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A STUDY OF THE ROLE OF MICROMETEOROLOGICAL CONDITIONS ON UPTAKE OF 3,4-DICHLOROANILINE IN MAIZE

DROULIA F.E.[1,*]
MATSOUKIS A.S.[1]
CHRONOPOULOU-SERELI A.G.[1]
GIANNOPOLITIS C.N. [2]
TSIROS I.X. [1]

[1]*Department of Crop Sciences, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece*
[2]*Department of Weed Science, Benaki Phytopathological Institute, St. Delta 8, 14561 Kifissia, Greece*

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*to whom all correspondence should be addressed:
e-mail: fdroulia@aua.gr

ABSTRACT
3,4-dichloroaniline (3,4-DCA) is a hardly biodegradable and hydrolysable compound, characterized as a persistent pollutant for water, soil and sediment and highly toxic for living organisms. In this work, the impact of different micrometeorological conditions on uptake of 3,4-DCA was investigated in maize (*Zea mays* L.) plants grown hydroponically. For this purpose, seedlings of a uniform vigor were developed in appropriate nutrient solution supplied with an initial amount of 3,4-DCA (growth solution) under controlled high light (HL) and low light intensity (LL) conditions and under high (HH) and low relative humidity (LH) conditions in the combinations of HL/HH, LL/HH and HL/LH. Plants grown under HL/LH were replenished with the initial amount of 3,4-DCA after 48 h from the application of the growth solution (AGS). The measurements which took place were related to the uptake of 3,4-DCA and of growth solution by plants. Also, the fresh weight of plants was measured.
Results showed that maize was capable of removing noticeably high amounts of 3,4-DCA (up to three quarters of the initial amount) from the growth solution after completion of the first 24 h period from AGS, irrespective of micrometeorological conditions. It was also demonstrated that almost the whole available amount of 3,4-DCA was removed from the growth solution after a 48 h period from AGS under HL/LH. Plants under these conditions removed a significantly higher volume of growth solution compared to the HH conditions, irrespective of measurement time. The significant increase of the 3,4-DCA uptake (almost the whole available amount) by maize plants which were replenished with this compound under LH compared to HH, indicated a considerable capability of these plants to remove high concentrations of 3,4-DCA from the growth solution after 72 h from AGS.
The fresh weight of maize plants under the examined micrometeorological conditions did not change significantly in the majority of the cases, as regards to different micrometeorological conditions. On the contrary, this plant parameter was significantly higher in 3rd compared to 1st measurement day in all examined conditions. The increase of 3,4-DCA uptake rates by maize plants grown under low relative humidity conditions, 72 hrs from AGS, could be associated with the expected acceleration of the plants transpiration rates and with the plant growth rate, as expressed by the fresh weight, as in these conditions plants remove considerably high volume of growth solution.
The information obtained from the aforementioned results on plant uptake of 3,4-DCA in maize plants could be a first step in designing suitable management practices such as phytoremediation strategies which might reduce environmental pollution.

KEYWORDS: 3,4-dichloroaniline, uptake, *Zea mays* L., light intensity, relative humidity, hydroponic culture.

1. INTRODUCTION

Chloroanilines are basically originated from widely applied herbicides and they constitute products of their microbial metabolism in soil or of their metabolism in plants. 3,4-dichloroaniline (3,4-DCA) is the aromatic moiety and principal biodegradation intermediate of acylanilides e.g. propanil (Zeyer and

