD (620 μE m^{-2} s^{-1}), but had little effect at 90 μE m^{-2} s^{-1}. Ninety-four percent of an initial 0.137 mM H_2O_2 had disappeared by 4 h after treatment of a *Raphidiopsis* culture. Apparently, H_2O_2 rapidly sensitized the plants to subsequent photo-induced injury. Sensitivity to H_2O_2 and phylogenetics were not related.

Key words: chemical control, phytoplankton, limited-contact herbicide, light potentiation, *Cabomba, Egeria, Hydrilla, Vallisneria, Myriophyllum, Anabaena, Raphidiopsis, Ankistrodesmus.*

INTRODUCTION

Management of noxious aquatic vegetation is accomplished primarily by chemical means. Very few new compounds have been registered for aquatic use for many years, and the majority of those previously registered are no longer available (Richard Comes, USDA-ARS, Prosser, WA, personal communication). Consequently, a definite need exists for the development and registration of safe, new compounds for herbicidal use in the aquatic system.

Recent literature has indicated that hydrogen peroxide (H_2O_2) may prove useful for the management of algae and submersed aquatic macrophytes. Hydrogen peroxide was suggested as a treatment for the control of slimes and algae in cooling towers (15) and as a non-corrosive algicide to replace copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) in

[1]Portions of this paper were reported previously in abstract form: Kay, S. H., P. C. Quimby, Jr., and J. D. Ouzts. 1982. Light enhances toxicity of H_2O_2 to aquatic weeds. Proc. 35th Ann. Meet., So. Weed Soc., 19-21 Jan., 1982, Atlanta, GA, pp. 290-291.

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systems constructed with aluminum alloys (17). Quimby (10) first provided evidence that H_2O_2 might be useful for control of submersed macrophytes, especially in flowing water where contact time would be limited. Kay *et al.* (7) reported a potential use for H_2O_2 as an algicide in aquaculture. Recent work by Kay *et al.* (8) demonstrated that the presence of low levels of Cu enhanced the toxicity of H_2O_2 to coontail (*Ceratophyllum demersum* L.).

Other workers have investigated the physiological and biochemical effects of H_2O_2 on higher plants and algae (5, 6, 9, 11, 12, 13, 14). The relationship between H_2O_2 and the mode of action (1) of dipyridylium quaternary ammonium herbicides, such as diquat (6,7-dihydrodipyrido [1,2-α:2′,1′-*c*] pyrazinediium ion), suggested that quantum flux density (QFD) might also influence the herbicidal activity of H_2O_2. This hypothesis is supported by the observation of Kay *et al.* (8) that the activity of H_2O_2 on coontail was reduced at low QFD (70 to 100 μE m^{-2} s^{-1}). The objective of this study was to determine the influence of various quantum flux densities on the toxicity of H_2O_2 to submersed vascular plants and algae.

METHODS AND MATERIALS

Macrophytes. Among the dicots we selected fanwort (*Cabomba caroliniana* Gray: Cabombaceae) and Eurasian watermilfoil (*Myriophyllum spicatum* L.: Haloragaceae). We also selected three monocots belonging to the Hydrocharitaceae: hydrilla [*Hydrilla verticillata* (L.f.) Royle], egeria (*Egeria densa* Planch.), and eelgrass (*Vallisneria americana* Michx.).

Fanwort, egeria, hydrilla, and watermilfoil were obtained from field populations, and eelgrass was purchased from a commercial supply house. Plant materials were washed, cut into 10-cm apical sections (rootstocks with 10-cm leaves in eelgrass), and planted in 57-mm plastic pots containing a mixture of 30 percent (by volume) sand and 70 percent sphagnum peat. Four pots, each containing one plant (two in the cases of hydrilla and watermilfoil), were placed into 3.79-liter glass jars containing three liters of tap water. Jars were covered with polyethylene wrap to retard evaporation and were placed in a growth chamber under combined incandescent and fluorescent lighting with 14 h light at a QFD of approximately 90 μE m^{-2} s^{-1} and 25/20 C day/night temperatures. Plants were allowed a minimum of two weeks to root prior to treatment.

At the outset of each test, four plants were placed into clean jars, each containing three liters of nutrient solution (8) and 0, 1, or 2 mM H_2O_2 solutions. Solutions were prepared using food grade, 35% H_2O_2. Hydrilla and watermilfoil were placed together in the same containers, whereas all other plants were tested separately. After a 1-h exposure period under laboratory lighting at approximately 20 μE m^{-2} s^{-1}, jars were drained and flushed with tap water, and each jar refilled with three liters of nutrient solution without H_2O_2.[3] Jars were recovered with the polyethylene wrap and returned to the growth chamber. QFD varying from near the compensation point to near saturation were obtained by placing the jars at varying distances from the light source. A heat shield was present between the lighting system and the jars; temperatures within the jars did not vary more than ±2C regardless of position within the growth chamber. The QFD varied slightly as two growth chambers were used and all studies could not be run simultaneously, due to space limitations. QFD was measured with a Lambda LI-185 quantum meter.[4] Plant injury was rated visually on a 0 to 10 scale where 0 represented no damage and 10 represented complete kill (8). Visual rating allowed the estimation of injury increase with time on the same plants. Previous work by the authors (8) indicated no significant differences between injury estimation by dry weights and visual ratings. Each study was established in a factorial design having three H_2O_2 treatments, four or five QFD, two to four evaluation periods, and three replications per treatment combination. Data were subjected to multiple linear regression analyses.

Algae. Three phytoplankton genera, *Anabaena* sp. (Cyanophyta: Oscillatoriales), *Raphidiopsis* sp. (Cyanophyta: Oscillatoriales), and *Ankistrodesmus* sp. (Chlorophyta: Chlorococcales), were selected for algal studies. *Anabaena* sp. and *Ankistrodesmus* sp. were obtained from Carolina Biological Supply and cultured in Carolina Alga-Gro medium prepared with Carolina Spring Water in an incubator with 12 h fluorescent lighting at 25 C.[4] *Raphidiopsis* sp. was taken from a monoculture bloom present in an aquarium containing goldfish (*Carassius auratus*) and which received constant illumination from two 15 W incandescent bulbs.

