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# Freshwater Community Responses to Mixtures of Agricultural Pesticides: Synergistic Effects of Atrazine and Bifenthrin

**K. Hoagland**
**R. Drenner**

**Texas Water Resources Institute**

**Texas A&M University**

RESEARCH PROJECT COMPLETION REPORT

# FRESHWATER COMMUNITY RESPONSES TO MIXTURES OF AGRICULTURAL PESTICIDES: SYNERGISTIC EFFECTS OF ATRAZINE AND BIFENTHRIN

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Kyle D. Hoagland
Ray W. Drenner

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Texas Water Resources Institute
Texas A & M University
College Station, Texas 77843-2118

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## Abstract

This study was an investigation of the effects of the herbicide atrazine and the insecticide bifenthrin on lake communities. The study was conducted in two phases: in phase one, we examined the effects of environmentally realistic levels of atrazine and bifenthrin, based on published data of concentrations measured in fresh waters; in phase two, the impacts of higher levels of atrazine and bifenthrin were investigated, based on concentrations used in previous studies. The factorial designed experiment included three levels of bifenthrin (0, 39, and 287 $ngL^{-1}$ in phase one and 0, 125, and 3,150 $ngL^{-1}$ in phase two) cross-classified with three levels of atrazine (0, 15, and 153 $ugL^{-1}$ in phase one and 0, 385, and 2,167 $ugL^{-1}$ in phase two), with duplicate replication of each treatment combination. Pesticides were added to 5,500-L fiberglass tanks containing natural plankton assemblages and bluegill. Tanks were sampled 7 and 14 d following the first pesticide treatment and 7 d after the second pesticide addition.

In phase one of the study, atrazine significantly reduced chlorophyll concentrations and turbidity on day 7 and had no significant impact on primary productivity or algal cell densities. Atrazine also had a significant negative effect on copepod nauplii and rotifers (days 7 and 14) and on *Bosmina* and particulate phosphorus in the 20-200 um size fraction (day 14). Bifenthrin significantly reduced *Bosmina* (days 7 and 14), cyclopoid copepodids (days 7 and 14), and copepod nauplii (day 14), however bifenthrin increased rotifers at day 7. Bifenthrin addition also increased colonial green algae and decreased particulate phosphorus in the 20-200 um size fraction on day 7 and decreased turbidity and particulate phosphorus in the >200 um size fraction on days 7 and 14. Only one fish mortality (in the high bifenthrin, no atrazine treatment combination) occurred during phase one of the study. Significant interaction effects were found

only for *Bosmina* (day 14), rotifers (day 7), and turbidity (days 7 and 14), indicating that at the concentrations used in phase one of this study, these agricultural pesticides did not act synergistically.

In phase two, higher levels of atrazine resulted in significant reductions in primary productivity, chlorophyll, green colonies, *Bosmina*, rotifers, and particulate phosphorus (>200 um and 20-200 um) on day 7. Bifenthrin had a negative impact on *Bosmina*, copepod nauplii, rotifers, primary productivity, chlorophyll, green colonies, and all particulate phosphorus fractions. In addition, 33% bluegill mortality was observed in treatment combinations with an average maximum concentration of 3,150 $ngL^{-1}$ bifenthrin. The interaction effects found indicated that when either compound was introduced at ecologically realistic levels, its effects were essentially masked if the other toxicant was present at high concentrations.

## Purpose of Research

This project was an experimental study of how individual and combined additions of an herbicide and an insecticide affect lake communities. The investigation focused on two important pesticides in Texas, the herbicide atrazine and the insecticide bifenthrin.

## Introduction

Despite decades of research on the toxicity of a variety of herbicides and insecticides, we have little knowledge of the synergistic effects of pesticides, because evaluation of their impacts has primarily focused on their individual effects (Vouk et al., 1987). In studying the impacts of toxicants in the natural environment however, one is inevitably dealing with mixtures of chemicals (Vouk et al., 1987).

Several mathematical models have been developed to predict synergistic pesticide effects (Colby, 1967; Akobundu et al., 1975; deMarch, 1987), but relatively few experimental studies have been reported. A majority of such studies have assayed only insecticide mixtures. Macek (1975) tested the toxicity of 29 pairs of pesticides on bluegill and found that the combined effects were generally more than additive (i.e. synergistic). Synergistic effects of pesticide mixtures have been shown for other fish species as well (Ludke et al., 1972; Fabacher et al., 1976; Koenig, 1977), but very few other aquatic organisms have been tested (E.I.F.A.C., 1987). In one of the few studies published, Mosser et al. (1974) reported that the combined effects of PCB's (polychloinated biphenyls) and DDE [1, 1-dichloro-2, 2-bis (p-chlorophynel) ethylene] on a marine diatom were more than additive.

Only two studies have been published on herbicide-insecticide mixtures. Lichtenstein et al. (1973) reported synergistic effects of parathion with several herbicides on adult mosquitos in

laboratory culture and Burrell et al. (1985) found that the combined effects of atrazine and Na-PCP (sodium pentachlorophenate) on single algal populations were additive, however the toxicants acted antagonistically in multi-species laboratory microcosms. Few studies have examined even individual toxicant effects on natural aquatic assemblages (Cairns, 1983; Cairns and Pratt, 1985), and no research has been published on the impacts of herbicide-insecticide mixtures on aquatic communities.

