

# The Effect of Cyanophages on the Growth and Survival of *Lyngbya wollei*<sup>1</sup>, *Anabaena flos-aquae*, and *Anabaena circinalis*

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## ABSTRACT

The effect of three newly isolated cyanophages on the growth and survival of the bloom-forming cyanobacteria *Lyngbya wollei*, *Anabaena flos-aquae*, and *Anabaena circinalis* was studied in laboratory culture experiments. Cyanophages LW 1, AF 1, and AC 1 were inoculated into cultures of their respective host species at different stages of growth (i.e. lag, mid-log, late-log, and stationary). Changes in cyanobacterial biomass were monitored using chlorophyll determination. The most rapid lytic effect was observed in exponential phases of cyanobacterial growth. Significant reductions in chlorophyll were observed within 7 days of cyanophage inoculation. When inoculated during the early growth stages, population expansion of cyanobacteria was reduced by 80 to 95% in all three species. Inoculation of cyanophage in stationary phase cultures significantly accelerated the decline of standing crop for *A. flos-aquae* and *A. circinalis*.

**Key words:** biocontrol, blue-green algae blooms, cyanobacteria.

## INTRODUCTION

Cyanophages, bacterial viruses which can infect and lyse cyanobacterial cells, have been isolated worldwide

<sup>1</sup>The genus *Lyngbya* is currently being reclassified by Dr. B. J. Speziale. Based on consultation with Dr. Speziale we have assigned the species name *L. wollei* to this benthic mat-forming cyanobacterium, which we previously referred to as *L. birgei* (Philips et al. 1990).

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(Martin and Benson 1988), and offer a potential alternative to chemical controls (Safferman and Morris 1964b). Most studies have focused on cyanophages of the LPP group, which infect *Lyngbya*, *Plectonema*, and *Phormidium* (Daft et al. 1970, Padan and Shilo 1967, Padan et al. 1970, Padan et al. 1971, Safferman and Morris 1964a, Safferman et al. 1969, Shane 1971, Singh and Singh 1967). Cyanophages which infect other cyanobacteria have been isolated, but not extensively studied (Stewart and Daft 1977).

With increasing cultural eutrophication and habitation of waterfront property, the need for cyanobacterial control is increasing. Cyanophages may provide an alternative or supplement to chemical algicides in the control of benthic or planktonic cyanobacteria (Safferman and Morris 1964b). This approach may also assist in controlling species of cyanobacteria which are relatively resistant to herbicides (Speziale et al. 1988). In addition, cyanophages are relatively specific to target cyanobacteria and are non-toxic to man, animals, and other microorganisms in the food chain (Brown 1972, Sladeckova and Sladeczek 1968).

In Florida, three of the most problematic cyanobacterial species are the planktonic forms *Anabaena flos-aquae* Breb ex Born. et Flah. and *Anabaena circinalis* Rabenh. ex Born. et Flah., and the benthic mat-forming species *Lyngbya wollei* (Farlow ex Gomont) Speziale Dyck. The two planktonic forms, *A. flos-aquae* and *A. circinalis* are heterocystous nitrogen-fixing species, and form extensive surface blooms in Florida lakes. *A. flos-aquae* has been associated with the production of toxins (Carmichael 1981, Mahmood et al. 1988, Shilo 1967). *L. wollei* is a filamentous, non-heterocystous cyanobacterium characterized by its foul odor, and is relatively resistant to currently registered

algicides (Beer et al. 1986). *L. wollei* has also been implicated with the production of toxins (Mynderse et al. 1976). This paper describes the effects of three newly isolated cyanophages (Phlips et al. 1990) on the growth and survival of *Lyngbya wollei*, *Anabaena flos-aquae*, and *Anabaena circinalis*. The hypotheses that cyanophages are most effective in early stages of blooms (Jackson and Sladeczek 1970) is examined using laboratory cultures inoculated with cyanophage at different cyanobacterial growth phases.

## MATERIALS AND METHODS

Cyanophages used for growth stage experiments were LW 1, AF 1, and AC 1 specific for *Lyngbya wollei* (local isolate), *Anabaena flos-aquae* (ATCC #18200), and *Anabaena circinalis* (local isolate), respectively. The cyanophages were isolated and concentrated using established methods (Safferman and Morris 1964a, Phlips et al. 1990). All three cyanophages were maintained at titers of approximately  $10^7$  PFU (Plaque Forming Units)/ml.

