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Balance between herbicidal activity and toxicity effect: A case study of the joint effects of triazine and phenylurea herbicides on *Selenastrum capricornutum* and *Photobacterium phosphoreum*

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ABSTRACT

The use of herbicide mixtures has become a cost-effective strategy against the evolution of herbicide resistance to protect global food production. Much research has focused on investigating either the herbicidal activities or the toxicity effects of herbicides; however, few of them have investigated both factors. This study investigates the balance between herbicidal activity for *Selenastrum capricornutum* and toxicity effect toward *Photobacterium phosphoreum* by determining the joint effects of triazine (simetryn, atrazine, prometon and prometryn) and phenylurea (fenuron, monuron, monolinuron and diuron) herbicides. The results showed that among the four triazines, only simetryn exhibited a unique effect (formation of a pi–sigma bond with the D1 microalga protein and an H-bond with the Luc photobacterial protein); and among 16 triazine–phenylurea binary mixtures, only the mixtures containing simetryn resulted in TU_1 values (herbicidal activities of mixtures on *S. capricornutum*) >TU_2 values (toxicity effects of mixtures on *P. phosphoreum*). However, the other 12 mixtures, which did not contain simetryn, showed the opposite result ($TU_1 < TU_2$). A comparison of TU_1 with TU_2 showed that additive effects occurred more frequently for TU_1, whereas antagonism effects occurred more frequently for TU_2. Based on these results, a preliminary mechanistic hypothesis of herbicide mixtures was proposed. Meanwhile, some suggestions are provided firstly for herbicide combinations based on the balance between herbicidal activity and toxicity effect, which will encourage thoughtful efforts for how to best combine herbicides in a sustainable way.

1. Introduction

Resistance to the use of herbicides has gained interest over the past few decades because the current usage of herbicides poses huge challenges for the sustainability of food production. To prevent or delay herbicide resistance, the use of herbicide mixtures for weed control has been proposed as one of the most cost-effective strategies (Gressel and Segel, 1990). However, this strategy runs contrary to the views of many environmental scientists because chemical mixtures in the environment may lead to additive, synergistic or antagonistic effects on target organisms, and they thus pose a greater potential threat to environmental safety and human health than the individual chemicals (Fatima et al., 2007; Kim et al., 2006). It is therefore necessary and important to investigate how to balance the herbicidal activity and toxicity for herbicide mixtures, i.e., how to create herbicide mixtures that have maximal herbicidal activity but minimal toxicity effect.

A wealth of data has shown that the use of herbicide mixtures can improve herbicidal activity. For instance, herbicide mixtures containing mesotrione and atrazine control not only grasses but also broadleaf weeds on corn (*Zea mays*) (Armel et al., 2005, 2003; Johnson et al., 2002), and they increase velvetleaf necrosis from 18% to 47% at their optimal rate on sunflowers (*Helianthus annuus*) (Abendroth et al., 2006). Meanwhile, certain classes of herbicides are extensively used commercially, making them ubiquitous in the environment and adverse to non-target organisms. Many studies have focused on the toxic side effects of herbicide mixtures (Benitez et al., 2006; Cavieres et al., 2002;

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Pape-Lindstrom and Lydy, 1997) and found that synergistic effects occurred on non-target organisms. For example, Salazar et al., 2005 demonstrated that the exposure to 3,4-dichloropropionanilide and 2,4-dichlorophenoxyacetic acid in mice produced a worse toxic effect on the immune system response to a bacterial vaccine than the individual chemicals. Although this and similar studies have focused their attention on either the herbicidal activity or the toxicity effect of herbicide mixtures, no study has considered both cases, let alone proposed how to obtain a balance between them. This question raises important issues that we focus on in the present study; that is, we seek a combination method for herbicide mixtures that enhances weeding activity but reduces toxic side effects.

It is well known that molecular docking has become a successful method for providing information on toxicological mechanisms over the last decade (De Wilde et al., 2013; Sheikha et al., 2011). Through the use of molecular docking, our previous study investigated the toxicological mechanisms in the chronic toxicity of sulfonamide antibiotics at the level of the receptors on *Photobacterium phosphoreum* and provided new insight into the hormesis mechanism (Deng et al., 2012). Additionally, we expanded molecular docking to the field of mixture toxicity, and revealed both acute and chronic toxicological mechanisms of mixtures containing sulfonamide antibiotics and their potentiators on *P. phosphoreum* (Zou et al., 2012). Consequently, if molecular docking can be used in the field of herbicide mixtures, it may be a reliable tool for researchers to find an optimal way of mixing herbicides, taking into consideration both the weeding activity and the toxicity effect.