## Objectives

The primary goal of the proposed project was to determine the individual and synergistic effects of an important herbicide (atrazine) and insecticide (bifenthrin) in Texas on lake communities. This was the first mesocosm (community-level) experiment to determine if the combined effect of these toxicants was greater than their additive effects. We used a factorial design, in which three levels of atrazine were cross-classified with three levels of bifenthrin (Zslonay et al., 1987). In phase one of the study, atrazine concentrations used were similar to those found in natural aquatic systems, based on a survey of the literature, and bifenthrin was added at levels based on industry bioassays and our recently completed research on this synthetic pyrethroid. In phase two, higher levels of atrazine and bifenthrin were introduced based on concentrations employed in previous studies [see Jurgensen and Hoagland (1990) for atrazine and Mokry and Hoagland (1990) for bifenthrin].

## Methodology

### *Mesocosm design*

The experiment was conducted at the Texas Christian University outdoor mesocosm facility, which consisted of 5,500-L fiberglass tanks, each 2.2 m high, and 1.8 m in diameter. The

factorial design of the study included three levels of atrazine (nominal concentrations of 0, 20, and 200 $ugL^{-1}$ in phase one and 0, 250 and 1,500 $ugL^{-1}$ in phase two) cross-classified with three levels of bifenthrin (nominal concentrations of 0, 20 and 200 $ngL^{-1}$ in phase one and 0, 20 and 1,600 $ngL^{-1}$ in phase two), resulting in nine treatment combinations. Actual pesticide concentrations are listed in Table 1. Each treatment combination was replicated twice, resulting in a total of 18 tanks. The tanks were arranged in three rows of six and sampled from walkways between the tanks.

Tanks were filled on May 6, 1990 with water containing a natural plankton assemblage from a small (0.7 ha) pond adjacent to the facility using a Honda centrifugal pump (model WB-30X) and mixed continuously during the experiment with an airlift mixer system (Drenner et al., 1986). Because zooplankton occur in low densities near the shore where the water was pumped, tank communities were supplemented with zooplankton collected from a local reservoir. Bluegill (*Lepomis macrochirus*) were electrofished from the pond and stocked at ten individuals per tank. All tanks were spiked with 10% of the initial ambient total phosphorus and total nitrogen concentration every other day throughout the experiment, to prevent nutrient depletion. Tank communities were allowed to develop for two weeks prior to the initial (pretreatment) sampling for phytoplankton, zooplankton, and water chemistry on May 20, and again on May 29, June 5, and June 19 (phase 2).

*Pesticide addition*

Pesticides were introduced into the tanks as a soil slurry to simulate a sediment plume entering a pond or stream following a storm runoff event. Sandy loam was obtained from a nearby soil pit and analyzed by the Texas A & M University Agricultural Experiment Station,

College Station, Texas (58.3% sand, 19.6% silt, 22.1% clay, 2.17% organic matter). Bifenthrin [Capture® 2E, 24.5% a.i. (w/v)] and/or atrazine [Hi-Yield®, 40.8% a.i. (w/v)] were added with 150 mL deionized water to 0.5 L of soil in an aluminum tray and stirred with a glass rod. An additional 125 mL of tank water was poured into each tray and stirred to produce a soil slurry just prior to addition to the tanks on May 22, 1990. Slurries were evenly distributed across the surface of the tanks with the airlift mixing system operating. Tanks appeared to reach uniform turbidity within ten minutes. Soil treated as above, but without pesticide addition, was also added to the control tanks. All treatment combinations were assigned to tanks at random. A second pesticide treatment was introduced on June 12, as a soil slurry using the same soil and procedure described above.

*Community monitoring*

Zooplankton were sampled by filtering 6 L of tank water (2 L from each of three locations from the surface of the tanks, collected with a plastic pitcher) through 54-um-mesh Nitex netting and preserving the filtrate with 5% sucrose-formalin (w/v). Zooplankton were identified to genus and counted with a Wildco counting wheel and a Wild M5 dissecting microscope. Phytoplankton samples (250 mL) were collected with a plastic pitcher from the center of each tank (without filtration) and preserved with 0.5% Lugol's iodine. A 4-mL subsample from each collection was settled overnight and ten random fields were counted using a Wild M-40 inverted microscope. To assess standing stocks of the different plankton community components (nano-, micro-, and macroplankton), a size fractionation technique was used (Mazumder et al., 1988) in which water was passed through a series of filters of decreasing porosity (200, 20, and 2 um) and the material retained on the filters analyzed for total phosphorus by acid persulfate hydrolysis (Menzel and

Corwin, 1965) and the orthophosphate malachite green method (Van Veldhoven and Mannaerts, 1987).

Basic limnological variables were also monitored either daily, including temperature (YSI model 43TD temperature meter) and visual observations of fish mortality, or weekly, including dissolved oxygen (YSI model 57 dissolved oxygen meter), turbidity (Hach model 2100A turbidimeter), total nitrogen (APHA, 1985), and total phosphorus (analyzed as indicated above) and pH (Orion model SA520). Algal chlorophyll was determined using the chloroform-methanol extraction method of Wood (1985). Primary productivity was measured weekly according to the light:dark bottle, dissolved oxygen method (Lind 1985) using a YSI dissolved oxygen meter.

*Pesticide analyses*

Pesticide samples were collected from the center of each tank on May 22 (approximately 1 h after the first pesticide introduction ), June 3 (12 d after the first introduction), June 12 (1 h after the second pesticide introduction), and June 25 (14 d after the second introduction), using a plastic pitcher (separate pitchers were dedicated to each treatment combination to prevent cross-contamination), decanted directly into a 1-L amber glass bottle, placed on ice in a cooler, and transported to Talem Inc. (Fort Worth, Tx) for extraction and analysis. Hexane extracts (EPA procedure 608) were analyzed using a Tracor 560 GC with a 1 m X 2 mm i.d. 4% SE-30/6% OV-210 on 100/120 Suplecoport-WV column, argon/methane as the carrier and a Tracor $^{63}N$ electron-capture detector. The limit of detection was 5 $ugL^{-1}$ for atrazine and 5 $ngL^{-1}$ for bifenthrin. Standards were prepared using the same pesticide lots used in the experimental treatments.