At the outset of the study, 100-ml aliquots of each algal suspension were placed into 250-ml erlenmeyer flasks and treated with 0.0, 0.2, or 0.5 mM H_2O_2. The study was conducted in the growth chamber in the same manner as for the macrophyte studies. Quantum flux densities measured at the level of the algal suspensions were 90, 140, 550, and 620 μE m^{-2} s^{-1}. After 24 and 48 h, 10-ml aliquots were removed from each flask, centrifuged 10 min at 12,100 x G, and extracted by resuspension of the algal pellet overnight in 90% methanol. Methanol extraction was used in lieu of the standard acetone procedure because of difficulty in obtaining complete extraction with acetone (2, 4). We prepared a standard curve by dissolving 1 mg chlorophyll *a* (Sigma No. C-6144, from *Anacystis nidulans*[4]) in 100 ml of 90% methanol and diluting to 0.1, 0.25, 0.5, 0.75, 1.0, 2.5, and 5.0 mg chlorophyll *a* liter^{-1}. Five replicates of each concentration were prepared, and absorbance was measured at 665 nm. Data for the standard curve were subjected to linear regression analysis. Chlorophyll *a* from the treated algae was measured spectrophotometrically at 665 nm. The study was established as a 4 x 3 x 2 factorial design with three replications per treatment combination. Algal genera were not included as variables in the statistical analyses, as initial cell densities were not measured. Data were subjected to multiple linear regression analysis.

[3]Previous (unpublished) work by the authors using coontail indicated that there was no significant difference in injury whether the 1-h exposure to H_2O_2 was in the laboratory at low QFD or in the growth chamber at high QFD.

[4]Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Dept. of Agriculture or Delta State University and does not imply its approval to the exclusion of other products or vendors that also may be suitable.

Degradation of H_2O_2 in an algal suspension. Suspensions of *Raphidiopsis* sp. were treated to provide calculated initial concentrations of 0.137 mM H_2O_2 and were placed in the growth chamber as described above. Four replications of the H_2O_2-treated algae and two untreated controls were analyzed for H_2O_2 immediately after treatment and at 4 and 24 h post-treatment using the procedure of Gupta (3). The sensitivity of this procedure is $\leq$ 0.003 mM H_2O_2.

RESULTS

Macrophytes. Numerous bubbles appeared in the leaves and stems of H_2O_2-treated plants, especially eelgrass, and were more prevalent at the higher QFD. These bubbles were presumably O_2 produced by decomposition of internally-absorbed H_2O_2, as H_2O_2 was no longer present in the external medium. Chlorophyll bleaching (not quantified) occurred at all QFD and appeared to be proportional to plant injury. Leaves of H_2O_2-treated plants were nearly colorless after 4 d at high QFD, but stems retained some green color for the entire 14-d experimental period. A bloom of filamentous, epiphytic algae also developed on all plants during this study and appeared to increase (visual estimation) in proportion to plant injury at all QFD.

Plant injury ratings increased with time following the 1-h exposure period (Fig. 1-5), especially at lower QFD. At the highest QFD, injury exceeded 80 percent in eelgrass (Fig. 1) and hydrilla (Fig. 2) 4 d after treatment with either 1 or 2 mM H_2O_2; injury also exceeded 80 percent following either H_2O_2 treatment after 7 d in egeria and 14 d in watermilfoil (Fig. 4). Maximum injury to fanwort was < 80 percent at 14 d (Fig. 5). Injury ratings following exposure to 2 mM H_2O_2 were slightly higher at all QFD than those observed following a 1 mM H_2O_2 treatment, but differences were consistently significant ($P<0.05$) throughout the 14-d study only for eelgrass (Fig. 1) at QFD <300 $\mu E\ m^{-2}\ s^{-1}$. Some regrowth from axillary buds occurred in hydrilla and watermilfoil 11 d after treatment at both H_2O_2 concentrations. Two months after treatment, some regrowth had occurred in all plants except egeria; only fanwort and eelgrass had regrown extensively (i.e., above-soil biomass visually $\geq$ 30 percent of original plant material).

Seven days after treatment, photo-induced injury (ratings $\leq$ 2) was observed in all control plants except fanwort (Fig. 5); after 14 d, photo-induced injury to controls exceeded 50 percent except in fanwort and eelgrass (Fig. 1), which had approximately 40 and 30 percent injury, respectively. Light potentiation of H_2O_2 toxicity occurred initially in all species but disappeared with time, except in eelgrass (Fig. 1). The apparent loss of light potentiation of H_2O_2 toxicity occurred after 7 d in egeria (Fig. 3) and watermilfoil (Fig. 4) and 14 d in hydrilla (Fig. 2) and fanwort (Fig. 5).

Phytoplankton. The standard curve prepared for chlorophyll *a* in 90% methanol was linear ($r^2 = 0.9900$, $y = 0.0483x + 0.003$).

Chlorophyll destruction increased with time and QFD in both control and H_2O_2 treatments. Maximum photo-induced injury to untreated controls was $\leq$ 15 percent after 24 h; after 48 h, injury had increased to 18, 20, and 28 percent, respectively, in *Anabaena* (Fig. 6), *Raphidiopsis* (Fig. 7), and *Ankistrodesmus* (Fig. 8). Light potentiation of H_2O_2 toxicity occurred in all three genera throughout the study. Treatment of *Ankistrodesmus* (Fig. 8) and *Raphidiopsis*