In the present study, triazines and phenylureas were evaluated as herbicides because they are in the top five herbicides using in China, and they account for 5% and 2% of the herbicide market, respectively (Agropages, 2013a, 2013b). Take atrazine for example: the annual sales reached 55,730.87 tons in 2011 (Duan, 2013). Meanwhile, the application of triazine–phenylurea mixtures has been promoted for the purpose of increased herbicidal activity, which positively affects the control over weeds in the main Chinese crop fields (maize and sorghum), such as crabgrass (*Digitaria sanguinalis*), goosefoot (*Chenopodium album*), green bristlegrass (*Setaria viridis*), etc. (Lei et al., 2007; Zhang and Chen, 1999). Furthermore, the herbicidal activity was determined using *Selenastrum capricornutum* (microalga) as the test organism, rather than by using conventional organisms. We choose this organism because research has suggested that the *S. capricornutum* is one of the most commonly tested species of alga and is also the most sensitive of the tested plant species (Fairchild et al., 1998). In addition, *P. phosphoreum* was employed to determine the toxicity of herbicides because it is used widely in toxicity assays to characterize chemical toxicity in the environment (Asami et al., 1996; Santos et al., 2004).

Therefore, the purposes of the present study are: (1) to determine the herbicidal activity (*S. capricornutum*) and toxicity effect (*P. phosphoreum*) of individual herbicides and their mixtures (triazine–phenylurea); (2) to reveal the possible joint effect mechanisms of the herbicidal activity and the toxicity effect; (3) and to propose suggestions for herbicide mixture compositions to maximize the herbicidal activity and minimize the toxicity effect.

2. Materials and methods

2.1. Test substances and microorganisms

Eight herbicides, including triazines (simetryn, atrazine, prometon and prometryn) and phenylureas (fenuron, monuron, monolinuron and diuron), were supplied by Sigma Co., Ltd. and used without repurifying (purity ≥99%). The Chemical Abstracts Service registry numbers (CAS-RN), IUPAC (International Union of Pure and Applied Chemistry) names, structures, molecular weights (MW) and logarithmic values of the partition coefficients (log Kow) of the four triazines and four phenylureas are provided in Table 1.

Due to its high sensitivity in photosynthesis, the freshwater microalga *S. capricornutum* was used here as a test organism for herbicidal activity. It was obtained from the Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, PR China and was maintained at 25 °C (1000 lx, 12 h:12 h light/dark cycle).

Due to its wide use for the aquatic toxic bioassay, the freeze-dried freshwater bacterium *P. phosphoreum* (T3 mutation) was used as a test organism for toxicity effect. It was supplied by the Institute of Soil Science, Chinese Academy of Science, Nanjing, PR China. It was reconstituted and maintained on fresh agar slants at 4 °C.

2.2. Chemical stability

Experimental conditions, e.g., light, temperature and period, can make chemicals unstable, which may cause them to corrode, decompose, polymerize, etc. Hence, stability analyses are developed here. The stabilities of the chemicals over the 96 h test period were determined using a Thermo Evolution 201 UV/Vis spectrophotometer (Thermo Fisher Scientific Inc., Shanghai, PR China) under the exact herbicidal activity assay conditions (illustrated in Section 2.3) on *S. capricornutum*. The changes in the UV absorbance before and after 96 h (the experimental period of the herbicidal activity assay on *S. capricornutum*) were used as indicators of the chemical stability (Zhu et al., 2009). The absorbance changes of the chemicals after 96 h were represented as the solute loss (see Supporting Information, Table S1). Because the solute loss for all of the substances was less than 7%, all of the dose responses were calculated using the nominal concentrations.

The stabilities of the test chemicals over the experimental period were not considered for the toxicity effect assay on *P. phosphoreum* because it was performed within a short period (15 min) at 22 °C in daylight.

2.3. Herbicidal activity assay on S. capricornutum

Herbicidal activity assays were conducted by determining the growth inhibition in the algal biomass as a result of exposure to the test herbicides or herbicide mixtures after a duration of 96 h, when the microalga *S. capricornutum* is in an exponential phase of growth. Experiments were conducted in 100 mL Erlenmeyer flasks containing 30 mL of the sterilized culture medium (see Supporting Information, Table S2). Then, the samples were diluted to approximately 2×10^4 cells mL^{-1} in each flask on a shaking incubator at 22 ± 1 °C and were subjected to 12 h:12 h light/dark (2500–3000 lx, cool-white fluorescence). A seven-step concentration series (see Supporting Information, Table S3) in geometric grade ranging from no effect to 100% growth inhibition was distributed among the flasks. The culture media without the test herbicides served as control. Triplicate measurements were performed simultaneously for each treatment. The data were expressed as the effective concentration and its 95% confidence intervals at 50% response. Based on the measurements of the algal biomass, the decrease in the algal density was used as a result of the exposure to the test herbicides. The herbicidal activity was quantified, and the median effective concentration (EC_{50}) was calculated using the probit model (Tian et al., 2013) ($I = a \times \log C + b$, where I and C denote the inhibition and concentration, respectively, and 'a' and 'b' denote the slope and the intercept) and reported as $\log(1/EC_{50})_{96\,h}$ in units of mol/L.

After obtaining the EC_{50} values of the individual herbicides, the activities of the herbicide mixtures were conducted in a similar manner as the individual herbicide tests. Sixteen binary mixtures containing one triazine and one phenylurea herbicide were tested at equal toxic ratios (i.e., identical fractions of EC_{50}). The herbicide mixtures were then combined using the initial individual chemical