*Data analyses*

The 16 response variables were analyzed using an ANOVA on individual sample dates (May 29, 1990 = day 7 and June 5 = day 14 of phase one; June 19 = day 7 of phase two), with SYSTAT (version 4.0; Systat, Inc., Evanston, IL). Treatment effects were examined on a date by date basis in order to evaluate starting conditions and ascertain when significant effects occurred. Due to the loss of one replicate of a treatment combination (tank 8, 0 $ugL^{-1}$ atrazine, 0 $ugL^{-1}$ bifenthrin), a significance level of $P \leq 0.1$ was chosen. Adoption of this level of significance was intended to reduce the probability of type II errors [accepting a false null hypothesis (i.e. "no effect")]. Significant atrazine X bifenthrin interactions were further analyzed for simple effects.

## Principal Findings

### Phase One

*Atrazine effects*

Atrazine significantly reduced chlorophyll and turbidity on day 7 (Fig. 1-2); however, none of the major algal groups declined significantly in the presence of even high concentrations of the herbicide during phase one (Fig. 1). Atrazine addition also had a significant effect on phosphorus concentrations in the 20-200 um size fraction and total phosphorus on day 14 (Figs. 2-3). Particulate phosphorus in the 2-20 um fraction (day 7) and total nitrogen (days 7 and 14) increased significantly in the presence of atrazine. The significant effect of atrazine on total nitrogen on days 7 and 14 may be due to nitrogen in atrazine. Atrazine had a significant negative effect on *Bosmina* on day 14, with highest *Bosmina* densities in the low atrazine treatment combinations (Fig. 4). Atrazine also had a significant effect on copepod nauplii and

rotifers on days 7 and 14, with the lowest densities in the high atrazine treatment combinations (Fig. 4).

*Bifenthrin effects*

Bifenthrin significantly reduced the zooplankters *Bosmina*, cyclopoid copepodids, and copepod nauplii on day 7, and the former two categories on day 14 (Fig. 4). Reductions in zooplankton densities were particularly evident at the highest bifenthrin concentration. This strong negative effect on the larger zooplankton was also reflected in significant reductions in phosphorus concentrations in the >200 um size fraction on days 7 and 14 and in the 20-200 um fraction on day 7 (Fig. 3). Conversely, bifenthrin increased rotifers on day 7. Bifenthrin also increased green algal colonies on day 7 (Fig. 1), decreased total phosphorus on day 14, turbidity on days 7 and 14, and total nitrogen on day 14 (Fig. 2). Only one fish died during phase one of the experiment (high bifenthrin, low atrazine treatment; June 12, 1990).

*Interaction effects*

Significant bifenthrin X atrazine interaction effects were found for *Bosmina* on day 14, rotifers on day 7, and turbidity on days 7 and 14 (Figs. 2, 4). Atrazine effects were most apparent on: (1) *Bosmina* at low bifenthrin levels; (2) rotifers at low and high bifenthrin levels; (3) turbidity on day 7 at low bifenthrin concentrations and on day 14 in the absence of bifenthrin.

**Phase Two**

*Atrazine effects*

The second atrazine addition significantly reduced primary productivity and chlorophyll on day 7 of phase two (Fig. 1), after the second pesticide addition. Atrazine also had a strong negative impact on green colonies. Phosphorus in the >200 um and 2-20 um fractions were also

significantly reduced by atrazine (Fig. 3) after the second addition, as were *Bosmina* and rotifers (Fig. 4). Atrazine addition had a significant effect on pH and total nitrogen, with the highest total nitrogen concentrations occurring at the highest atrazine levels (Fig. 2).

*Bifenthrin effects*

In the second phase, bifenthrin addition significantly reduced *Bosmina*, copepod nauplii, and rotifers on day 7 (Fig. 4). In addition, bifenthrin input reduced primary productivity, chlorophyll levels, and green colonies (Fig. 1), as well as each of the three phosphorus size categories (Fig. 3). Total nitrogen and turbidity were also significantly reduced by bifenthrin after the second addition. A total of 21 bluegill mortalities were observed in phase two, all of which occurred in high bifenthrin treatments (Table 3).

*Interaction effects*

Following the second pesticide treatment, significant atrazine X bifenthrin interaction effects were found for numerous additional parameters including: primary productivity, chlorophyll, green colonies, particulate phosphorus >200 um and 20-200 um, *Bosmina*, and pH (Figs. 1-4). Further analysis of atrazine and bifenthrin simple effects (for variables with significant interaction effects) indicated that atrazine effects were generally significant at zero and/or low bifenthrin levels, but not at high bifenthrin concentrations (Table 2). Similarly, bifenthrin effects tended to be significant at zero or low atrazine levels, but not at high atrazine concentrations. Notable exceptions to these trends were atrazine effects on rotifers on day 7 and bifenthrin effects on pH on day 7.

## Conclusions and Recommendations

This project was an experimental assessment of how individual and combined additions of

an herbicide and an insecticide affect lake communities. Atrazine is the second most widely used pesticide in North America, with over two million acres of crops in Texas treated annually (Gianessi and Puffer, 1988). Bifenthrin is a new pyrethroid insecticide for use on cotton, which accounts for nearly 25% of all cropland in Texas. Despite the heavy use of atrazine and insecticides such as bifenthrin in Texas and throughout the midwestern U.S., the impacts of mixtures of these and similar toxicants on aquatic systems remains virtually unaddressed.