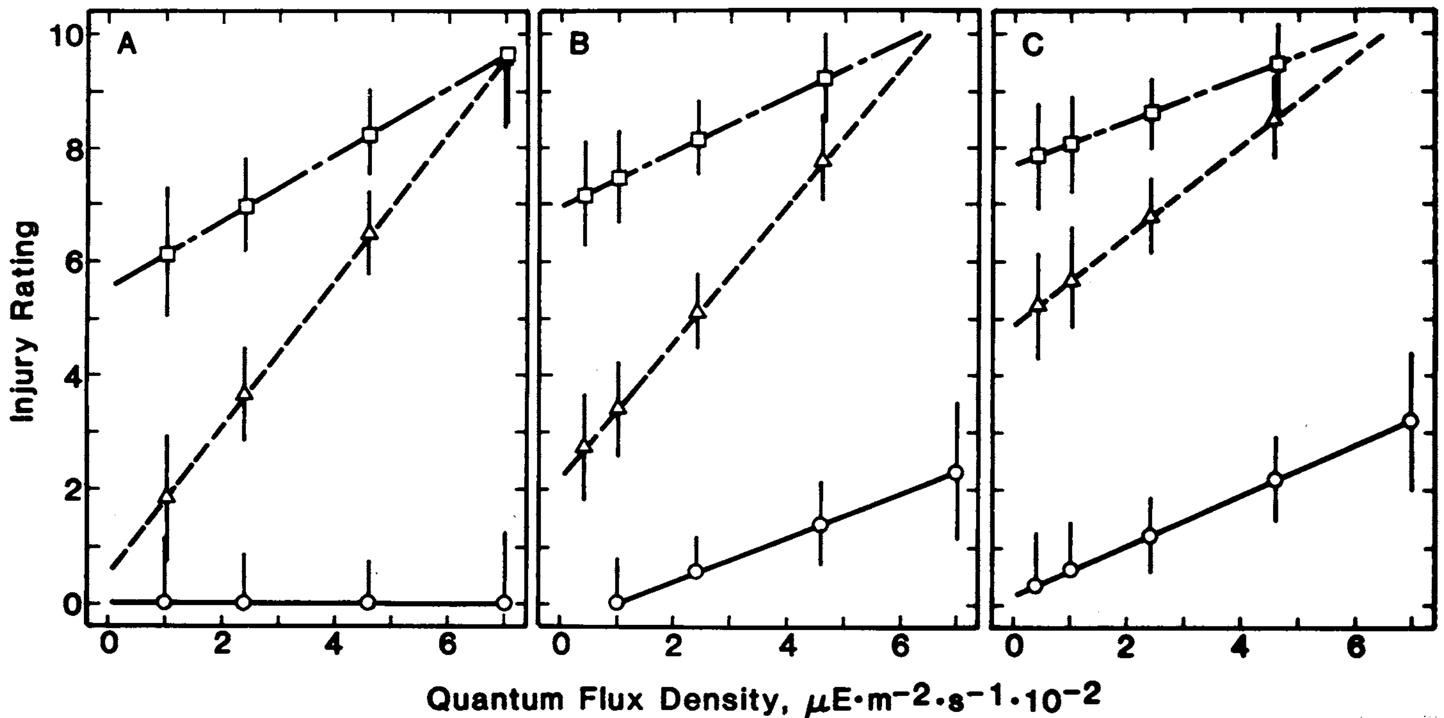

Figure 1. Toxicity of H_2O_2 to *Vallisneria americana* at different QFD. o——o control; △——△ 1 mM H_2O_2; □——□ 2 mM H_2O_2. (A) 4 days; (B) 7 days; (C) 14 days. Vertical bars represent the 95% confidence intervals for predicted mean injury ratings at the actual QFD encountered in the study.

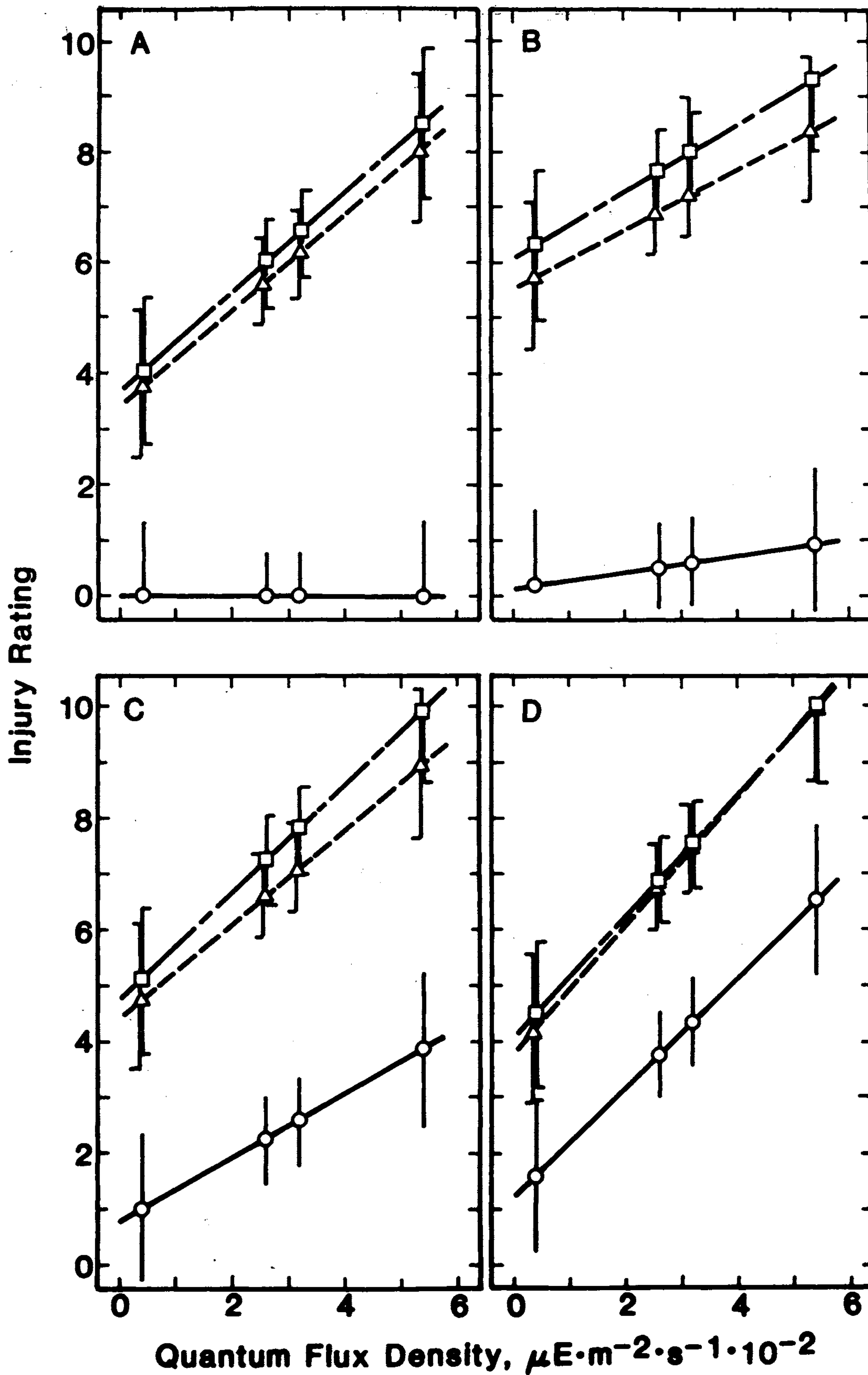

Figure 2. Toxicity of H_2O_2 to *Hydrilla verticillata* at different QFD. (A) 4 days; (B) 7 days; (C) 11 days; (D) 14 days. Symbols and confidence intervals as in Fig. 1.