Kearney, 1982; Hirase and Hoagland, 2006), phenylureas e.g. diuron and linuron (Tixier *et al.*, 2002; Sørensen *et al.*, 2009) and phenylcarbamates e.g. swep (Bartha and Pramer, 1969; Kaufman and Blake, 1973). The aforementioned substances are herbicides of financial importance widely used in horticulture in various countries including Greece. Water and soil are affected in a high degree by 3,4-DCA due to its physicochemical properties and modes of emission (European Commission, 2001). The toxic and genotoxic effects of 3,4-DCA on various aquatic and terrestrial organisms are well known and documented, e.g., negative effects of 3,4-DCA on growth, reproduction and survival parameters of representative genders of the crustaceans (Klüttgen *et al.*, 1996; Pascoe *et al.*, 2000; Oda *et al.*, 2007), reduction of population, biodiversity, colonization as well as elimination of sweet water and ocean invertebrates (Taylor *et al.*, 1994; Schmitz and Nagel, 1995), peroxidation of vital organs (Li *et al.*, 2003; Monteiro *et al.*, 2006), adverse effects on growth and reproduction of fish even in very low 3,4-DCA concentrations (Schäfers and Nagel, 1991) and on changes of specific biochemical and cellular blood parameters of male Wistar rats (Guilhermino *et al.*, 1998).

By contrast, there is substantial lack of information on the 3,4-DCA uptake ability of various plant species and particularly of plants developed under hydroponic culture conditions. Most studies focus on the metabolism mechanisms of 3,4-DCA in plant cell suspension cultures (Schmidt *et al.*, 1994), in individual organs (Schmidt *et al.*, 1995) and more rarely in whole plants (Lao *et al.*, 2003; Brazier-Hicks *et al.*, 2007) where the formation of 3,4-DCA conjugates as well as of 3,4-DCA non extractable-insoluble bound residues predominates. The existence of 3,4-DCA metabolism and bonding in plants gives helpful signs on the manipulation of plant species which are able to tolerate and transform 3,4-DCA and consequently remediate this organic contaminant from water and soil.

Phytoremediation is a promising technique based on the ability of several green plants to remove, contain, or render harmless an environmental contaminant (Cunningham and Berti, 1993). This technique uses natural processes to break down, stabilize or accumulate environmental pollutants (Pilon-Smits and Freeman, 2006). Maize (*Zea mays* L.) is the third most important cereal crop cultivation in the world after *Triticum* sp. and *Oryza* sp. cultivations. The maize plant is used for both human and animal nutrition (Papakosta-Tasopoulou, 2008) and shows considerable potential to remove dangerous environmental pollutants, e.g. hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) from both soil and hydroponic culture (Chen *et al.*, 2011). The purpose of this paper is to study 3,4-DCA uptake by maize under different environmental conditions. To our knowledge there is lack of information on this topic. The experimental plants were grown in hydroponic culture which allows the elimination of interfering soil factors that may impede root uptake.

2. MATERIALS AND METHODS

2.1. Chemicals

3,4-dichloroaniline, $C_6H_5CL_2N$ (purity 99%) and sulfamic acid, H_3NO_3S (purity 99.3%) were purchased from Fluka Chemicals, sodium nitrite, $NaNO_2$ (purity 98%) from British Drug Houses Ltd, methanol, MeOH (analytical grade 99.9%) from Lab-Scan Analytical Sciences and N-(Naphthyl)ethylene-diamine-dihydrochloride ($C_{12}H_{16}Cl_2N_2$) from Merck.

2.2. Experimental Set-up

Seeds of maize (*Zea mays* L.) were germinated in plastic (filled with moist perlite) pots (38 x 15.5 x 12.5 cm) in a growth chamber with controlled environmental conditions, that is to say, a day/night temperature of 28/20 ^{0}C, relative humidity and light intensity of 80% and 24 Klux, respectively and a photoperiod of 14-hr in a 24-hr period. Seedlings of a uniform vigour were selected from the germination pots at the stage of the 1st leaf where the perlite was removed by the roots very carefully in a large beaker containing deionized water. After the removal of all root-bond perlite, roots were immersed in a large beaker containing 1‰ water solution of sodium hypochloride and immediately rinsed with deionized water. Excess solution was removed by gentle wiping on a soft towel paper and the seedlings were placed in plastic cups (7 cm height, 6 cm upper diameter) containing 80 ml of standard 1/4 (3 volumes of water to 1 volume of Hoagland's solution) strength Hoagland's solution (Hoagland and Arnon, 1950).