**Phase one**

*Atrazine effects*

In phase one of the present study, we found that atrazine reduced chlorophyll levels one week after herbicide addition, but had no significant effect by day 14 (Fig. 1). This supports previous findings by deNoyelles et al. (1982), who found that 20 $ugL^{-1}$ atrazine had a negative impact on algal productivity and biomass in experimental ponds, followed by recovery to control levels or higher after 7 days. Larsen et al. (1986) reported similar recovery times after introducing 60 and 100 $ugL^{-1}$ of atrazine into microcosms.

The lack of atrazine effects on chlorophyll after 14 days or any significant effects on primary productivity or algal cell densities at 153 $ugL^{-1}$ is also consistent with previous studies. Lay et al. (1984) found little evidence of a significant reduction in phytoplankton density at 200 $ugL^{-1}$ atrazine. Similar results have generally been reported for attached algal communities (Goldsborough and Robinson, 1986; Lynch et al., 1985; Jurgensen and Hoagland, 1990), although Krieger et al. (1988) reported declines in biomass and chlorophyll with 134 $ugL^{-1}$ and Hamala and Kollig (1985) at 100 $ugL^{-1}$ atrazine.

The recovery by phytoplankton and the apparent tolerance to 153 $ugL^{-1}$ is meaningful in light

in light of atrazine concentrations typically found in nature. Based on several aquatic surveys, concentrations in streams and rivers rarely exceed 100 $ugL^{-1}$, with peak values often occurring below 50 $ugL^{-1}$ (Jurgensen and Hoagland, 1990). That is, the concentrations employed in phase one of the present study were somewhat higher than those occurring in aquatic systems, yet few effects on the primary producers were noted. We conclude that higher atrazine levels than those found in nature may have significant effects for approximately 7 days of exposure, followed by rapid recovery of the algal community.

Atrazine concentrations in the tanks were somewhat variable between 1 h and 10 d and between replicates (Table 1). Five of 13 atrazine concentrations decreased from 1 h to 10 d, indicating no definite trend. Additional variation between replicate tanks could have resulted from sampling error, heterogeneous distribution of sediment in the water column, and/or sample analysis error. Replicate atrazine analyses on June 25 (tank 14) indicated that up to 8% of the variability was likely due to sample extraction and GC analysis variability. Extraction and analysis of deionized water blanks showed that bifenthrin values were accurate to $\pm 15$ $ngL^{-1}$ and analysis of samples held for varying lengths of time prior to extraction indicated that this source of error accounted for up to 10% of the variability for bifenthrin and 5% for atrazine.

*Bifenthrin effects*

We detected a significant reduction in most zooplankton groups on days 7 and 14 following the first exposure to bifenthrin, while rotifers were enhanced by day 7 (Fig. 4). This enhancement was likely due at least in part to an indirect effect, resulting from the concomitant decline in larger zooplankton taxa. Kaushik et al. (1985) reported similar reductions in zooplankton diversity and the density of larger cladocerans and copepods in limnocorrals exposed

to 0.5-50 $ugL^{-1}$ of the pyrethroid permethrin. Yasuno et al. (1988) found that permethrin reduced cladocerans and increased *Keratella* at 1.5 $ugL^{-1}$. The positive impact of bifenthrin on green algal colonies on day 7 was also likely an indirect effect, resulting from the decline of large zooplankters in the tanks.

The lack of fish mortality in the high bifenthrin treatment ($\bar{x}_{1h}$ = 287 $ngL^{-1}$) after 14 days is consistent with previous reports on the toxicity of pyrethroid insecticides to *L. macrochirus*. The 48-h $LC_{50}$ for fenvalerate is 900-1,890 $ngL^{-1}$ (Dyer et al., 1989), the 96-h $LC_{50}$ for fenpropathrin is 1,950 $ngL^{-1}$, for phenothrin 18,000 $ngL^{-1}$, for resmethrin 2,620 $ngL^{-1}$, and tetramethrin 21,000 $ngL^{-1}$ (cited in Smith and Stratton, 1986).

*Interaction effects*

Significant interaction effects for biological parameters were found only for *Bosmina* (day 14) and rotifers (day 7), indicating that the two pesticides were, in general, not acting synergistically or antagonistically on the aquatic biota. The significant interaction effect on turbidity (both dates) is likely a combined result of a negative bifenthrin impact on zooplankton densities and a negative atrazine impact on chlorophyll concentrations, although the latter effect is equivocal on day 14 (Fig. 1).

**Phase two**

*Atrazine effects*

Additions of much higher levels of atrazine resulted in significant reductions in several algal parameters, including chlorophyll, primary productivity, and green colonies. Similar reductions were observed in numerous other studies which employed high levels of the herbicide (e.g., Brockway et al., 1984; Larsen et al., 1986). Based on lower concentrations typically reported

from field surveys (e.g., Goolsby et al., 1989), these dramatic impacts on the algae are likely applicable only to spill incidences or high-level point source inputs of the herbicide. While lower concentrations affected the algal community on a short-term basis, followed by recovery between 7 and 14 days, higher levels remained inhibitory to primary productivity and chlorophyll levels after 14 d in phase two (Fig. 1). This concentration-dependent temporal response suggests that mechanisms such as chemical degradation of atrazine in the tanks (Sirons et al., 1973) or metabolism of the herbicide by bacteria (Kaufman and Kearney, 1970) or perhaps algae are more important to algal recovery following exposure than induced resistance to the toxicant by the algal community as suggested by previous authors (Hamilton et al., 1987). The lack of a significant decline in diatoms and green unicells, even at high levels of atrazine, is interesting to note and potentially of great importance considering their dominance in many freshwater systems. While various algal taxa have been found to exhibit differential responses to atrazine (e.g., Patterson and Wright, 1988), their have been no reports of high levels of atrazine resistance by diatoms, unicellular green algae, or other algal groups.

