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In Vitro Cultured Cocklebur (*Xanthium strumarium* L.) Responses to Dimercaptopropanesulfonic Acid and Monosodium Methanearsonate

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Abstract. Monosodium methanearsonate (MSMA) is an effective herbicide used for the control of *Xanthium strumarium* (cocklebur), a serious weed problem in cotton production. Recently, MSMA-resistant cocklebur was reported, which reduced MSMA effectiveness in the cocklebur control strategy. The mode of action of MSMA may involve the reduction of the pentavalent arsenic in MSMA to a trivalent form, which may then form a complex with sulfhydryl-containing enzymes. Dimercaptopropanesulfonic acid (DMPS) chelates trivalent arsenic, resulting in a nontoxic complex. The objective of this study was to determine the effect of DMPS and MSMA on the growth of *in vitro* grown cocklebur shoot and callus tissues. Lateral and terminal shoots were grown on basal Murashige and Skoog medium containing 6.25 mg L^{-1} (0.0428 mM) MSMA alone and with various concentrations of DMPS. Callus was cultured on basal callus medium also containing 6.25 mg L^{-1} MSMA alone and an equimolar concentration of DMPS. Shoot injury symptoms, dry root weight, and fresh weight of callus were recorded. Susceptible cocklebur shoots showed severe discoloration and death when treated with MSMA. Resistant shoots and cultured shoot tips were not adversely affected. DMPS alone did not inhibit growth of these tissues. Callus induced from susceptible tissue was inhibited by MSMA and DMPS alone. In all combinations of DMPS and MSMA tested, DMPS did not counteract the MSMA response. Thus, the pentavalent form of arsenic in MSMA apparently is not reduced to the trivalent form by the plant and hence is not involved in the mechanism of action

Xanthium strumarium L. (cocklebur) is a major weed species in the world. Its control is important to crop management (especially cotton), and resistance to a commonly used herbicide, monosodium methanearsonate (MSMA) has been documented (Haigler et al. 1988). MSMA is a cheap, relatively nontoxic organic arsenic herbicide. Even though this chemical has been used for more than 35 years, the mode of action has not been established clearly. Methanearsonate herbicide injury symptoms include foliar chlorosis followed by browning and death, rapid wilting of the plant, inhibition of the sprouting of rhizomes and tubers, inhibition of growth in general, and aberrant cell division. MSMA has also been reported to inhibit photosynthesis and respiration, induce contact poisoning, and inhibit nucleic acid or protein synthesis (Wauchope 1983).

In plants, pentavalent arsenate is a competitive inhibitor of phosphate and can be taken up and translocated in its place. Arsenate also acts to uncouple mitochondrial oxidative phosphorylation (Sachs and Michael 1971). Knowles and Benson (1983) proposed that the methanearsonates are photochemically reduced from pentavalent to trivalent arsenic by photosystem I and subsequently interact with compounds containing sulfhydryl groups. One trivalent compound formed, arsenomethane, can react with sulfhydryl groups of carbon fixation enzymes and inhibit carbon fixation.

In animals, the toxicity of arsenic is dependent on its oxidation state with trivalent inorganic arsenic more toxic than the inorganic pentavalent species, which are more toxic than the organic arsenicals. The reaction of inorganic arsenide with sulfhydryl groups appears to be the mechanism of toxic action (Hatch 1988). 2,3-Dimercapto-1-propanesulfonic acid (DMPS), a water-soluble analogue of dimercaprol (British anti-Lewisite, BAL), is an antidote for heavy metal poisoning (Petrunkin 1956). DMPS has

Abbreviations: MSMA, monosodium methanearsonate; DMPS, dimercaptopropanesulfonic acid; SM, shoot medium; CM, callus medium; ANOVA, analysis of variance.