The maize seedlings were held on sponge plugs (applied to the cups' upper diameter) supporting their stems (2 plants per cup), and therefore only the roots were in contact with the solution. After the stay of seedlings for 3 days to the aforementioned environmental conditions, the sponge plugs with the seedlings were transferred to new cups containing 80 ml of the standard ¼ Hoagland's solution

and of the treatment solution with 3,4-DCA, (containing an initial amount of 3,4-DCA) after a rinsing of the roots with deionized water. Treatment solution had the concentration of 100 μM of 3,4-DCA (1296 μg of 3,4-DCA per cup).

The plants were grown in the growth solution (standard ¼ Hoagland's solution and the treatment solution) for a maximum period of 72 hrs under different micrometeorological conditions (table 1). Growth solution analysis was made every 24 hrs after the application of growth solution (AGS). Therefore, there were three analyses (1st, 2nd and 3rd day's after 24, 48 and 72 hrs, respectively, from AGS).The measurements which took place were related to the uptake of 3,4-DCA as well as the uptake of growth solution by plants. Also, the fresh weight of the plants (shoots and leaves) was measured.

The maize plants absorbed almost all the available 3,4-DCA in HL/LH conditions after the first 48 hrs from AGS. For this reason, in the aforementioned conditions, the remaining growth solution was replenished with the initial concentration of 3,4-DCA, after a rinsing of the roots with deionized water, up to the volume of 80 ml, after 48 hrs from AGS. Thus, in this case, 3rd day's analysis was performed in the basis of the new solution in which plants were grown for 24 hrs.

2.3. Quantitative Determination of 3,4-DCA Plant Uptake

The amount of the total 3,4-DCA taken up by the maize plants was calculated by subtracting the remained amount in the growth solution (medium) from that in the initial solution. After the medium removal from the roots, plants were gently withdrawn from the sponge plugs and weighed (shoots and leaves). The remaining medium was transferred into a volumetric cylinder and brought to the initial volume (80 ml) by the addition of deionized water.

Two replicates of 3 ml aliquots were taken from each cylinder and the concentration of 3,4-DCA was determined by the spectrophotometric method (Hoagland *et al.*, 1974) First, 0.5 ml of a freshly prepared 1% solution of $NaNO_2$ was added to the aliquot with immediate mixing. After 10 min, 1.0 ml of a 10% solution of H_3NO_3S, followed by 1.0 ml MeOH was added to the aliquot with immediate mixing. This mixture was allowed to stand an additional 10 min period followed by the addition of 0.5 ml $C_{12}H_{16}Cl_2N_2$. Fifteen minutes were allowed for colour development, after which the absorbance at 555 nm was determined. Concentration of 3,4-DCA was calculated by comparison of the absorbance to a standard curve. Plant uptake was then calculated by subtracting the determined concentration from the initial one.

Table 1. Micrometeorological conditions during maize growth

Maize	T[1] ($^0C_{day}/^0C_{night}$)	LI[2] (Lux)	RH[3] (%)	P[4] (hrs)	Designation
		24,000	80		HL/HH[5]
	28/20	10,000	80	14	LL/HH
		24,000	40		HL/LH

[1]T: Temperature, [2]LI: Light intensity, [3]RH: Relative humidity, [4]P: Photoperiod
[5]HL/HH: High light intensity/High relative humidity, LL/HH: Low light intensity/High relative humidity, HL/LH: High light intensity/Low relative humidity.

2.4. Statistical Analysis

The experiment was carried out according to the two-factor completely randomized design. The first factor (time of analysis) had three levels (1st, 2nd and 3rd day), and the second factor had three levels too, corresponding to the micrometeorological conditions (HL/HH, LL/HH and HL/LH). For the 3,4-DCA data, means were calculated for each experimental plant and used for the analysis of variance. The aforementioned analysis was repeated for the growth solution and fresh weight data. Where appropriate, means were compared using Tukey's HSD test. Statistical analysis was performed using SPSS version 8.0 for Windows. Results were considered to be significant at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