The cyanobacteria were cultured in modified Hoagland's media (Phlips et al. 1990). *A. flos-aquae* and *A. circinalis* were cultured without the addition of combined nitrogen. Cultures were grown at irradiance of  $80 \mu\text{mol photons m}^{-2}\text{s}^{-1}$  and 30 C. In order to examine how the growth stage of cyanobacteria affects the impact of cyanophage infection, replicate time series inoculations of phage were performed. Axenic cultures of the three target cyanobacteria were inoculated with axenic cyanophage at four different stages of culture, i.e. lag phase (cyanophage and cyanobacteria inoculated into fresh media simultaneously), early log phase (day 5-7 of culture), late log phase (day 14 of culture), and stationary phase (day 21 of culture). Control cultures were inoculated with sterile media treated in the same manner as phage samples. Cyanophage was added to a final starting concentration of  $10^5$  PFU. After inoculation of phage, replicate control and test cultures were harvested weekly for chlorophyll determination.

Changes in cyanobacterial biomass were estimated as chlorophyll *a* (A.P.H.A. 1989). Samples were filtered onto 47 mm Gelman AE glass fiber filters. Filters were then macerated in a tissue grinder at 500 rpm with 90% acetone, and extracted for 24 hours at 4 C in the dark. Chlorophyll *a* was measured spectrophotometrically and expressed as  $\mu\text{g ml}^{-1}$ . Chlorophyll *a* determinations for *A. flos-aquae* samples were determined fluorometrically according to established methods (A.P.H.A. 1989). Fluorometric readings were calibrated using spectrophotometrically determined samples.

Results were analyzed using analysis of variance and paired Tukey's range tests. Significance level was set at  $p < 0.05$ .

## RESULTS AND DISCUSSION

*L. wollei*, *A. flos-aquae*, and *A. circinalis* were all clearly affected by their respective cyanophages (Figures 1-3). The exact timing of lytic activity appeared to vary between species and the age of the cultures.

Cyanophage LW 1 significantly reduced the growth and survival of *L. wollei* (Figure 1). In cultures inoculated

with phage in lag phase, inhibition of growth was observed within 7 days of inoculation (Figure 1A). Fourteen days after inoculation chlorophyll concentrations in inoculated flasks were reduced by 95% over control flasks. In cultures inoculated during mid-log phase it took between one to three weeks for the inoculum to reduce chlorophyll by 37% (Figure 1B). Within 28 days chlorophyll levels were reduced by 85%. In cultures inoculated with cyanophage in stationary (Figure 1C) phase, standing crops of cyanobacteria were in decline in both control and infected cultures.

Cyanophage AF 1 reduced chlorophyll levels of *A. flos-aquae* cultures within five days of inoculation into lag phase cultures (Figure 2A). Within 32 days chlorophyll concentrations in infected cultures were 80% less than in control cultures. In mid/late-log phase cultures (Figure 2B) the cyanophage reduced chlorophyll levels by 65% after 22 days of incubation. The effect of cyanophage AF 1 appeared to be delayed with increasing culture age. The effects of AF 1 were not significant until 32 days after stationary phase inoculation (Figure 2C), when inoculated flasks exhibited 40% less chlorophyll than control flasks. Although viral inoculation accelerated the decrease in chlorophyll, both control and inoculated flasks were in decline.

Cyanophage AC1 lowered chlorophyll concentrations in *A. circinalis* cultures by 75% within 21 days of inoculation into lag phase cultures (Figure 3A). At 28 days chlorophyll concentration was reduced by 95%. The most rapid impact of cyanophage AC 1 was observed when the phage was inoculated into mid-log phase cultures (Figure 3B). There was a 50% reduction in chlorophyll within 7 days of inoculation. Cyanophage AC 1 continued to lower chlorophyll through 28 days of culture, with a maximum inhibition of 70%. In stationary phase cultures, standing crop of *A. circinalis* declined in both controls and infected cultures (Figure 3C), but the addition of virus accelerated the decline. Within 14 days inoculated flasks exhibited 50% less chlorophyll than control flasks.

These results indicate that cyanophages LW 1, AF 1, and AC1 all reduce cyanobacterial biomass of their respective host species. Growth was suppressed up to 80 to 95%. The exact timing and magnitude of the effect was subject to the physiological condition of the cyanobacteria. Inoculation of cyanophage into actively growing cultures of cyanobacteria yielded the greatest reductions in biomass. In terms of potential biocontrol, this implies that treating cyanobacterial blooms early in their development would yield the best results.