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been studied extensively for its ability to chelate trivalent inorganic and organic arsenic (Aposhian 1983, Aposhian et al. 1981, 1983, Maiorino and Aposhian 1985, Tadlock and Aposhian 1990). Evidence indicates that trivalent arsenic strongly binds to the vicinal sulfhydryl groups of DMPS, forming a nontoxic complex (Aposhian et al. 1981). Wildenauer et al. (1982) reported facilitated transport of DMPS mediated by an anion carrier protein into red blood cells, where it would be able to form sulfhydryl complexes.

The objective of this study was to determine the effects of DMPS and MSMA on the growth of cocklebur shoot and callus tissues in vitro. An in vitro culture protocol for cocklebur had been established (Ellis and Camper 1994) and was viewed as a model system for studies of herbicide mechanism of action studies as suggested earlier (Camper and McDonald 1989).

Materials and Methods

Plant Materials

Seeds of susceptible and resistant *X. strumarium* L. were excised from the bur and surface disinfested for 2 min in 95% ethanol followed by 10 min in 0.68% sodium hypochlorite with Tween 20 (three drops/100 ml). Two seeds each were then placed in a sterile 95- × 25-mm shell vial containing 20 ml of sterile distilled deionized water and covered with a plastic cap. Seed coats were removed aseptically after 2 h. The seeds were placed two to a 60- × 15-mm sterile, disposable Petri dish containing 15 ml of a germination medium [4.30 g L^{-1} Murashige and Skoog salts (1962), 30 g L^{-1} sucrose, pH adjusted to 5.7] Six g L^{-1} Difco Bacto-agar was added, and the medium was autoclaved at 104 kPa and 121 °C for 20 min. After 7 days the seedlings were placed four to a 100- × 15-mm sterile disposable Petri dish containing 25 ml of basal medium to which various concentrations of MSMA had been added.

Leaves and shoots were obtained from 2- to 7-month-old resistant and susceptible plants kept in a culture chamber under fluorescent lights (25 mol $m^{-2}s^{-1}$ Photosynthetic Photon Flux, 12-h photoperiod) at 25 °C. All subsequent growth chamber conditions used for culture growth were the same. These plants were grown in commercial potting soil and fertilized every 2 weeks with Peter's 20-20-20 fertilizer. Leaves and shoots were surface disinfested in 0.525% sodium hypochlorite with Tween 20 (three drops/100 ml) for 10 min and then placed in sterile distilled deionized water for 5 min. Under aseptic conditions leaf discs were excised with a No. 6 cork borer avoiding the midrib vein. Leaf discs were placed five to a 100- × 15-mm sterile disposable Petri dish containing 25 ml of medium. Lateral and terminal shoots were cut to include from two to four small young leaves. From one to three shoots were placed in a 100- × 15-mm sterile disposable Petri dish containing 25 ml of medium. Shoots and leaves were also obtained from field-grown plants and treated as above. Micropropagated shoots were obtained from shoot tips grown in culture for 6 weeks. Methods for micropropagation of *X. strumarium* follow Ellis and Camper (1994).

Culture Medium and Conditions

Shoot and seedling basal medium (SM) consisted of 4.30 g L^{-1} Murashige and Skoog salts (1962) and 30 g L^{-1} sucrose, pH adjusted to 5.7. Six g L^{-1} Difco Bacto-agar was added, and the medium was autoclaved at 104 kPa and 121 °C for 20 min. For shoots and leaf discs the basal medium was supplemented with the following concentrations of filter-sterilized MSMA (mg L^{-1}): 2,400, 1,200, 600, 400, 200, 100, 50, 25, 12.5, 6.25, and 0. For seedlings the basal medium was supplemented with these concentrations of filter-sterilized MSMA (mg L^{-1}): 2,400, 1,200, 600, 400, 200, 100, 50, 25, 8, 4, 2, 1, and 0. Seven seedlings, 10 leaf discs, and six shoots were cultured at different concentrations of MSMA. Experiments were repeated at least once at concentrations of 100 mg L^{-1} and lower. Cultures were examined once a week for 4 weeks with indications of herbicidal injury recorded.

