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Root to shoot transfer and distribution of endosulfan in the wetland macrophyte *Bidens laevis* L.

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Abstract- Endosulfan (EDS) is genotoxic in somatic cells of *Bidens laevis* and reproduction could be affected if translocated from roots to flower buds. Hydroponic experiments were conducted to quantify this transfer. While the root uptake of [^{14}C] EDS and its transfer to aboveground tissues was relatively low, the resulting average flower buds concentration (1.01 ± 0.76 ng/g) after 30 d of exposure to an aqueous concentration of 5 µg/L could still represent a genotoxic risk for germ cells.

Key words- Endosulfan, *Bidens laevis,* Transpiration stream concentration factor, Bioconcentration factor, Wetland macrophyte

INTRODUCTION

Endosulfan (EDS) is an organochlorine insecticide extensively used throughout the world on food and non-food crops [1]. Despite recommendations for banning its application starting in 2012 [2], several countries including Argentina have extended its use until July 2013 [3]. Endosulfan has been detected worldwide in soils, sediment, invertebrates, fishes and macrophytes [4,5] and in run-off water from agricultural fields at concentrations as high as 100 μg/L [6]. The toxic effects of this compound have been demonstrated in various animals [7-9] and in aquatic and wetland macrophytes [10,11].

Because of the frequent association between wetlands and agricultural lands and their ability to accumulate agrochemicals, submerged and emergent macrophytes are used as *in situ* bioindicators of water quality [12]. They comprise an important component of benthic primary production in wetlands providing oxygen, nutrient cycling, sediment stabilization, habitat and shelter for aquatic life [13]. *Bidens laevis* is a common macrophyte with an extensive distribution throughout the Americas, including the USA, Mexico, Colombia, Chile, Uruguay and Argentina [14]. Like *Bidens cernua* in Canada, this species is a representative plant in many of the ecologically important areas [15] that are repeatedly exposed to agrochemicals [16]. In Argentina, *B. laevis* inhabits marsh and stream edges [17] in several provinces, including Buenos Aires Province, where extensive agricultural activities occur. Previous studies have shown that EDS is genotoxic to mitotic chromosomes from somatic root cells of *B. laevis* [11]. However, meiotic chromosomes of germ cells are generally 10 times more susceptible to breakage than mitotic chromosomes [18] and sublethal effects, like somatic and hereditable mutations, occurring on a few plant species may have repercussions at the community or ecosystems levels [19]. If EDS is translocated from roots to flowers of *B. laevis*, germ cells could suffer adverse effects potentially impacting the

125 reproductive success of natural populations. Thus the focus of this study was to examine the
126 potential root uptake of [^{14}C] EDS and the subsequent transfer to flower buds by *B. laevis.*
127
128 **MATERIALS AND METHODS**
129 *Chemical*
130 Endosulfan, [2, 3 ^{14}C] (specific activity 1.83 mCi/mL, radioactive purity > 95%),
131 hereafter [^{14}C] EDS, was purchased from the Institute of Isotopes Co., Ltd (Budapest, HU) as
132 a mixture of the α- and β- isomers (α-endosulfan, CAS:959-98-8 and β-endosulfan, CAS:
133 33213-65-9) dissolved in acetonitrile. It was used as received from the vendor without any
134 additional purification.
135 Environmentally relevant physical-chemical properties of the α- and β- isomers of
136 endosulfan (molecular weight 406.9 g/mol) respectively at 25º C are: octanol-water partition
137 coefficients (log K_{ow}) 4.74 and 4.79, aqueous solubilities 0.0063 and 0.089 mol/m^3, vapor
138 pressures 0.0044 and 0.0040 Pa and Henry's law constants 0.70 and 0.045 Pa m3/mol units
139 [20, 21].
140
141 *Plants*
142 Seeds of *Bidens laevis* were collected in La Brava lake (37º 53' South, 57º 59' West),
143 Argentina and were sent to the Research Greenhouse at Utah State University (Logan, USA)
144 after obtaining legal authorization from the USDA (United State Department of Agriculture)
145 and SENASA (Argentinean National Service of Agricultural Health and Quality).
146 Seeds were placed in a plastic box with a damp filter paper for germination at 20ºC.
147 Rooted seedlings were transferred into pots containing a mixture of 50% vermiculite and
148 50% peat and grown within a controlled environment chamber (12- h light at 25˚C and 12-h
149 dark at 20˚C) for 1 mo. The plants were then transferred to a hydroponic environment and

grown in a greenhouse (16-h day at 25 ºC and 8-h night at 20˚C) for 3 to 4 wk prior to the start of the exposure study.