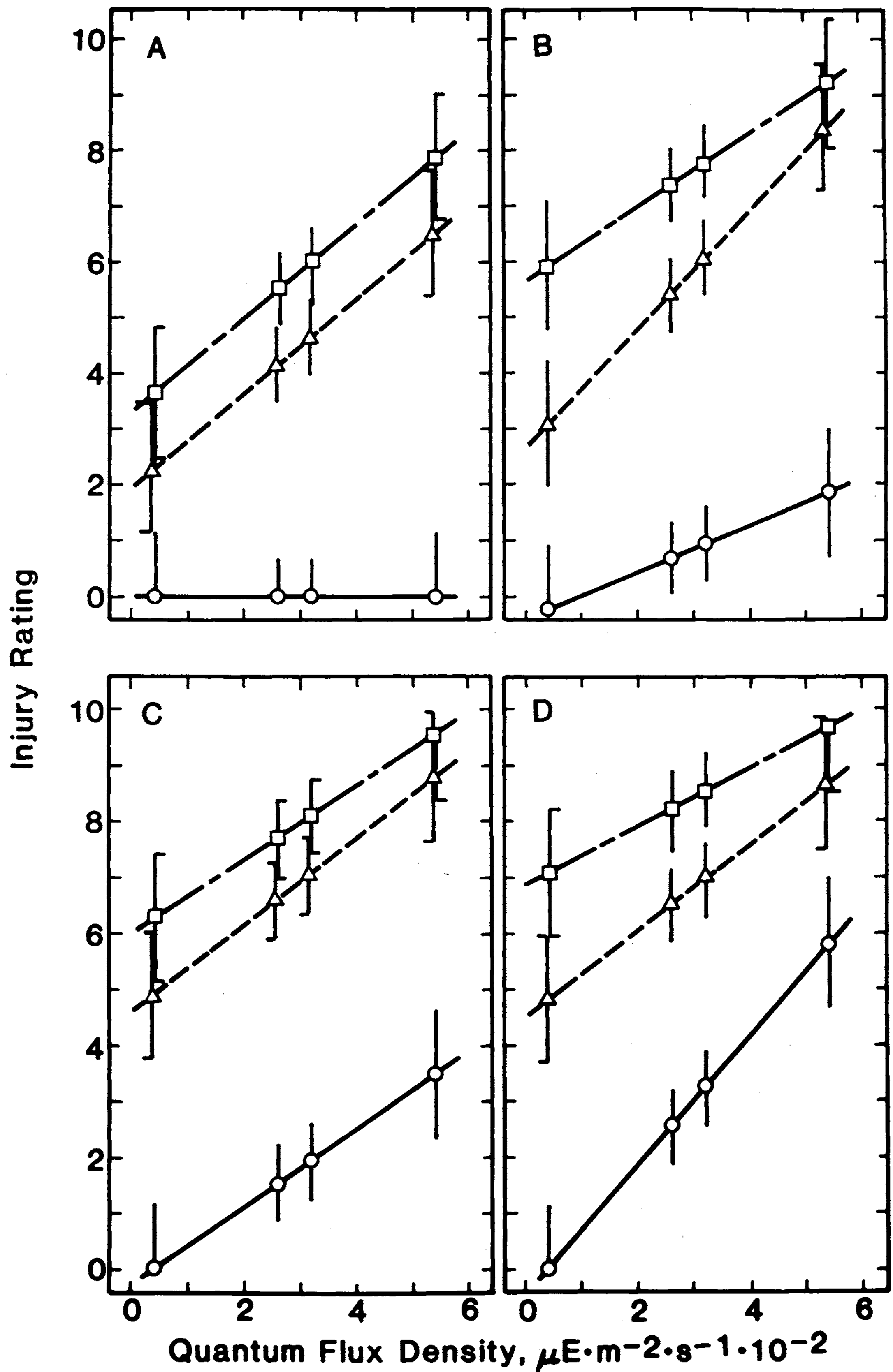

Figure 3. Toxicity of H_2O_2 to *Egeria densa* at different QFD. (A) 4 days; (B) 7 days (C) 11 days; (D) 14 days. Symbols and confidence intervals as in Fig. 1.

