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SHORT COMMUNICATION

Effects of asplanchnin allelochemical on the toxicity of triasulphuron herbicide to rotifer *Brachionus patulus* (Rotifera: Brachionidae)

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ABSTRACT

We evaluated the effects of 4 concentrations (15, 30, 60 and 120 µg L^{-1}) of triasulphuron on the population growth of rotifer *Brachionus patulus*. The rotifers were cultured at an algal density of 0.25× 10^6 cells mL^{-1} of *Chlorella vulgaris* for 15 days and with and without the presence of asplanchnin allelochemical obtained from the predator *Asplanchna sieboldi*. Regardless of the presence of asplanchnin, the population density of *B. patulus* decreased with increasing concentration of triasulphuron and its populations drastically reduced when grown with triasulphuron herbicide at 120 µg mL^{-1}. At any given triasulphuron concentration, *B. patulus* + asplanchnin showed significantly higher peak abundances and higher growth rates than populations cultured without asplanchnin. The population growth rates varied from +0.23 to -0.13 per day, depending on the presence or absence of asplanchnin and the concentration of the herbicide. Our results suggest that infochemicals from *Asplanchna* may influence the population abundances of the prey brachionids in ponds and lakes that receive effluents containing herbicides.

Key words: Asplanchnin, rotifers, *Brachionus*, allelochemical, population growth, herbicide, triasulphuron

INTRODUCTION

Many factors influence the dynamics of freshwater zooplankton spp., including the infochemicals released by various organisms in aquatic ecosystems (4,7,10). Though food and temperature control the dynamics of herbivorous species, but other factors such as predation significantly influence the zooplankton population structure (3). In many freshwater ponds and lakes, the ciliates, rotifers and crustaceans (cladocerans and copepods) are the main components of zooplankton. Among rotifers, the predatory genus *Asplanchna* induces the spine elongation in rotifer prey [*Brachionus* and *Keratella* (6)] through an allelochemical, asplanchnin. Besides spine elongation, asplanchnin also

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increases the body size of prey. Therefore in the presence of *Asplanchna*, the population of brachionid prey consists of large bodied individuals with well developed spines (9).

Anthropogenic influence on freshwater ecosystems is mainly through release of toxic chemicals originating from industrial and agricultural operations (8). Triasulphuron (1-[2-(2-chloroethoxy) phenyl] sulfonyl-3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl) urea) is major herbicide for weeds control in wheat and rice fields world wide (18). In Mexico this herbicide (commercially known as Amber® 75 GS), is used in Guanajuato and Mexico states. It persists in soil for 3 to 24 months and with rains reaches the water bodies. It is also persistent in aquatic systems (12) and affects the survival and reproduction of plankton. In laboratory tests, triasulphuron is toxic to phytoplankton and its lower concentrations (10 $\mu g\ L^{-1}$) adversely effects the green algae (such as *Chlorella*), however, zooplankton (such as cladocerans) are resistant to this herbicide (the median lethal concentration for *Daphnia magna* is 48 mg L^{-1}) (19).

Rotifers are often exposed to the combined effects of several natural and man-made chemicals and are susceptible to herbicides (17). The effects of infochemicals from *Asplanchna* are reported on brachionids (6,11), but very little is known about the combined influence of asplanchnin and herbicide. This study aimed to evaluate the combined effects of triasulphuron herbicide and asplanchnin allelochemicals on the population growth of *Brachionus patulus*.

MATERIAL AND METHODS

The green alga *Chlorella vulgaris* Beijerinck was used as the exclusive diet for the herbivorous rotifer *Brachionus patulus* (O. F. Müller). *C. vulgaris* (strain CL-V-3, CICESE, Ensenada, Baja California, Mexico) was mass cultured using 2L transparent bottles on Bold' basal medium (1) at 20°C under continuous fluorescent illumination. Log phase algae were harvested by centrifuging at 4000 rpm for 5 min, rinsed and resuspended in distilled water. The algal density was estimated using a haemocytometer. For the population growth experiments, we used one low algal food level (0.25×10^6 cells mL^{-1}) to avoid confounding effects of high food density on *B. patulus*.

B. patulus was isolated from the wetland Chimaliapan, Mexico State and mass cultured on moderately hardwater (the EPA medium) and *C. vulgaris* at a density of 0.25×10^6 to 0.5×10^6 cells mL^{-1} in 2L jars. The predatory rotifer *Asplanchna sieboldi* (Leydig) was isolated from the Lake Chapultepec (Mexico City) and cultured in 1L jars using the EPA medium. The EPA medium was prepared by adding $NaHCO_3$, $MgSO_4$, $CaSO_4$ and KCl to distilled water (20). The medium containing asplanchnin was daily prepared based on literature (11). We maintained 100 individuals of *A. sieboldi* in 500 mL EPA medium for 24 h. It was then filtered using Whatman filter paper (1 µm) and the resulting medium was used in the experiments.