Experimental design

Five plants, selected for size uniformity, were transplanted into individual 1.8 L glass jars containing a complete nutrient solution [22] and fitted with aeration tubes. A 10 cm thick closed-cell foam seal was used to provide support for the plants. The jars also served as the root zone exposure chambers for the uptake experiment. The jars were placed in a constantly ventilated greenhouse with no humidity control. Conditions during the experiment were summer natural photoperiod and temperature (16-h day at 25 ± 5˚C and 8-h night at 18± 5˚C).

A single dose of [^{14}C] EDS dissolved in acetonitrile was added to four of the jars to yield an initial nominal exposure concentration of 5 μg/L. This concentration was selected because it was the lowest concentration that caused genotoxicity effects in *Bidens laevis* during a previous study [11]. The fifth jar was used as an untreated control, no added EDS or acetonitrile. Pumps were used to draw atmospheric air through the root zone chamber at a flow rate between 40 and 50 mL/min to maintain adequate mixing and oxygen levels in the root zone. The air exiting the root zone was directed through charcoal traps to reduce volatilized EDS from entering the greenhouse atmosphere.

The nutrient solution in the root zone jars was replenished daily to replace the water lost due to transpiration. Root zone solution samples were collected and analyzed every 1 to 2 d throughout the duration of the studies. The amount of [^{14}C] in the root zone solution samples was determined directly by liquid scintillation counting (Beckman LS1701, Beckman Instruments) after adding 2 to 3 mL of sample to 7 mL of Ready Gel scintillation cocktail (Beckman Instruments). Based on the results of preliminary kinetic experiments,

[^{14}C] EDS was added to the dosed systems daily to maintain the root zone concentrations at 5 µg/L.

Collection of xylem sap

After 30 days of exposure, the plants were cut at the base of the stem while keeping roots in the root zone chamber. The tissues were immediately processed for [^{14}C] analysis as described in next section. Xylem sap was collected as it exited the stem using 1 ml disposable polypropylene syringes. A 1 to 2 mL sample of xylem sap was needed to collect enough [^{14}C] for direct liquid scintillation counting.

[^{14}C] tissue distribution

The concentrations of the [^{14}C] equivalents of EDS within the various plant tissues (leaves, steam, flower buds, bracts and roots) were determined by combusting of triplicate samples of tissue (1 to 2 g wet weight) at 900ºC using a biological oxidizer (R.J. Harvey Model OX-600). Prior to combustion, the tissues were cut into small pieces with a stainless steel knife or scissors and thoroughly mixed. The evolved [^{14}C] CO_2 was collected in a solution of 50% Ready Gel, 40% methanol, 10% monoethanolamine and analyzed directly by liquid scintillation counting.

Root lipid content

The lipid content of the *Bidens laevis* roots was determined by soxhlet extraction procedure described by Dettenmaier (2008) [23]. Data are presented as in lipid percentage by wet weight root tissue.

RESULTS AND DISCUSION

Plant transpiration and growth

During the 30 d exposure period, no difference in plant growth and transpiration rates was observed between exposed plants and the control. Average shoot length increased from 20 to 130 cm and the average amount of water transpired was 35 L. No phytotoxicity (i.e. necrosis or chlorosis) was observed in any of the plants.

Additional considerations

The biotransformation of EDS within plants yield several metabolites including the corresponding sulfate, diol, ether, and lactone [24]. Due to [^{14}C] analytical methodology used, we cannot discriminate between EDS and metabolites. However, depending on the metabolite(s) formed, the toxicological risk may be increased. For example, EDS-sulfate is more toxic and more persistent than the α– and β- isomers [25].

Root uptake and transfer to shoots

Experimental data quantitatively describing the extent of chemical uptake by plants roots are often expressed as bioconcentration factors (BCFs) or ratios of chemical concentrations in the plant (e.g., roots, shoots, xylem sap) to that in the exposure medium (soil, soil pore water, hydroponic solution) measured at the time the samples are collected. The terminology used can vary depending on the type of plant tissue analyzed. For example, the ratio between the chemical concentration in the roots and that in the exposure media (water or soil) is referred to as Root Concentration Factor (RCF) [26,27]. The transpiration stream concentration factor (TSCF) specifically describes the ratio of the contaminant concentration in the xylem sap to that in water taken up by the root.

Bioconcentration factors for each tissue type (leaves, flower buds, bract, stem and roots) were calculated from the ratio between the [^{14}C] concentrations in the tissue ([^{14}C]

equivalents of EDS) divided by the average [^{14}C] concentration in the root zone samples collected over the last 48 h of the exposure period. A TSCF was calculated using the [^{14}C] concentration in the xylem sap divided by the average [^{14}C] concentration in the root zone samples collected over the last 48 h exposure period, the period of time where the concentration in the root zone solution was close to the nominal concentration.