*Bifenthrin effects*

The high concentrations of bifenthrin used in phase two had a devastating effect on zooplankton densities, including rotifers, 7 d after the second pesticide treatment (Fig. 4). The negative effects on primary productivity, chlorophyll and green colonies of the second pesticide addition were significant. Insecticides can have a direct negative impact on algae (Lal, 1984), however these effects have typically been found at much higher pesticide concentrations than those in the present study. Bifenthrin resulted in corresponding reductions in particulate phosphorus concentrations in each of the three size categories.

Higher fish mortalities in phase two of the study were anticipated based on known toxicities of pyrethroids to bluegill (Dyer et al., 1989). The approximately 33% mortalities observed in the treatment combinations with high bifenthrin (Table 3) occurred primarily within the first four days after pesticide addition, indicating that the average maximum bifenthrin concentration (3,150 $ngL^{-1}$) was below the 96-h $LC_{50}$ for bluegill in the present study. In contrast, gizzard shad (*Dorosoma cepediatum*) is much more susceptible to bifenthrin under very similar conditions, with an 96-h $LC_{50}$ of 207-550 $ngL^{-1}$ (Drenner et al., 1988).

*Interaction effects*

Despite the fact that a greater number of significant interaction effects were observed after the addition of higher levels of the two pesticides (Fig. 1-4), further analysis of atrazine and bifenthrin simple effects indicated that the impacts of both compounds were significant only when the other pesticide was absent or present at low concentrations (Table 2). Consequently, the effects of ecologically realistic concentrations of either pesticide were not apparent in the presence of high concentrations of the other toxicant. These results have important implications for interpreting past and future studies in which unrealistically high levels of pesticides are added in combination. In addition, the interaction effects found do not indicate that atrazine and bifenthrin acted synergistically, rather these pesticides impacted the aquatic biota independently.

**Summary**

This study shows that levels of atrazine commonly found in the environment had short-term effects on algal biomass and minimal effects on algal species composition and primary productivity. Bifenthrin affected the plankton at concentrations much lower than atrazine and had a greater impact on the entire system, particularly at higher concentrations such as those used

in phase two of the study. At the concentrations used in phase one, these two agricultural pesticides did not act synergistically, rather they impacted the aquatic community in an independent manner. If either compound was introduced at ecologically realistic levels, then its effects were masked when the other toxicant was present at higher concentrations.

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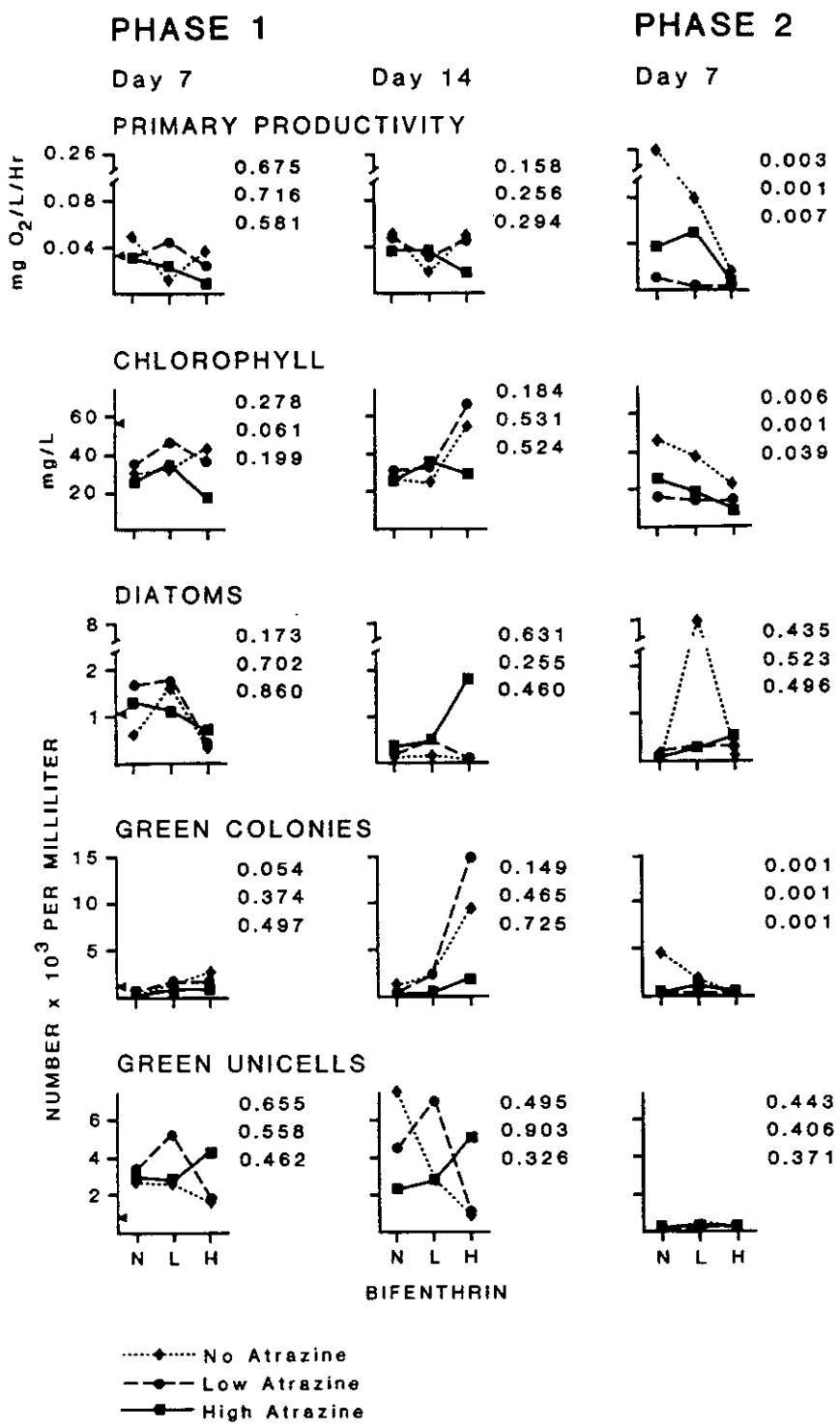