Our results are in agreement with Jackson and Sladeczek's (Jackson and Sladeczek 1970), and Desjardins and Olsen's (1983) experiments with *Plectonema boryanum* and LPP-1 virus. They concluded that cyanophage application is most effective when applied before host populations are well established. The impact of cyanophages on established populations of cyanobacteria may be more difficult to predict. The results for *A. flos-aquae* and *A. circinalis* indicate that the addition of cyanophage may accelerate the decline of standing crops in non-expanding populations. This effect appears, however, to be less pronounced in *L. wollei*.

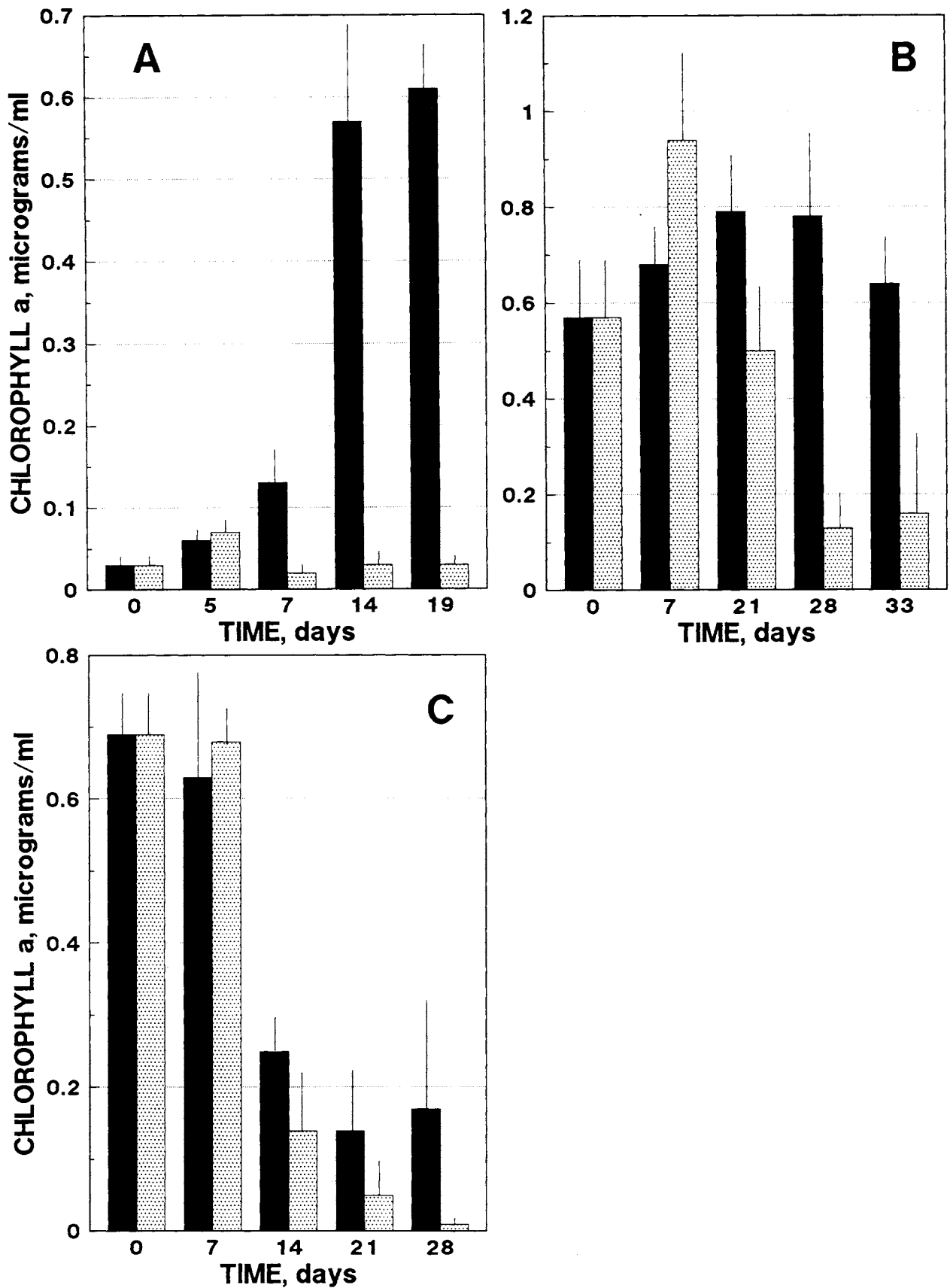


Figure 1. Effect of cyanophage LW 1 on the growth and survival of *Lyngbya wollei*. Cyanophage was added to cultures in three growth stages, A - lag, B - log, and C - stationary. Time is expressed as days after cyanophage addition. Dark bars are mean chlorophyll a values for control flasks and shaded bars are for flasks inoculated with phage. Extended lines are standard deviations.