The uptake of 3,4-DCA and of growth solution by maize plants were significantly affected by both the time and micrometeorological conditions as well as by their interaction (table 2). The time and its interaction with micrometeorological conditions had a significant effect on the fresh weight of plants. Maize was capable of removing noticeably high amounts of 3,4-DCA (up to three quarters of the initial amount) from the growth solution after completion of the first 24 h period from AGS, irrespective of micrometeorological conditions (table 3). Maize plants grown under HL/LH conditions presented significantly higher uptake rates of 3,4-DCA compared to LL/HH and HL/HH in the majority of the cases. The increase of 3,4-DCA uptake under HL/LH was such that plants had taken up almost all of the available dichloroaniline from the second day. In this case, as already mentioned, a replenishment of the plants with the initial dichloroaniline concentration was made. It is interesting to note the considerably increased uptake of 3,4-DCA by maize plants at HL/LH conditions at the 3rd day's analysis (the plants had already been replenished with 3,4-DCA) where these plants accumulated almost the two-fold concentration of this dichloroaniline in relation to its concentration at the previous two days (table 3). On the other hand, plants grown under high relative humidity conditions (HL/HH and LL/HH) presented no significant change in the uptake of 3,4-DCA in most cases (1st and 2nd day).

As regards to the uptake of growth solution (GS) by maize plants, it was demonstrated that the plants absorbed significantly higher GS volume in conditions of low relative humidity (HL/LH) compared to high relative humidity (HL/HH and LL/HH) irrespective of measurement time (table 4). The GS volume in HL/LH conditions was approximately two-fold and over compared to LL/HH in 1st, 2nd and 3rd day's analyses (1, 2 and 3 days respectively). The pronounced effect of low relative humidity conditions on uptake of 3,4-DCA (table 3) and of GS by maize plants (table 4), especially at the 3rd day's analysis, could be attributed to the expected increase of plant transpiration under these conditions (Forbes and Watson, 1992; Ward and Trimble, 1995). Also, plants grown under conditions of high relative humidity absorbed, in general, significantly higher volume of GS in high light intensity compared to low light intensity conditions, independently of day's analysis (table 4). To our knowledge, no comparable studies on uptake of 3,4-DCA and of GS have been conducted with other plant species.

Table 2. Analysis of variance for effects of time and micrometeorological conditions on daily uptake of 3,4-DCA and of growth solution by maize and on its fresh weight

		3,4-DCA[1] (μg/plant)	Growth solution (ml/2 plants of cup)	Fresh weight (g/plant)
	df[2]		F(P)[3]	
T[4]	2	397.00***	476.73***	28.26***
MC[5]	2	272.19***	197.58***	1.69ns
T x[6] MC	4	97.37***	25.87***	3.45*
Residual mean square	45	$0.18 \cdot 10^4$	7.58	0.03

[1]3,4-DCA: 3,4-dichloroaniline, [2]df: degrees of freedom, [3]F(P): variance ratio(probability), [4]T: time, 1st, 2nd and 3rd day, [5]MC: micrometeorological conditions, high light intensity/high relative humidity, low light intensity/high relative humidity and high light intensity/low relative humidity, [6]x: interaction, ***,*: significant at p≤0.001 and 0.05, respectively, ns: not significant (p>0.05).

Table 3. Influence of time and micrometeorological conditions on total uptake of 3,4-DCA by maize

Micrometeorological conditions	Uptake of 3,4-DCA[1] (μg/plant)		
	Time (days)		
	1	2	3
HL/HH[2]	376.1±25.67[3] a[4] a[5]	555.7±3.05 ab[4] b	619.0±0.45 b[4] c
LL/HH	360.1± 6.94 a a[5]	94.5±35.86 a b	571.5±8.51 a b
HL/LH	488.6±17.61 b a[5]	630.8±6.43 b b	1220.0±16.62 c c

[1]3,4-DCA: 3,4-dichloroaniline, [2]HL/HH: high light intensity/high relative humidity, LL/HH: low light intensity/high relative humidity, HL/LH: high light intensity/low relative humidity, [3]mean±standard error of mean, n=6, 2 plants/replication, [4]In each column entries with different letters indicate significant differences between different micrometeorological conditions at p≤0.05 according to Tukey's HSD test, [5]In each row entries with different letters indicate significant differences between different days at p≤0.05 according to Tukey's HSD test.