Shoots and Callus

Shoots were obtained from 7-month-old plants kept in a culture chamber as specified above. These plants were grown in commercial potting soil and fertilized every 2 weeks with Peter's 20-20-20 fertilizer. Lateral or terminal shoots (containing two to four small leaves) were surface disinfested in 0.525% sodium hypochlorite with Tween 20 (three drops/ 100 ml) for 10 min and then placed in sterile distilled deionized water for 5 min. Three shoots were placed in a 100- × 15-mm sterile disposable Petri dish containing 25 ml of medium described above.

Callus of susceptible cocklebur was maintained on a basal callus medium (CM) consisting of 34.5 g L^{-1} MSMO (Murashige and Skoog minimal organics medium, Life Technologies, Inc.), pH adjusted to 5.7, and supplemented with 2,4-dichlorophenoxyacetic acid (0.1 mg L^{-1}) and kinetin (2.0 mg L^{-1}). Eight g L^{-1} Difco Bacto-agar was added, and the medium was autoclaved at 104 kPa and 121 °C for 20 min. Callus was incubated in a growth chamber and subcultured every 4–6 weeks.

Culture Medium and Conditions

SM was prepared as above with MSMA and DMPS filter sterilized before adding to autoclaved, cooled medium. Treatments included SM with 6.25 mg L^{-1} (0.0428 mM) MSMA; SM with 9.00 mg L^{-1} (0.0428 mM) DMPS; SM with 6.25 mg L^{-1} (0.0428 mM) MSMA and 9.00 mg L^{-1} (0.0428 mM) DMPS; SM with 6.25 mg L^{-1} (0.0428 mM) MSMA and 18.00 mg L^{-1} DMPS; SM with 6.25 mg L^{-1} (0.0428 mM) MSMA and 36.00 mg L^{-1} DMPS; SM with 9.00 mg L^{-1} (0.0428 mM) DMPS; SM with 18.00 mg L^{-1} DMPS; SM with 36.00 mg L^{-1} DMPS; SM with 0 mg L^{-1} MSMA and 0 mg L^{-1} DMPS.

Susceptible callus (0.3 g) was placed in 60- × 15-mm sterile disposable Petri dishes containing 15 ml of medium. Treatments included CM supplemented with 6.25 mg L^{-1} (0.0428 mM) MSMA; CM plus 9.00 mg L^{-1} (0.0428 mM) DMPS; CM plus 6.25 mg L^{-1} (0.0428 mM) MSMA and 9.00 mg L^{-1} (0.0428 mM) DMPS; CM with 0 mg L^{-1} MSMA and 0 mg L^{-1} DMPS.

Shoot cultures were examined at the end of 4 weeks, and indications of herbicidal injury were recorded. Root tissue was freeze dried and then weighed. Callus was weighed at 4, 7, 11, 14, 18, 21, 24, and 28 days. There were from four to six replicates/treatment in each experiment, and each experiment was

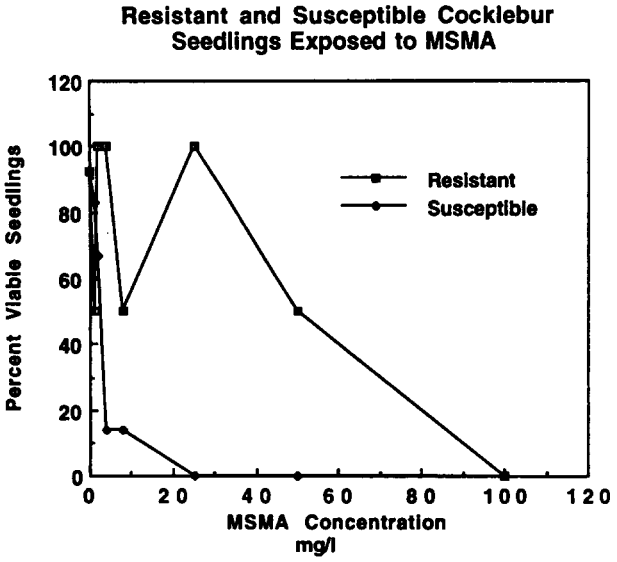

Fig. 1. Percent of living resistant and susceptible cocklebur seedlings after exposure to various concentrations of MSMA (mg L^{-1}) in culture for 4 weeks.