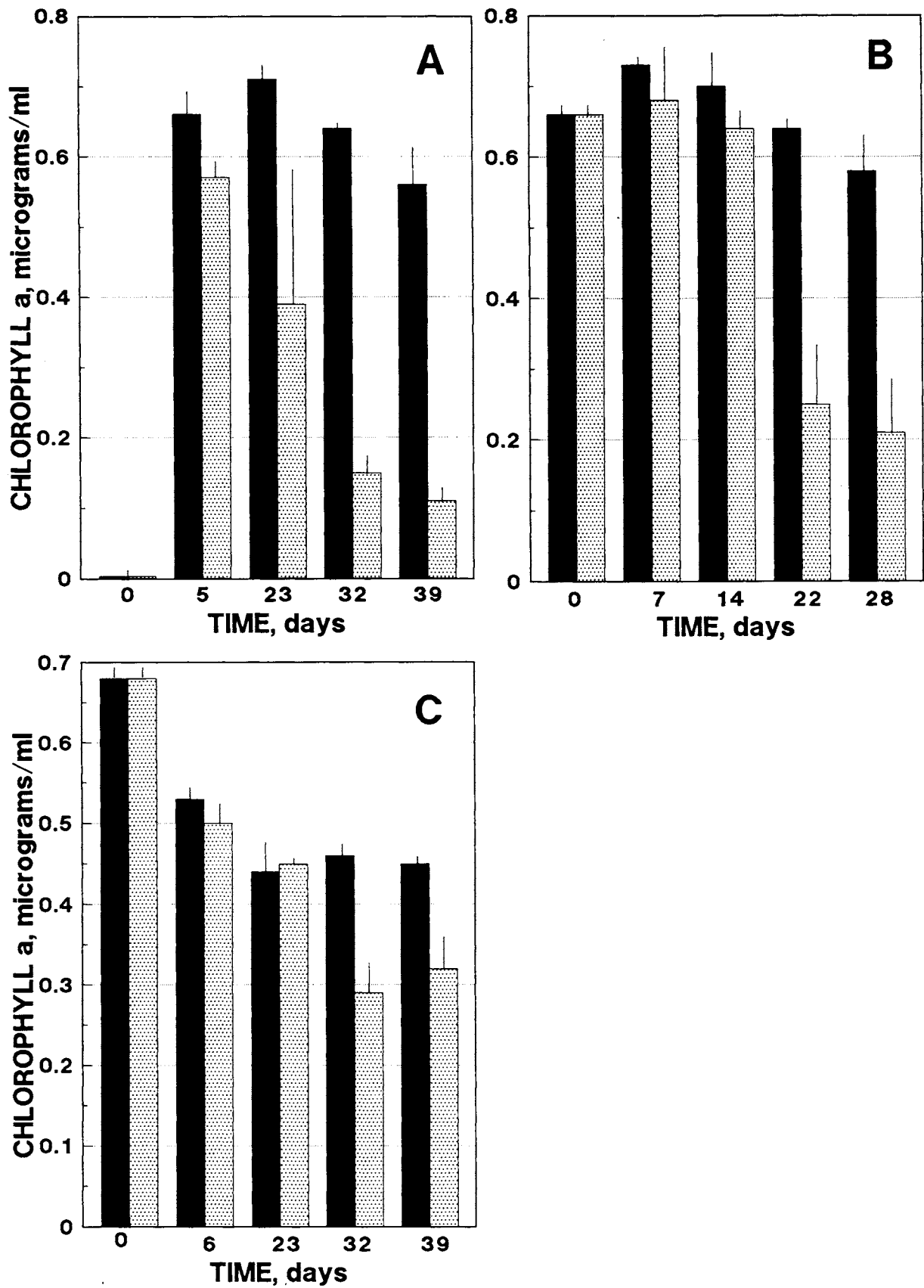


Figure 2. Effect of cyanophage AF 1 on the growth and survival of *Anabaena flos-aquae*. Cyanophage was added to cultures in three growth stages, A - lag, B - log, and C - stationary. Time is expressed as days after cyanophage addition. Dark bars are mean chlorophyll a values for control flasks and shaded bars are for flasks inoculated with phage. Extended lines are standard deviations.