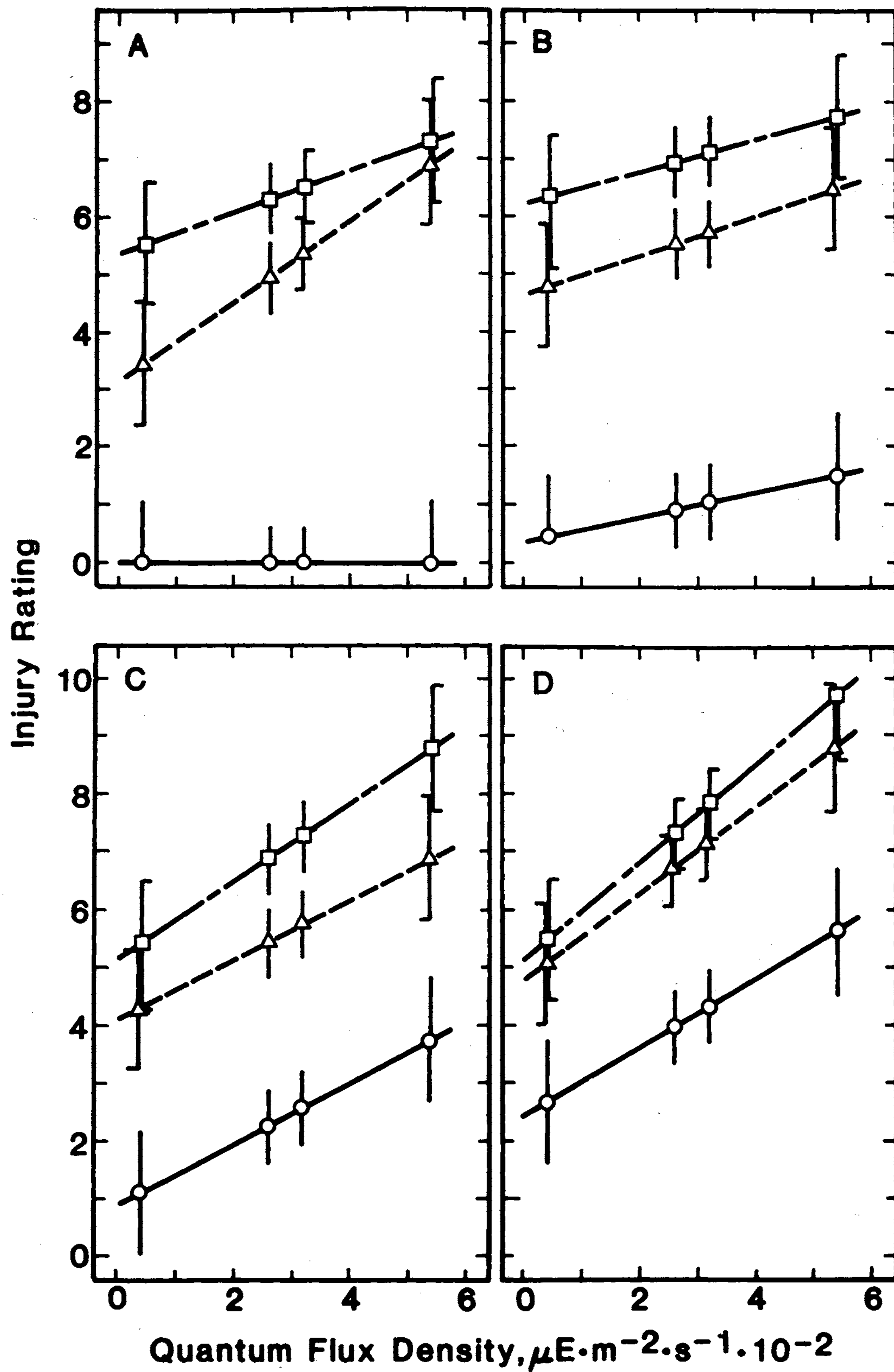

Figure 4. Toxicity of H_2O_2 to *Myriophyllum spicatum* at different QFD. (A) 4 days; (B) 7 days; (C) 11 days; (D) 14 days. Symbols and confidence intervals as in Fig. 1.

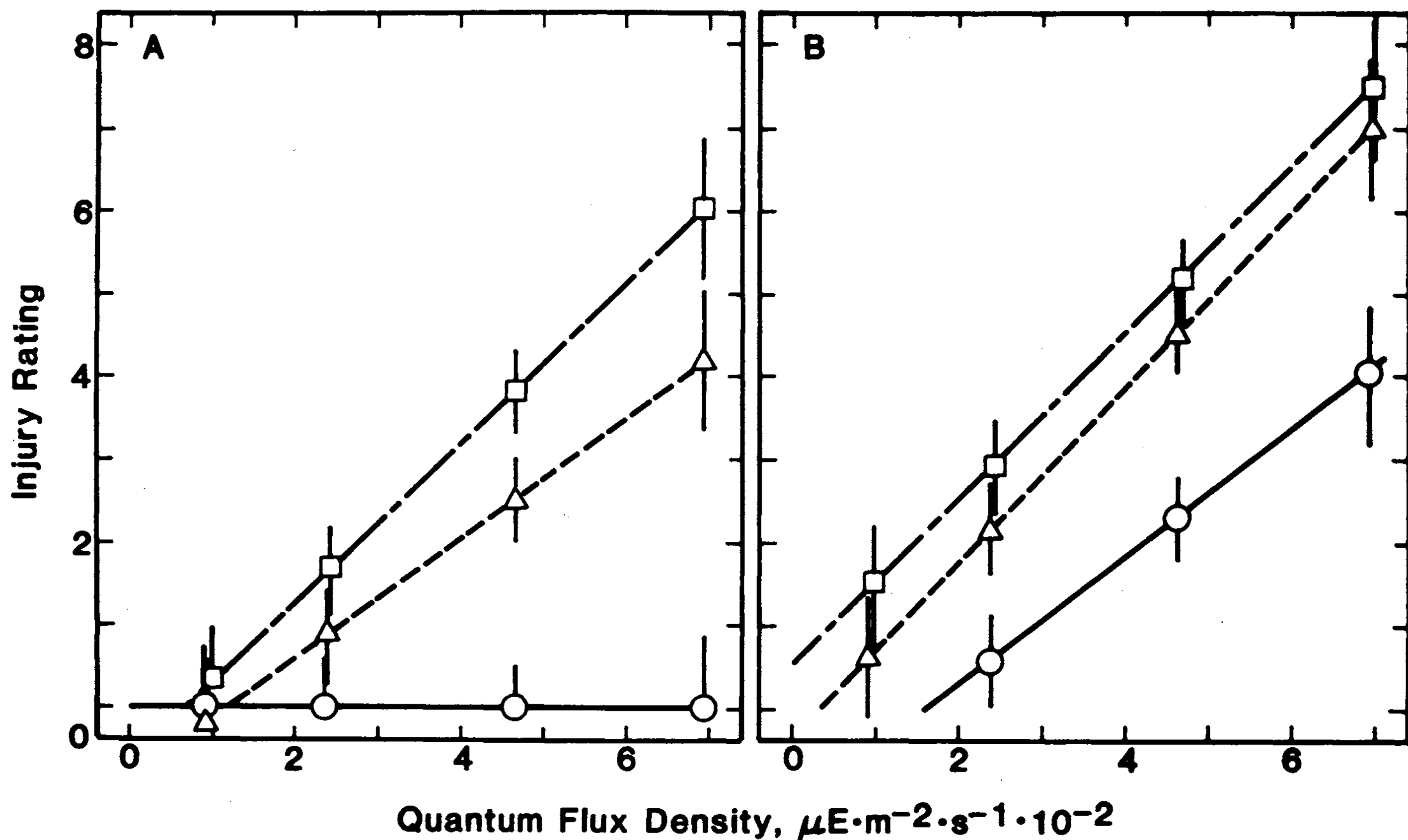

Figure 5. Toxicity of H_2O_2 to *Cabomba caroliniana* at different QFD. (A) 7 days, (B) 14 days. Symbols and confidence intervals as in Fig. 1.