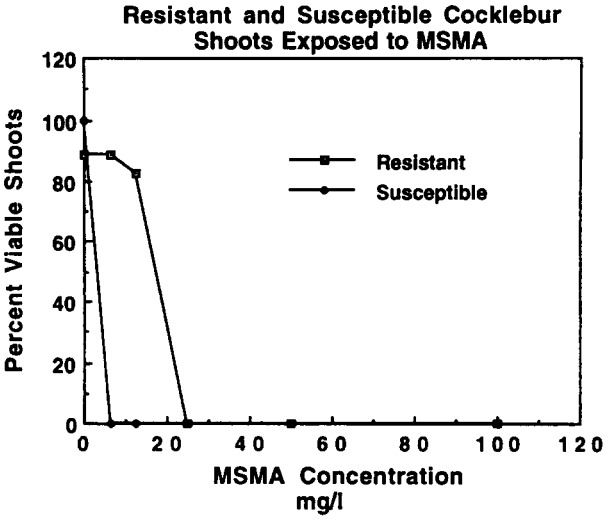

Fig. 2. Percent of living resistant and susceptible cocklebur shoots after exposure to various concentrations of MSMA (mg L^{-1}) in culture for 4 weeks.

repeated four times. The callus experiment was arranged in a randomized complete block split plot design with day of measurement as the subplot effect and treatment as the whole plot. Blocks were replications of all of the treatments. ANOVA was done, and means were compared using paired comparisons.

Results and Discussion

The *in vitro* culture of cocklebur has provided a model system to compare resistant and susceptible tissue responses and learn more about the mode of action and resistance. Week-old seedlings of susceptible cocklebur grown on SM supplemented with MSMA after 4 weeks turned brown, and no new growth was noted at concentrations as low as 2 mg L^{-1}. Only 15% of the plants survived at this concentration. One hundred percent of the susceptible seedlings died when exposed to concentrations of 25 mg L^{-1} MSMA and above (Fig. 1). Roots that were originally present turned brown, and very little root growth was seen. Resistant seedlings continued to grow with new leaves and roots produced. At concentrations of 6.25 and 12.5 mg L^{-1} lateral and terminal shoots taken from resistant culture chamber-grown plants produced new leaves and roots *in vitro*. Leaves of the shoots taken from susceptible culture chamber-grown plants turned brown, and very few if any roots developed (Fig. 2). Resistant and susceptible shoots from field-grown plants and micropropagated shoots reacted in the same manner. Leaf discs proved unreliable as an indicator of herbicide resistance. As a result of the above studies of MSMA at 6.25 mg L^{-1} was chosen for further in vitro studies.

In studies of the responses of DMPS and MSMA in susceptible tissue, MSMA-treated shoot cultures produced significantly lower root weights per shoot than the controls (Fig. 3). There was no significant difference in the root weight/shoot produced in cultures containing MSMA and equimolar concentrations of DMPS (Fig. 3). Even when the concentrations of DMPS tested were doubled and quadrupled in combination with MSMA at 6.25 mg L^{-1}, there was no difference compared with MSMA alone (Fig. 3). Similar trends in MSMA damage to the leaves were observed. The leaves turned brown, and very few if any new leaves were formed in all cultures with MSMA. When DMPS was used alone there was no difference in this treatment compared with the control in root weight/shoot and amount of leaf damage (Figs. 3 and 4). The control and DMPS-exposed shoots continued to grow and produce new leaves.