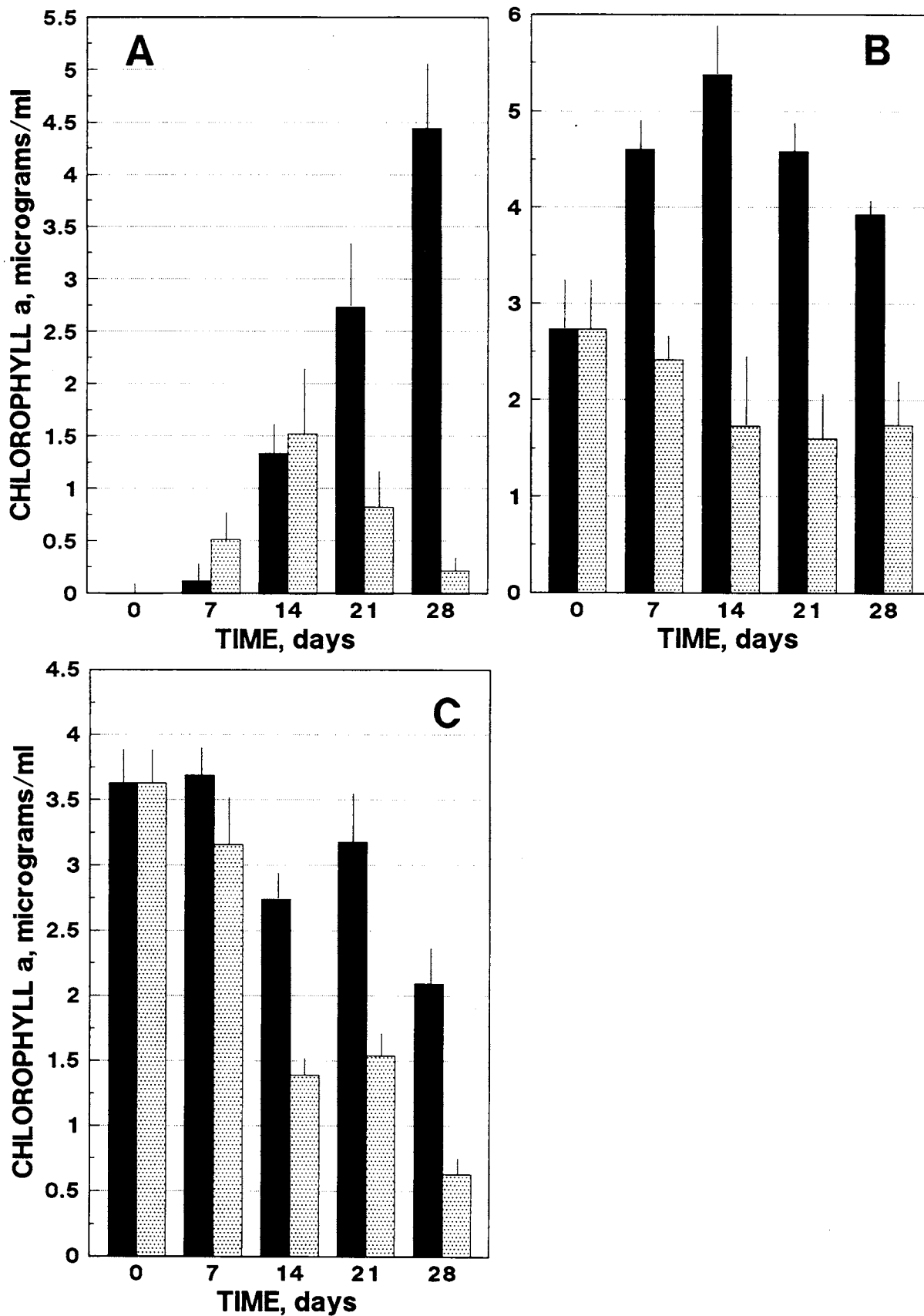


Figure 3. Effect of cyanophage AC 1 on the growth and survival of *Anabaena circinalis*. Cyanophage was added to cultures in three growth stages, A - lag, B - log, and C - stationary. Time is expressed as days after cyanophage addition. Dark bars are mean chlorophyll a values for control flasks and shaded bars are for flasks inoculated with phage. Extended lines are standard deviations.

## ACKNOWLEDGEMENTS

We thank Drs. Ken Langeland and Paul Thayer for reviewing this manuscript. This research was supported by a grant through the Center for Aquatic Weeds, Institute for Food and Agricultural Science, University of Florida. This grant is part of the United States Department of Agriculture, Agricultural Research Service, Cooperative Agreements Nos. 58-7B30-3-570 and 58-43YK-9-90001. This publication is part of the Florida Agricultural Experiment Station Journal Series Number R-01457.

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