(Fig. 7) with 0.5 mM H_2O_2 caused significantly ($p < 0.05$) greater chlorophyll loss after 24 h at low QFD than did 0.2 mM H_2O_2. Complete chlorophyll destruction from H_2O_2 treatments occurred in *Ankistrodesmus* (Fig. 8) and *Raphidiopsis* (Fig. 7) at high QFD after 24 h and after 48 h in *Ankistrodesmus* at low QFD. In *Raphidiopsis,* chlorophyll destruction at low QFD exceeded 80 percent 48 h after H_2O_2 treatment (Fig. 7). Treatment of *Anabaena* with either level of H_2O_2 produced no significant ($p < 0.05$) chlorophyll loss at low QFD; as QFD increased beyond 500 $\mu E\ m^{-2}s^{-1}$, however, treatment with 0.5 mM H_2O_2 produced significantly ($p < 0.05$) greater chlorophyll loss than did 0.1 mM H_2O_2 (Fig. 6). Complete chlorophyll destruction by either H_2O_2 treatment did not occur in *Anabaena* at any QFD after 48 h. *Degradation of H_2O_2 in an algal suspension.* Table 1 shows that H_2O_2 disappeared rapidly in the presence of a suspension of *Raphidiopsis.* Initial recovery of H_2O_2 following treatment with 0.137 mM H_2O_2 was 0.115 mM, or 84% of the initial treatment level. Four h after treatment, the H_2O_2 concentration had decreased to 0.008 mM, or 6% of the initial level. After 24 h, the H_2O_2 concentration in treated algal suspensions was the same as background levels observed in the controls.

TABLE 1. DEGRADATION OF H_2O_2 IN A TREATED ALGAL SUSPENSION OF *Raphidiopsis.*

Treatment[a]	Measured Concentration of H_2O_2 (mM)		
	Initial	4h	24h
Control	0.001 ± 0.000	0.002 ± 0.000	0.003 ± 0.001
0.137 mM H_2O_2	0.115 ± 0.002	0.008 ± 0.001	0.003 ± 0.001

[a]Measured values for controls and H_2O_2-treated suspensions are the means ± sd of 2 and 4 replications, respectively.

DISCUSSION

Senescence of the macrophytes and loss of chlorophyll following H_2O_2 treatment occurred over a similar time span as that reported previously for excised rice leaves (9). A buildup of epiphytic algae in direct proportion to plant damage suggested leakage of nutrients due to altered membrane permeability following H_2O_2 exposure, as reported previously for beet roots (14). Chlorophyll destruction following treatment of the algal suspensions with H_2O_2 occurred over a similar time period as that for *Scenedesmus* exposed to diquat (16). The apparent light potentiation of H_2O_2 activity and its bleaching effect on chlorophyll *a* may be similar to that of diquat and very likely involves the generation of free radicals within the plant cells (1, 16).

The data presented demonstrate that H_2O_2 injury to submersed vascular plants and phytoplankton is light potentiated. This photo-enhancement effect occurred primarily during the first 4 d after treatment in the macrophyte studies and within the first 24 h for the phytoplankton; further injury thereafter appeared to be largely due to the effect of light. The action of H_2O_2 on the macrophytes ap-