Fig. 1. Mean responses of primary productivity, chlorophyll, and phytoplankton to atrazine and bifenthrin additions. Probability values from ANOVA of bifenthrin (top) and atrazine (middle) effects and their interaction effects (bottom) given to the right of each graph, for days 7 and 14 of phase one and day 7 of phase two. Initial concentrations or densities are indicated by an arrow. N = no bifenthrin; L = low bifenthrin; H = high bifenthrin.

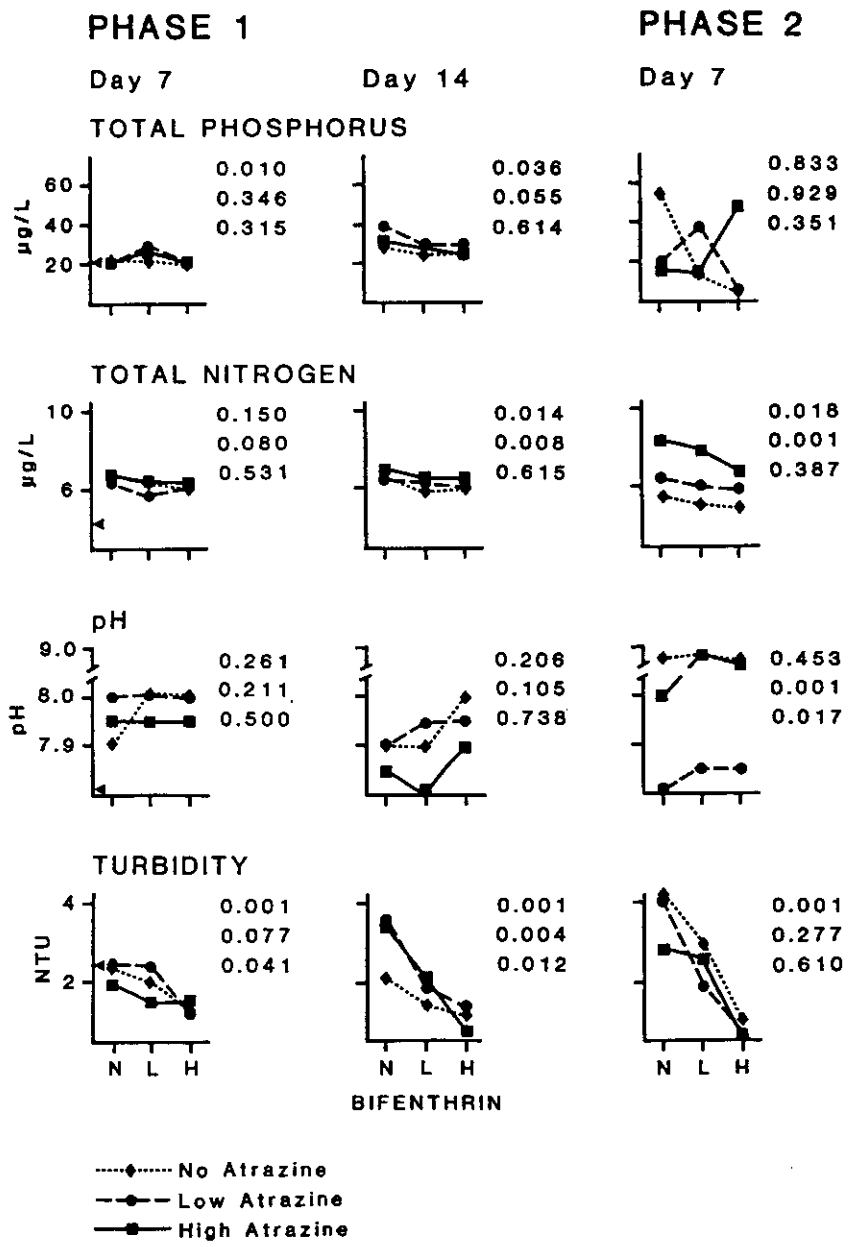


Fig. 2. Mean responses of limnological parameters to atrazine and bifenthrin additions. Probability values from ANOVA of bifenthrin (top) and atrazine (middle) effects and their interaction effects (bottom) given to the right of each graph, for days 7 and 14 of phase one and day 7 of phase two. Initial concentrations or densities are indicated by an arrow. N = no bifenthrin; L = low bifenthrin; H = high bifenthrin.