Herbicide concentrations

Triasulphuron (Analytical grade, batch 7310x, Sigma-Aldrich, USA) was dissolved in a small volume of acetone and later diluted using distilled water. Based on range finding tests, the final concentrations of triasulphuron selected for the experiments were: 15, 30, 60 and 120 $\mu g\ L^{-1}$. Daily while preparing the herbicide levels, we mixed equal volumes of know concentrations of triasulphuron and *Chlorella vulgaris* to obtain the final chosen herbicide concentration with the algal density of 0.25×10^6 cells mL^{-1}. Since the actual quantities of acetone reached the test jars was extremely low (a few µL), which had no effect on *Brachionus* spp.(17), hence additional controls (i.e., negative controls) containing acetone alone were not kept.

Experimental design and data collection

Population growth experiments were conducted in 50 mL transparent jars containing 50 mL medium with $0.25 X 10^6$ cells mL^{-1} of *Chlorella*. In each of 27 test jars (= 4 herbicide levels X 2 treatment types (with or without asplanchnin) X 3 replicates + 3 controls), we introduced 25 individuals of *B. patulus* using finely drawn Pasteur pipette under stereo-microscope (Nikon SMZ 645, Japan). The test conditions were: temperature 23±1°C, pH 7.1 - 7.3, continuous but diffused fluorescent (1000 lux) illumination.

After starting the growth experiment, every day we estimated the density of living *B. patulus* in each jar using total count or 2 to 3 aliquots of 1 to 2 ml each. After recording the rotifer density, we transferred the zooplankton to new test jars containing 0, 15, 30, 60 or 120 µg L^{-1} of herbicide and alga ($0.25 X 10^6$ cells mL^{-1}). When the density of *B. patulus* in the test jars reached stationary or declining phase (i.e., after 15 days), the experiments were discontinued.

Based on the data collected, we obtained peak population densities and rate of population increase (r). The r was derived using the following formula (16):

$r = (\ln N_t - \ln N_0)/t$, where, N_0 = initial population density, N_t = density of population at time t in days.

Differences in the peak population densities and the growth rates were statistically evaluated using two-way ANOVA and Tukey tests for multiple comparisons (Statistica ver. 5, StatSoft, Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

Regardless of the presence of asplanchnin, the population density of *B. patulus* decreased with increasing concentration of triasulphuron in the medium (Fig. 1). However, at any given concentration of herbicide, when asplanchnin was present in the medium, *B. patulus* showed higher population growth. The rotifer population was drastically decreased when grown on herbicide at 120 µg mL^{-1} concentration, regardless of the presence of allelochemical in the medium.

The peak population densities of *B. patulus* decreased with increasing concentration of triasulphuron in the medium (Fig. 2). Statistically, the concentration of herbicide, presence of asplanchnin had a significant impact on the peak population abundances of *B. patulus* ($p<0.01$, 2-way ANOVA). The interaction of toxicant concentration X presence of asplanchnin was not statistically significant ($p>0.05$).

Population growth rates varied from +0.23 to -0.13, depending on the presence or absence of asplanchnin and the concentration of herbicide in the medium (Fig. 3). The growth rates were negative at the herbicide concentration of 20 µg mL^{-1}. Two way