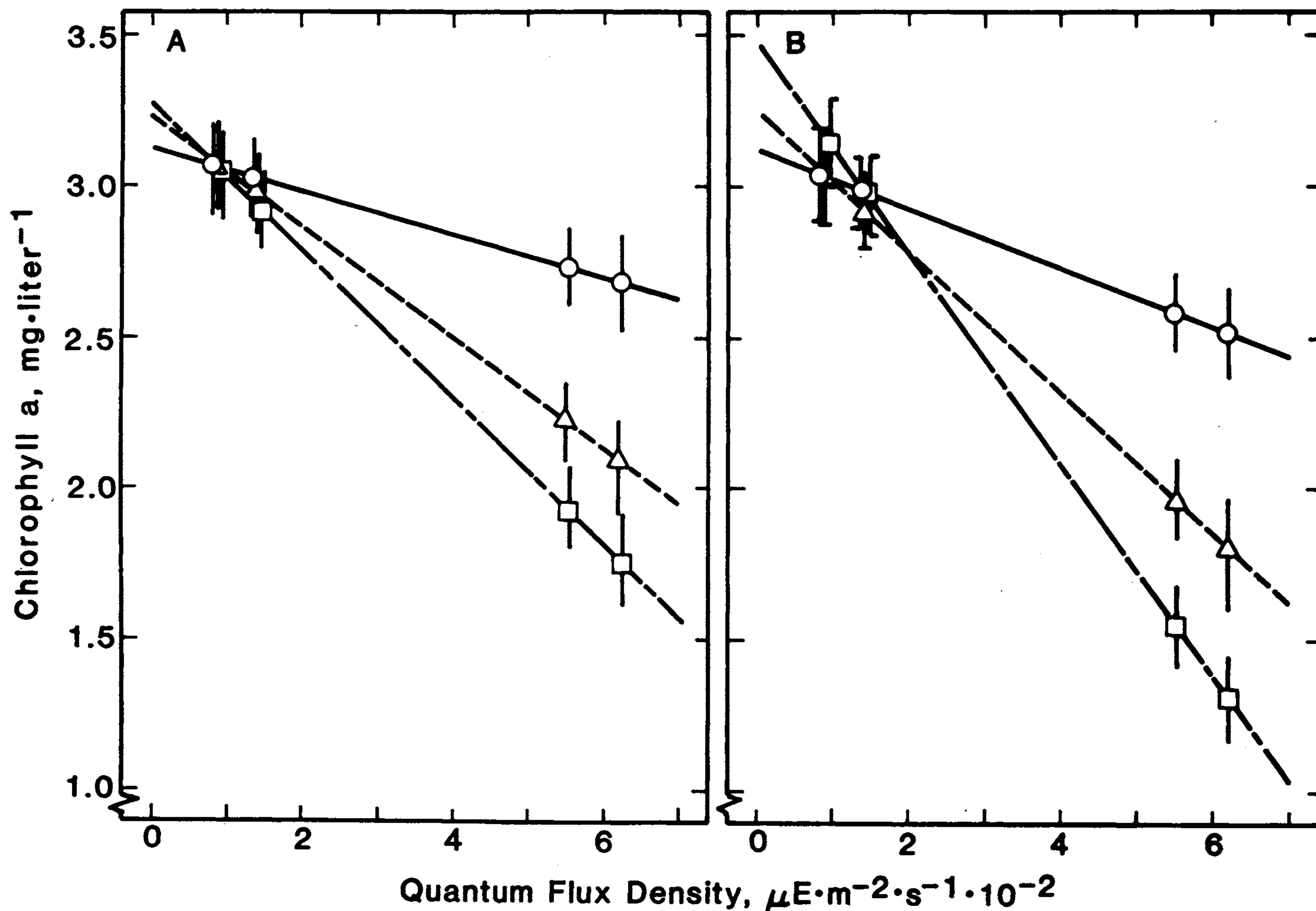

Figure 6. Decrease in chlorophyll following treatment of *Anabaena* sp. with H_2O_2 at different QFD. (A) 24 hours, (B) 48 hours. ○——○, control; △– – –△, 0.2 mM H_2O_2; □– – –□, 0.5 mM H_2O_2. Vertical bars represent 95% confidence intervals for predicted mean chlorophyll at the actual QFD encountered in the study.

parently occurred very rapidly, as the plants were exposed to H_2O_2 for only 1 h. Data on the disappearance of H_2O_2 in a culture of *Raphidiopsis* (Table 1) suggest that the actual exposure of the algae to toxic levels of H_2O_2 was also of very short duration. The elicitation of injury after short-term exposures and the dependence of plant injury upon QFD suggest that H_2O_2 sensitizes the plants to subsequent photoinduced injury.

Our data indicate that fanwort may be more tolerant than the other macrophyte species tested. Injury to fanwort (Fig. 5) was less than 80 percent, which is the minimum control level usually deemed acceptable in aquatic herbicide screening tests. Submersed vegetation control with H_2O_2 may be better under field conditions than in the laboratory, as QFD may be greater under full sunlight than in the growth chamber. A preliminary study (authors' unpublished data) at Lake Bolivar County, MS, demonstrated that H_2O_2 could control coontail under field conditions.[5]

[5]Two 75-liter plastic barrels with the bottoms removed were staked in place in a dense mat of coontail and were treated with approximately 2 mM H_2O_2. Two hours after treatment, the barrels were removed, leaving the stakes in place to mark the area. Three weeks after treatment, plants in the treated area were brown and totally defoliated, but plants in adjacent (control) areas remained green.

The potential effects of competing substances (e.g., organic matter, Fe, nitrite, etc.) that are oxidized by or catalyze the decomposition of H_2O_2 must be considered. The activity of H_2O_2 on phytoplankton under simulated field conditions in commercial catfish ponds (plastic tanks containing pond water and catfish, placed within the ponds) actually exceeded that observed under growth chamber conditions, however (7). The pond water was high in organic matter, due to the presence of a continual dense algal bloom, uneaten fish food, and fish excrements. High levels of Fe were present, as evidenced by the formation of a reddish-brown deposit soon after fresh water was pumped from shallow wells into the pond. This study and subsequent work by the authors (P. C. Quimby, Jr., and S. H. Kay, manuscript in preparation) suggest that the effects of competing substances on efficacy of H_2O_2 as an herbicide may not be significant in the field, perhaps due to rapid absorption of the H_2O_2 and the short effective contact time.

Water chemistry and other factors, such as time of day for treatment, plant morphology, population densities and growth habit, stage of growth, water temperature, and light quality (i.e., spectrum), may influence the efficacy of H_2O_2 as a new compound for the management of noxious aquatic

