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Effects of sublethal herbicides on offspring germination and seedling growth: Redroot pigweed (*Amaranthus retroflexus*) vs. velvetleaf (*Abutilon theophrasti*)

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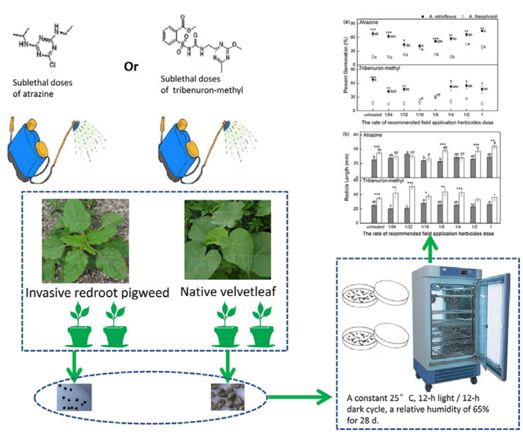
HIGHLIGHTS

- The first study to compare the offspring of invasive and native plants following sublethal exposure of parent to herbicides.
- Sublethal atrazine and tribenuron-methyl had effects on the germination and seedling growth of the F1 generation of plants.
- The response of the F1 generation of invasive plants and native plants to herbicides was different.
- The herbicide inhibition effect on the F1 velvetleaf and redroot pigweed did not increase as the sublethal dose increased.

GRAPHICAL ABSTRACT



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ABSTRACT

The effects of sublethal doses of herbicides on plants cannot be ignored, yet little is known about the effects of sublethal doses of herbicides on the F1 generation of plants. Seed germination and seedling growth of native and invasive plants following the sublethal exposure of parent plants to herbicides were comparatively analyzed in this study. Sublethal atrazine and tribenuron-methyl had carry-over effects on the germination and seedling growth of the F1 generation of invasive redroot pigweed (*Amaranthus retroflexus* L.) and native velvetleaf (*Abutilon theophrasti* Medicus), both of which had different responses to the carry-over effects of sublethal herbicide. The germination percentage of the F1 redroot pigweed (decreased) was greater than that of the F1 velvetleaf (increased or not significantly changed) following parental exposure to atrazine or tribenuron-methyl. Atrazine reduced the radical growth of 7-day-old velvetleaf seedlings and decreased the difference in seedling length between velvetleaf and redroot pigweed, while tribenuron-methyl had no significant effects on the growth of 7-day-old velvetleaf seedlings. The herbicide inhibition effect on the germination and growth of F1 velvetleaf and redroot pigweed did not increase as the sublethal dose increased. This study suggests that carry-over effects of sublethal herbicides weaken the growth advantage of the F1 velvetleaf at the seedling stage and may have a more negative influence on progeny population development of native velvetleaf compared with invasive redroot pigweed.

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1. Introduction

Herbicides are widely used in farmlands around the world to raise crop yields and increase farm labor efficiency (Gianessi, 2013). The global area cultivated with genetically modified herbicide-resistant crops has dramatically increased leading to greater herbicide use (Liang et al., 2005; Davis et al., 2012). There is no acceptable weed control substitute for herbicides in current crop production (Keller et al., 2014). In China, the arable field area treated with herbicides has increased at an annual rate of 2×10^6 hm^2, and it now exceeds 0.6×10^8 hm^2 (Zhang et al., 1998; Zhang, 2004; Hong et al., 2011). Several wild plant species in cropland areas are almost extinct or regionally extinct in China (Guo and Li, 1996), and similar situations have occurred in other countries (Grundy et al., 2011; Rassam et al., 2011). In addition, wild plant diversity has been reduced in both cropland and non-crop habitats of agricultural lands (Jose-Maria et al., 2011; Schmitz et al., 2014a).

The ecological risk of herbicides on native plant survival, seed production, long-term seedbank replenishment and eventual species composition has not been adequately assessed (Boutin et al., 2014; Schmitz et al., 2015). Fortunately, much attention has been paid to the effects of sublethal doses of herbicides on non-target terrestrial and wetland plants through drift, runoff and/or volatilization, in agricultural plant ecosystems (Carpenter and Boutin, 2010; Olszyk et al., 2013; Olszyk et al., 2017; Reeg et al., 2017). Many studies have focused on the sublethal herbicide effects on the biomass and reproduction of native plants (Pan et al., 2009; Carpenter and Boutin, 2010; Carpenter et al., 2013; Boutin et al., 2014; Rotches-Ribalta et al., 2015), while there has been limited information on the effects of sublethal dose of herbicides on plant progeny (Tanveer et al., 2009; Qi et al., 2016).

Most of the studies on the ecological risk of herbicides were based on the individual plant level, although the risk assessment of herbicides is gradually developing specific protection goals on the population and community level (EFSA, 2014). Studies at the individual level are still the basis for analyzing the effects of herbicides on plant species interactions (Reeg et al., 2017). Comparing the responses of two plant species provides a better understanding of the impact of herbicides (Damgaard et al., 2008; Carpenter et al., 2013). Moreover, invasive plants that compete with native plants for light, nutrients, and moisture negatively affect native communities (Harvey and Fortuna, 2012), and both invasive plants and native plants in agro-ecosystems are often affected by herbicide applications. Thus, studies are needed to compare the response of native plants and invasive plants to herbicides in order to better understand the ecological risk of herbicides, as there are few studies on this subject.