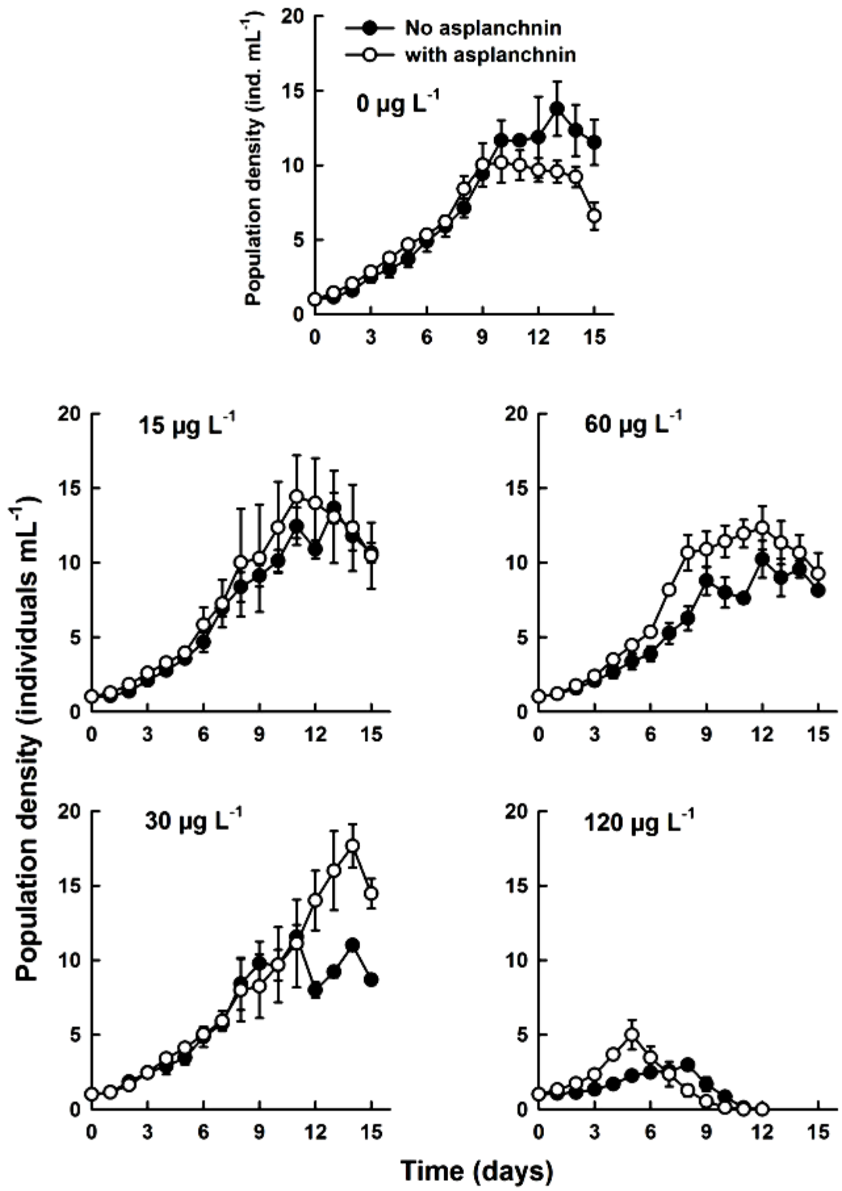

Figure 1. Population growth curves of *B. patulus* grown at 0.25 X 10^6 cells mL^{-1} of *Chlorella vulgaris*, with and without the presence of asplanchnin and under different concentrations (µg L^{-1}) of the herbicide triasulphuron. Values represent mean±standard error based on three replicates.

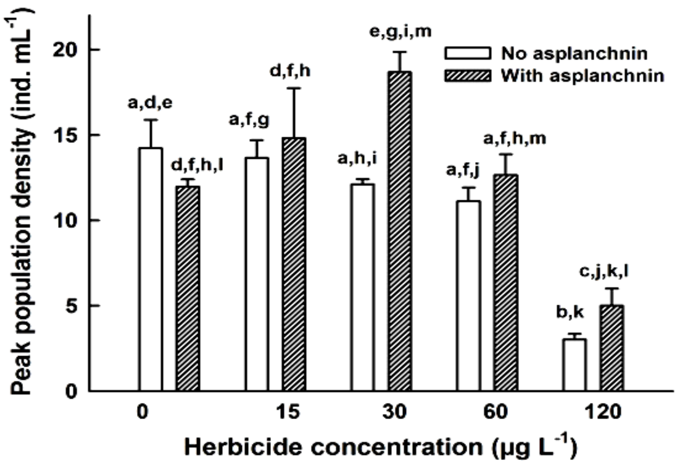

Figure 2. Peak population abundances (individuals mL^{-1}) of *B. patulus* grown at 0.25 X 10^6 cells mL^{-1} of *Chlorella vulgaris*, with and without the presence of asplanchnin and under different concentrations (µg L^{-1}) of the herbicide triasulphuron. Values represent mean±standard error based on three replicates. Data bars carrying identical alphabets are not significantly different (p>0.05 Tukey test).

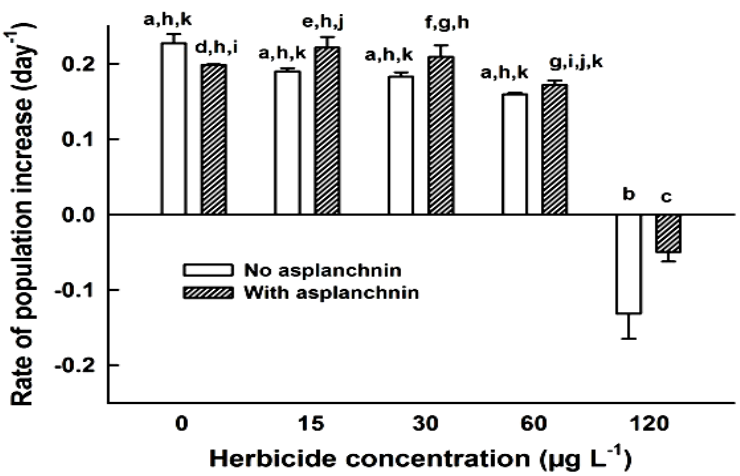

Figure 3. Rate of population increase (day^{-1}) of *B. patulus* grown at 0.25 X 10^6 cells mL^{-1} of *Chlorella vulgaris*, with and without the presence of asplanchnin and under different concentrations (µg L^{-1}) of the herbicide triasulphuron. Values represent mean±standard error based on three replicates. For the different treatments the data bars carrying similar alphabet are not significantly different (p>0.05 Tukey test).