Lipophilicity, often described by the octanol-water partition coefficient (K_{ow}), is thought to be the most important physicochemical property governing root uptake and translocation to aboveground tissues for neutral organics [28,29]. The log K_{ow} of the EDS α- and β- isomers are 4.74 and 4.79, respectively [20] suggesting a relatively low translocation potential. The concentrations of EDS and BCFs in the various plant tissues and xylem sap are summarized in Table 1. The highest concentration of EDS was found in the roots yielding a RCF of 148.00 ± 26.50 mL/g. This compares favorably with the RCF value of 142 predicted from log K_{ow} using the relationship by Briggs et al. (1982) [26]. The lipid percent in roots was 0.058% on wet weight basis. That is similar to other species like soybean (0.047%) and tomato (0.062%) [23].

Transfer from roots to shoot was minimal with the stems, leaves and flowers having concentrations roughly 150 times less than found in the roots (Table 1). The average TSCF calculated from [^{14}C] EDS measurements in the xylem sap and hydroponic exposure solution was 0.14 ± 0.02 mL/g. This compares favorably to the TSCF value of 0.1 estimated from Log K_{ow}, [29] suggesting that the model previously developed using terrestrial plants could be extended to wetlands macrophytes.

No [^{14}C] EDS was detected in the control plant xylem sap. However, [^{14}C] was detected in the foliar tissue of the control plant (0.18 ± 0.01 ng [^{14}C] equivalents of EDS/g fresh plant tissue) suggesting EDS volatilization from the nutrient solution followed by deposition/sorption to the leaves and/or the uptake [^{14}C] CO_2 generated from the exposed to

the control plant [30]. This fact is possible because the isomers of EDS are semi-volatile, with vapor pressures of 0.0044 and 0.0040 Pa for the α- and β- isomers of EDS, respectively making them susceptible to volatilization to the atmosphere with subsequent atmospheric transport and deposition [21].

Based on the EDS measure in the control plant, a fraction of EDS measured in the leaves and flower buds of exposed *B. laevis* plants could come from the deposition of EDS volatilized from the hydroponic solution. Assuming that the EDS in control leaves comes only from volatilization/ deposition (0.18 ± 0.01 ng/g), the percentage due to volatilization/ deposition in EDS treated plants (1.10 ± 0.56 ng/g in leaves and 1.01 ± 0.76 ng/g in flower buds) would be estimated to be about 10%.

In the present study the transfer of EDS into the flower buds was minimal, 1.01 ± 0.76 ng [^{14}C] equivalents/g fresh plant tissue (Table 1). Although we do not know the genotoxic concentrations for each tissue, the concentration detected still could represent a potential genotoxic risk for germ cells in chronic exposures. This hypothesis is based on the significant increase of aberrations frequency observed in the genotoxicity assays with somatic root cells of *B. laevis* exposed during 48 h to environmentally relevant concentrations from 1 to 100 μg/L EDS (acute exposure) [11]. Therefore, chronic exposure could be a worse scenario because deleterious effects could accumulate affecting the development and growth, influencing the energetic metabolism and the reproductive success of the populations [19,31].

CONCLUSION

The present study showed that the EDS or its metabolites can translocate from root to flowers in *B. laevis* resulting in flower concentrations that are a potential genotoxic risk. With the conservation of genetic diversity emerging as one of the central issues in conservation biology, evaluation of acute and chronic effects in germinal cells (i.e. chromosome

aberrations and/or pollen viability assays) should be included in future risk assessment studies.

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Table 1: Concentration of [^{14}C] EDS in tissues and xylem sap of *Bidens laevis*

	EDS concentration [a]	BCF [b]
Leaves	1.10 ± 0.56	0.28 ± 0.14
Flower buds	1.01 ± 0.76	0.26 ± 0.19
Bract	1.01 ± 0.37	0.26 ± 0.09
Stem	0.31 ± 0.29	0.08 ± 0.07
Roots	575.00 ± 103.00	148.00 ± 26.50
Xylem sap	0.56 ± 0.06	0.14 ± 0.02[c]

[a] ng [^{14}C] equivalents of EDS/ g fresh plant tissue

[b] [^{14}C] equivalents of EDS concentration/ average solution phase concentration

EDS: endosulfan

BCF: Bioconcentration Factor (mL/g)

[c] Transpiration Stream Concentration Factor (TSCF) value.

All values are average ± standard deviation.