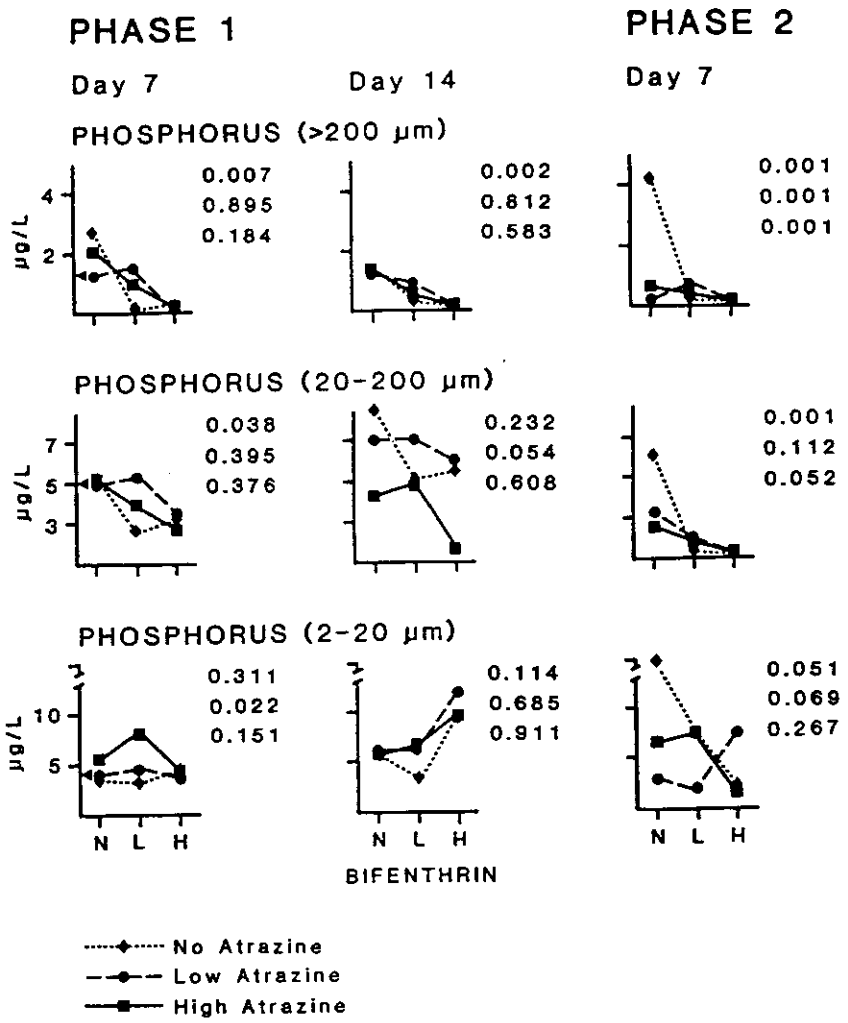


Fig. 3. Mean responses of particulate phosphorus size fractions to atrazine and bifenthrin additions. Probability values from ANOVA of bifenthrin (top) and atrazine (middle) effects and their interaction effects (bottom) given to the right of each graph, for days 7 and 14 of phase one and day 7 of phase two. Initial concentrations are indicated by an arrow. N = no bifenthrin; L = low bifenthrin; H = high bifenthrin.