ANOVA showed that presence of allelochemical or the concentration of herbicide had a significant effect on the *r* ($p<0.01$). However, their interaction was not significant ($p>0.05$).

The adverse affect of herbicides on the rotifer populations as reported in earlier studies was also observed in our work (15). Thus, as the herbicide concentration in the medium was increased, there was a reduction in both the peak population density and the rate of population increase. Triasulphuron at 120 µm mL^{-1} did not support *B. patulus* for long and the rotifer population decreased drastically after about a week. Not much is known about the impact of triasulphuron on the population growth of zooplankton in literature. However, acute toxicity tests showed that the cladoceran *Daphnia magna* showed 50% mortality, when exposed to triasulphuron at 48 mg L^{-1} (19). Our study showed that *B. patulus* was very sensitive to triasulphuron. Because firstly, in most acute toxicity tests on zooplankton algal diet is not added to the experimental containers (20) and hence, herbicides, which are designed specifically for the plants, do not affect zooplankton at low concentrations. In our study, we added algal food to the test jars so that population grows for evaluating the chronic effects. Consequently, in the test jars the green alga *Chlorella vulgaris*, which is also very sensitive to triasulphuron (19), was possibly first adversely affected and this could have influenced the rotifer populations in the jars. There is some information on the biosorption of this herbicide on the alga cells (5) which is eventually transferred to zooplankton via food ingestion causing declined rotifer growth.

The differences in the peak population abundances and the growth rates of *B. patulus* in the herbicide concentrations when cultured on the medium with and without asplanchnin reflected the combined influence of asplanchnin and triasulphuron. Generally, in presence of asplanchnin, brachionid rotifers including *B. patulus* produce large-bodied individuals (6) and due to the high energetic requirements of large sized individuals, the population density becomes lower than the treatments containing no asplanchnin (10). We did not quantify the body size of *B. patulus* in this study, although while estimating the population abundances daily we observed relatively large individuals in treatments containing no herbicide but with asplanchnin as compared to those that did not receive asplanchnin. While toxicants causes a reduction in the body size of rotifers (14), asplanchnin induces production of large sized individuals (6). Consequently, the strategy of the population is to maintain an energetic balance between these two stresses. In this study the impact of asplanchnin possibly promoted the larger individuals, which better resisted the stress from triasulphuron compared to the smaller individuals from the population grown in at the comparable herbicide levels. Therefore, *B. patulus* when simultaneously exposed to stress from allelochemical of *Asplanchna* and the herbicide level showed higher population abundances and growth rates compared to the rotifers exposed only to triasulphuron. In addition, toxicants such as heavy metals, pesticides and herbicides under low levels caused enhanced offspring production compared to controls (2) and this enhanced offspring production under stressful conditions is called the hormesis. Thus, hormesis may be responsible for higher population abundances and growth rates of *B. patulus* in treatments containing triasulphuron as compared to the populations not exposed to the herbicide.

Regardless of the triasulphuron levels and the presence or absence of asplanchnin in the medium, in our study in controls the peak population density and the rate of population increase are within the range reported for this species in literature. For example, when cultured on *Chlorella* at density of 1.5 X 10^6 cells mL^{-1}, *B. patulus* has reached a peak population abundances of about 100 ind. mL^{-1} (13). In our study we used one-sixth of this food levels and consequently the peak population densities of *B. patulus* were about 20 ind. mL^{-1}. The rate of population increase of *B. patulus* varies from 0.17 to 0.24 per day depending on the food level (13). In this study, the growth rates of *B. patulus* are thus within the range known for this species.

CONCLUSIONS

Regardless of the presence of asplanchnin, the population density of *B. patulus* decreased with increasing concentration of triasulphuron in the medium. At any given triasulphuron concentration, *B. patulus* cultured in the presence of asplanchnin showed significantly higher peak abundances and higher growth rates compared to the populations cultured without asplanchnin. This implies infochemicals from *Asplanchna* have an indirect influence on the population abundances of the prey brachionids in ponds and lakes that receive effluents containing herbicides.

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