Table 4. Influence of time and micrometeorological conditions on total uptake of growth solution (ml/2 plants of cup) by maize

Micrometeorological conditions	Uptake of growth solution (ml/2 plants of cup)		
	Time (days)		
	1	2	3
HL/HH[1]	10.5±0.41[2] a[3] a[4]	27.5±1.06 b[3] b	37.5±0.47 b[3] c
LL/HH	9.5±0.26 a a[4]	18.8±0.36 a b	27.8±0.33 a c
HL/LH	18.0±0.68 b a[4]	34.5±1.84 c b	57.7±2.62 c c

[1]HL/HH: high light intensity/high relative humidity, LL/HH: low light intensity/high relative humidity, HL/LH: high light intensity/low relative humidity, [2]mean±standard error of mean, n=6, 2 plants/replication, [3]In each column entries with different letters indicate significant differences between different micrometeorological conditions at p≤0.05 according to Tukey's HSD test, [4] In each row entries with different letters indicate significant differences between different days at p≤0.05 according to Tukey's HSD test.

Table 5. Influence of time and micrometeorological conditions on fresh weight (g/plant) of maize

Micrometeorological conditions	Fresh weight (g/plant)		
	Time (days)		
	1	2	3
HL/HH[1]	0.91±0.10[2] a[3] a[4]	1.33±0.11 a[3] b	1.39±0.01 ab[3] b
LL/HH	0.98±0.02 a a[4]	1.20±0.02 a b	1.24±0.04 a b
HL/LH	1.03±0.04 a a[4]	1.13±0.02 a a	1.57±0.13 b b

[1]HL/HH: high light intensity/high relative humidity, LL/HH: low light intensity/high relative humidity, HL/LH: high light intensity/low relative humidity, [2]mean±standard error of mean, n=6, 2 plants/replication, [3]In each column entries with different letters indicate significant differences between different micrometeorological conditions at p≤0.05 according to Tukey's HSD test, [4]In each row entries with different letters indicate significant differences between different days at p≤0.05 according to Tukey's HSD test.

The fresh weight of maize plants under the examined micrometeorological conditions did not change significantly in the majority of the cases (Table 5) as regards to different micrometeorological conditions. On the contrary, this plant parameter was significantly higher in the 3rd compared to the 1st day in all examined conditions. As already said, an increased transpiration is expected by maize plants under low relative humidity conditions at the 3rd day. This hypothesis is strengthened by the significantly increased fresh weight of plants at the 3rd day, under the aforementioned conditions, compared to 1st and 2nd day (Table 5). Therefore, the expected increased transpiration seems to be associated with the maize growth rate. The produced information, from our research, on plant uptake of 3,4-DCA in maize plants, could be a first step in designing suitable management practices such as phytoremediation strategies which might reduce environmental pollution.

4. CONCLUSIONS

From the present study the following conclusions can be drawn:

1. Maize plants were capable to remove noticeably high amounts of 3,4-DCA (up to three quarters of the initial amount) from the growth solution even after completion of the first 24 h period from application of this solution, irrespective of the examined micrometeorological conditions.
2. Almost the whole amount of 3,4-DCA was removed from the growth solution after a 48 h period from AGS under HL/LH. Plants under these conditions removed a significantly higher volume of growth solution compared to the HH conditions, irrespective of measurement time.
3. The significant increase of the 3,4-DCA uptake by maize plants which were replenished with this dichloroaniline under LH, compared to HH conditions, indicated a considerable capability of these plants to remove high concentrations of 3,4-DCA from the growth solution after 72 h from AGS.
4. The fresh weight of the experimental maize plants was significantly higher in the 3rd compared to the 1st day in all examined micrometeorological conditions.
5. The increase of 3,4-DCA uptake rates by maize plants grown under low relative humidity conditions, 72 hrs from AGS, could be associated with the expected acceleration of the plants transpiration rates and with the plant growth rate as in these conditions plants remove considerably high volume of growth solution.

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