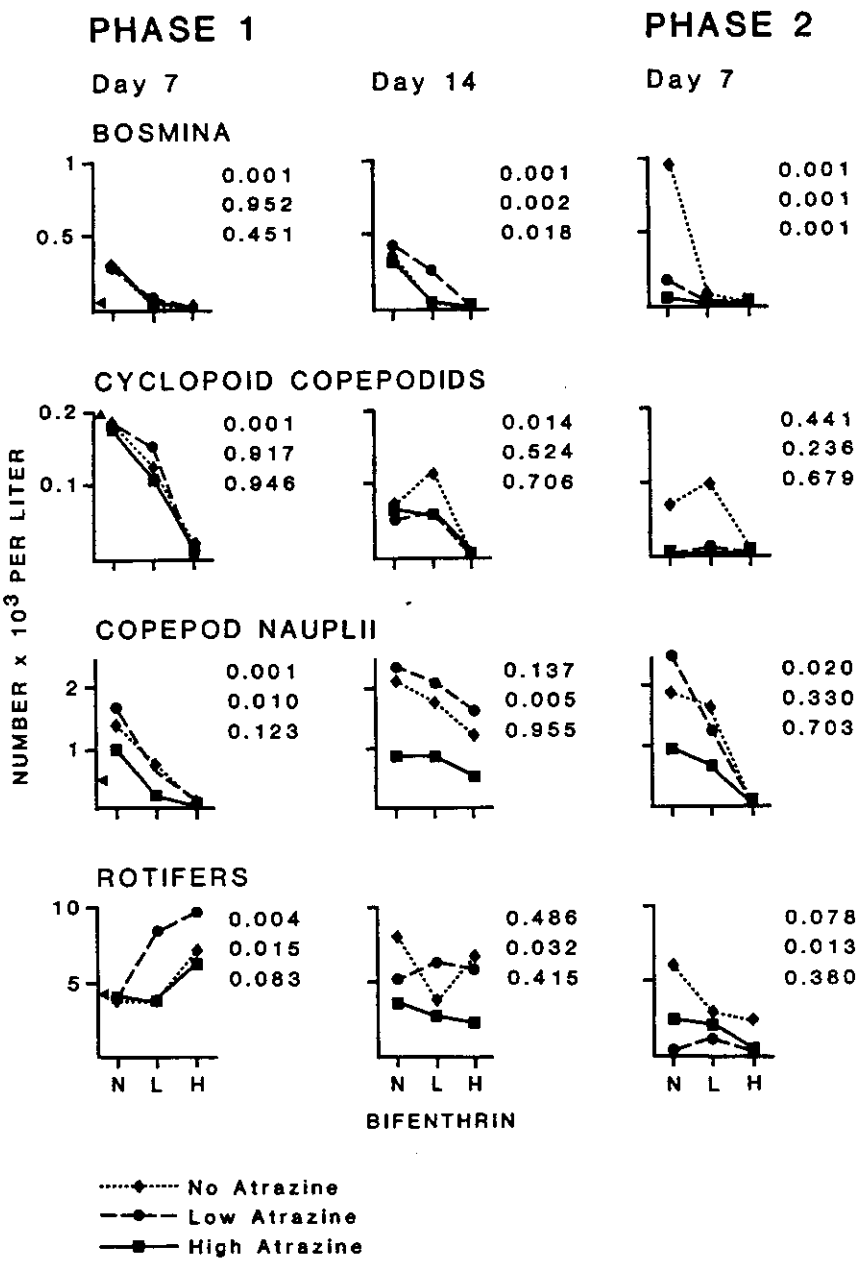


Fig. 4. Mean responses of zooplankton to atrazine and bifenthrin additions. Probability values from ANOVA of bifenthrin (top) and atrazine (middle) effects and their interaction effects (bottom) given to the right of each graph, for days 7 and 14 of phase one and day 7 of phase two. Off-scale initial densities are indicated by an upward-pointing arrow. N = no bifenthrin; L = low bifenthrin; H = high bifenthrin.

Table 1. Bifenthrin (B) and atrazine (A) concentrations in tanks 1 h (5/25) and 10 d (6/3) after the first pesticide introduction and 1 hr (6/12) and 14 d (6/25) after the second introduction.

| Treatment Combinations[1] | | Tank # | Bifenthrin ($ngL^{-1}$) | | | | Atrazine ($ugL^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Phase I | | Phase II | | Phase I | | Phase II | |
| | | | 1 h | 10 d | 1 h | 14 d | 1h | 10d | 1h | 14d |
| OB,OA | rep. 1 | 8 | 14 | na | na | na | <5 | na | na | na |
| | 2 | 18 | <5 | na | na | na | <5 | na | na | na |
| LB,OA | rep. 1 | 6 | 36 | 27 | 60 | <5 | na | na | na | na |
| | 2 | 17 | 66 | 13 | 42 | 29 | na | na | na | na |
| HB,OA | rep. 1 | 12 | 100 | 32 | 2900 | 190 | na | na | na | na |
| | 2 | 4 | 330 | 83 | 2000 | 100 | na | na | na | na |
| OB,LA | rep. 1 | 13 | na | na | na | na | 17 | 21 | 490 | 500 |
| | 2 | 11 | na | <5 | na | na | 9.5 | 6.9 | 370 | 360 |
| LB,LA | rep. 1 | 16 | 32 | 15 | 67 | 100 | 7.8 | 18 | 470 | 480 |
| | 2 | 10 | 27 | 28 | 420 | <5 | 22 | 22 | 320 | 350 |
| HB,LA | rep. 1 | 3 | 280 | 65 | 3400 | 2000 | 25 | <5 | 390 | 230 |
| | 2 | 5 | 150 | 71 | 3500 | 180 | 7.6 | 17 | 270 | 250 |
| OB,HA | rep. 1 | 15 | na | na | na | na | 110 | 170 | 2700 | 1100 |
| | 2 | 7 | na | na | na | na | 190 | 160 | 1800 | 1400 |
| LB,HA | rep. 1 | 9 | 30 | <5 | 86 | <5 | 160 | 96 | 1800 | 1400 |
| | 2 | 1 | 40 | 18 | 77 | <5 | 130 | 220 | 2000 | 2200 |
| HB,HA | rep. 1 | 2 | 510 | 31 | 4700 | 180 | 180 | 200 | 1800 | 2400 |
| | 2 | 14 | 350 | 140 | 2400 | 88 | 150 | 160 | 2900 | 2700 |

[1]O = No pesticide; L = low; H = high
na = not analyzed

Table 2. Summary of atrazine (A) and bifenthrin (B) simple effects corresponding to significant atrazine X bifenthrin interactions (entries are P-values).

| Parameter [Day] | Atriazine Simple Effects | | | Bifenthrin Simple Effects | | |
|---|---|---|---|---|---|---|
| | OB | LB | HB | OA | LA | HA |
| Day 7 | | | | | | |
| Rotifers | .831 | .008 | .047 | .062 | .003 | .208 |
| Turbidity | .081 | .018 | .274 | .006 | .001 | .234 |
| Day 14 | | | | | | |
| *Bosminia* | .063 | .001 | .990 | .000 | .000 | .000 |
| Turbidity | .002 | .044 | .134 | .041 | .000 | .000 |
| Day 7 (Phase Two) | | | | | | |
| Primary Productivity | .000 | .088 | .813 | .000 | .911 | .279 |
| Chlorophyll | .001 | .090 | .438 | .002 | .965 | .253 |
| Green Colonies | .000 | .006 | .361 | .000 | .935 | .027 |
| Phosphorous: >200 um | .000 | .373 | a | .000 | .222 | .016 |
| Phosphorous: 20-200 um | .018 | .347 | .462 | .001 | .013 | .030 |
| *Bosminia* | .000 | .759 | .998 | .000 | .178 | .748 |
| pH | .001 | .000 | .001 | .143 | .785 | .007 |

[a]Due to zero variance in one cell, analysis was not possible.

Table 3. Bluegill mortality following the second addition of atrazine (A) and bifenthrin (B), indicating corresponding treatment combination[1].

| Treatment | | #Individuals |
|---|---|---|
| HB,HA | rep. 1 | 5 |
| | rep. 2 | 5 |
| HB,LA | rep. 1 | 1 |
| | rep. 2 | 4 |
| HB,OA | rep. 1 | 2 |
| | rep. 2 | 4 |

[1]O= No pesticide; L = low; H = high