We studied the effects of two commonly used herbicides in China on the F1 generation of velvetleaf (*Abutilon theophrasti* Medicus) and redroot pigweed (*Amaranthus retroflexus* L.), which often co-occur in fields and orchards in China. Velvetleaf is a native plant of China, while redroot pigweed is a noxious invasive plant in China. Previous studies have found that redroot pigweed and velvetleaf both can evolve resistance to atrazine (Anderson and Gronwald, 1991; Jing et al., 2014). Therefore, redroot pigweed and velvetleaf were collected from parent plants growing in an herbicide-free area in this study, in order to avoid different viability of the resistant plant and the sensitive plant under the effect of herbicide (Vila-Aiub et al., 2009). The major objectives were to: (i) evaluate possible effects of sublethal atrazine and tribenuron-methyl on the offspring of velvetleaf and redroot pigweed, (ii) evaluate differences in F1 generation germination and seedling growth responses between velvetleaf and redroot pigweed following sublethal exposure of parent plants to herbicides, and (iii) determine if increased sublethal dose exposure to the parent plant increases toxic effects on the F1 generation of velvetleaf and redroot pigweed.

2. Materials and methods

2.1. Plant material

Velvetleaf (*A. theophrasti*), an annual subshrublike species native to China, 1–2 m tall, is a member of the family Malvaceae (Editorial Committee of Flora of China, 2007). It is a useful plant serving as a trap plant of *Bemisia tabaci* in cabbage, cotton, and soybeans (Jian et al., 2006; Tan et al., 2011), and as a source of high quality natural cellulose fibers (Reddy and Yang, 2008), and it has medicinal value (Matlawska and Sikorska, 2005; Kunming et al., 2015). Redroot pigweed (*A. retroflexus*), a common annual C4, monoecious dicotyledonous weed, 1–1.5 m tall, is a member of the family Amaranthaceae (McWillia et al., 1968; Ghorbani et al., 1999). It is one of the world's worst weeds (Horak and Loughin, 2000; Costea et al., 2004), and it has been listed on *the third list of the China exotic invasive species* (http://www.zhb.gov.cn/gkml/hbb/bgg/201408/t20140828_288367.htm). Seeds of velvetleaf and redroot pigweed, used for the outdoor pot experiment, were collected from parent plants growing in an herbicide-free area at the experiment station of Chinese Research Academy of Environmental Sciences.

2.2. Culture conditions

The outdoor pot experiment was carried out at the Chinese Research Academy of Environmental Sciences located in Zhaoquanying, Shunyi District, Beijing, China (115.7°-117.4°E, 39.4°-41.6°N; 20-60 MASL). On May 6, 2014, seeds of redroot pigweed and velvetleaf, were sown in 78 plastic pots per species outdoors at a rate of approximately 10 seeds per pot. The potting soil used was Fluvo-aquic soil collected from an herbicide-free area at the experiment station of Chinese Research Academy of Environmental Sciences. The pH of the soil is 7.5, and the soil organic carbon content is 6.93 g kg^{-1}. After seedlings had developed 2–3 true leaves, they were thinned to one per pot. Seedlings were watered daily. All plants were supplemented with 50 mL of a prepared solution consisting of 2.5 mL L^{-1} of 20-20-20 "All Purpose Plant Food fertilizer" (America chemcore biochemistry technology group CO.,LTD.) at approximately 30 d and 60 d after seedling emergence (Carpenter and Boutin, 2010).

2.3. Herbicide treatments

This experiment used two herbicides, commonly used in China, with different chemistry and modes of action. Atrazine (2 Chloro-4-ethylamino-6-isopropylamino-1, 3, 5 triazine) (GREEN LAND® Shandong shengbang greenland Chemical Co., Ltd., China) binds to the plastoquinone binding site (Qb) in the photosynthetic electron transport system and inhibits photosynthesis (Arntz et al., 1998). Atrazine is a triazine herbicide used for soil and leaf treatments, and atrazine is persistent in the environment (Li et al., 2012). The recommended application rate of atrazine in north China is 1200 g ai ha^{-1}. Tribenuron methyl (methyl 2-[4-methoxy-6-methyl-1, 3, 5-triazin-2-yl (methyl) carbamoylsulfamoyl] benzoate) (QCC® Shandong Qiaochang Chemical Co., Ltd., China) inhibits acetolactate synthase (ALS), a key enzyme in the biosynthesis of branched chain amino acids (Tang et al., 2012). It is rapidly absorbed by plant leaves (Duman et al., 2010). The recommended application rate of tribenuron-methyl in north China is 22.5 g ai ha^{-1}. For each of the two herbicides, six concentrations were tested: 1/2, 1/4, 1/8, 1/16, 1/32 and 1/64 of the recommended field application concentrations (RFAC) (i.e. full strength). No additional surfactants or other adjuvants were used in the treatments.

At 30 d postemergence, redroot pigweed with 12–14 true leaves (20 cm) and velvetleaf with 8–10 true leaves (30 cm) were sprayed with different rates of atrazine (600,300,150,75,37.5,18.75 g ai ha^{-1}) or tribenuron-methyl (11.25,5.63,2.81,1.41,0.70,0.35 g ai ha^{-1}) using a manual sprayer with a cone-shape spray nozzle (Worth® NS-5,