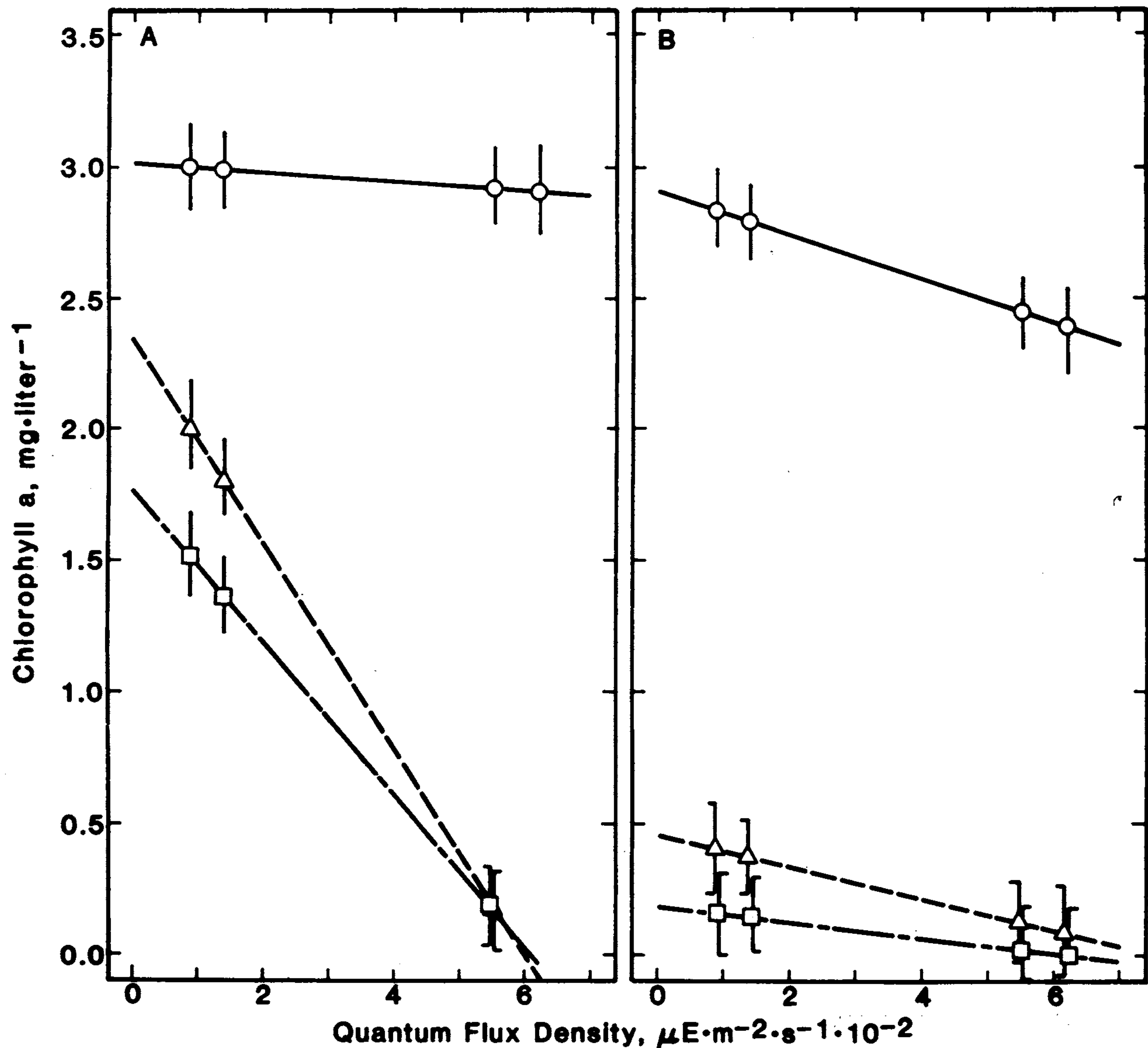

Figure 7. Decrease in chlorophyll following treatment of *Raphidiopsis* sp. with H_2O_2 at different QFD. (A) 24 h (B) 48 h. Symbols and confidence intervals as in Fig. 6.

vegetation and should be investigated. The photo-enhancement effect reported here affirms that screening of herbicides for submersed vegetation control should be done at QFD approximating those found in nature.

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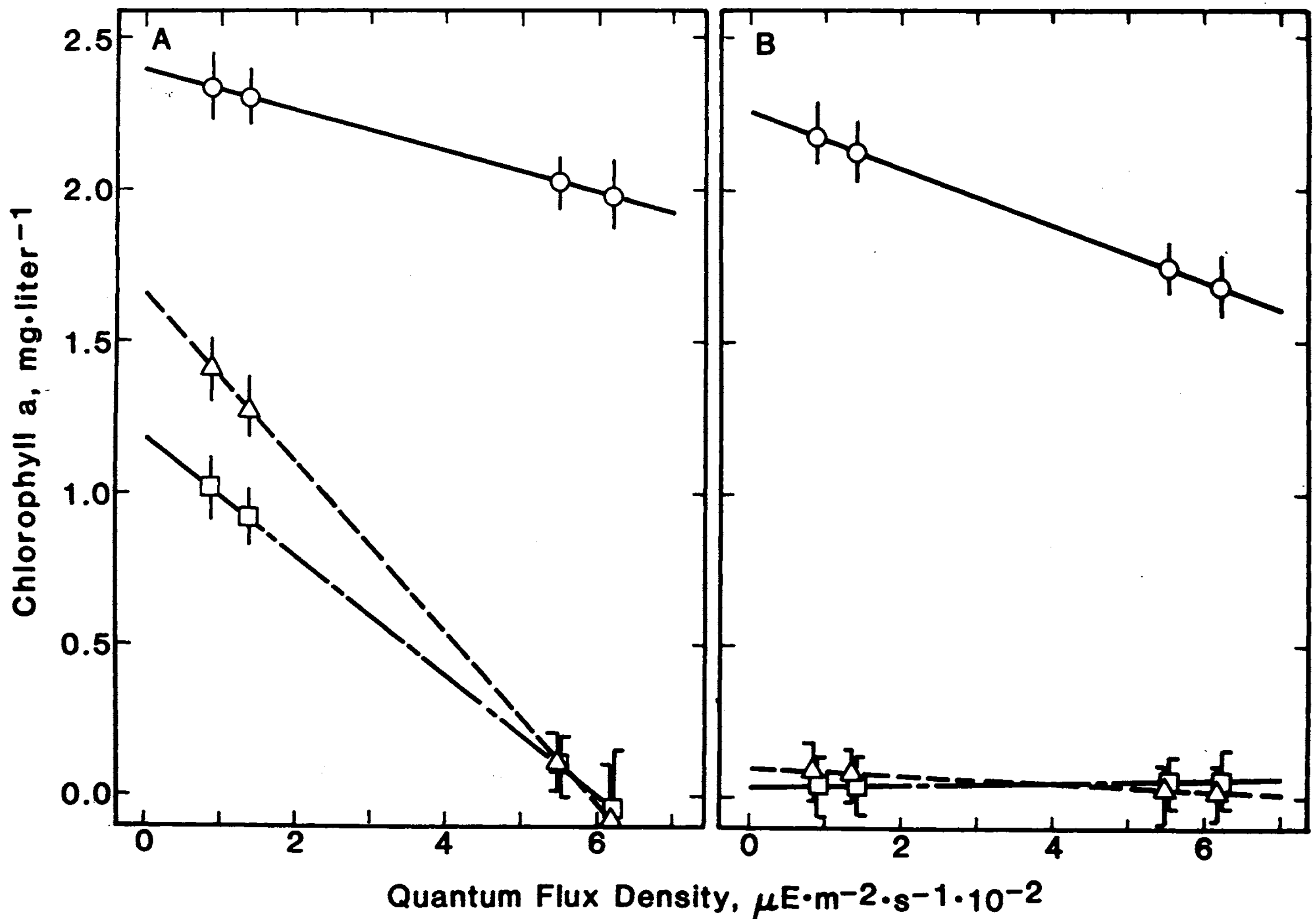

Figure 8. Decrease in chlorophyll following treatment of *Ankistrodesmus* sp. with H_2O_2 at different QFD. (A) 24 h (B) 48 h. Symbols and confidence intervals as in Fig. 6.

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