No differences in callus tissue (initiated from susceptible cocklebur biotype) fresh weight were found over 4 weeks among the MSMA, DMPS, and MSMA/DMPS treatments. The control was significantly different from the MSMA, DMPS, and MSMA/DMPS (Fig. 5). Thus, similar responses were observed between callus and shoot tissues. Several authors suggest that there can be significant correlation between whole plant responses to a phytotoxin and calli and cell suspension cultures (Zilkah and Gressel 1977; Zilkah et al. 1977). DMPS-treated shoots showed the same growth pattern as controls even at the three concentrations used.

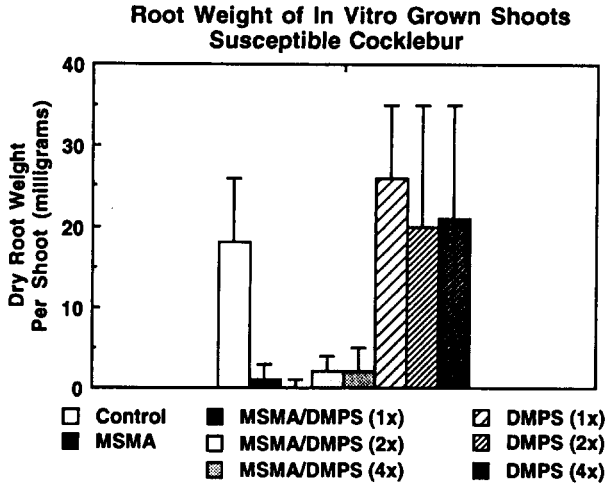

Fig. 3. Root weight (mg dry weight)/shoot from susceptible cocklebur grown in culture with MSMA and DMPS for 4 weeks. Treatments consisted of a control, MSMA and DMPS tested alone, and various combinations of MSMA/DMPS as described in the Materials and Methods section. *Bars* indicate ±SE.

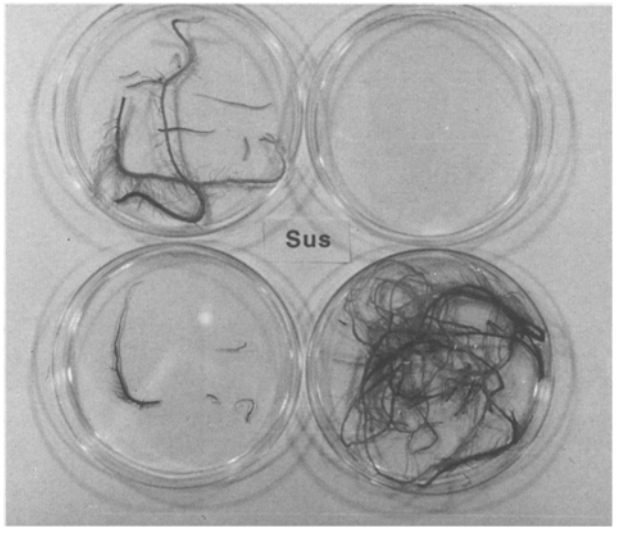

Fig. 4. Root tissue excised from susceptible cocklebur shoots grown in culture for 4 weeks. *Upper left,* control [MSMA and DMPS at 0 mg L]; *upper right,* MSMA [6.25 mg L^{-1} (0.0428 mM) MSMA]; *lower left,* MSMA/DMPS (1×) [6.25 mg L^{-1} (0.0428 mM) MSMA and 9.00 mg L^{-1} (0.0428 mM) DMPS]; *lower right,* DMPS (1×) [9.00 mg L^{-1} (0.0428 mM) DMPS].

DMPS does not appear to be inhibitory to the growth of shoots *in vitro;* however, the growth of callus exposed to DMPS alone was inhibited. Any shoot treatment containing MSMA and MSMA/DMPS showed significantly lower root weight/shoot and exhibited herbicidal leaf damage. The callus cultures with MSMA and MSMA/DMPS were not different but had a lower average fresh weight increase as compared with the controls.

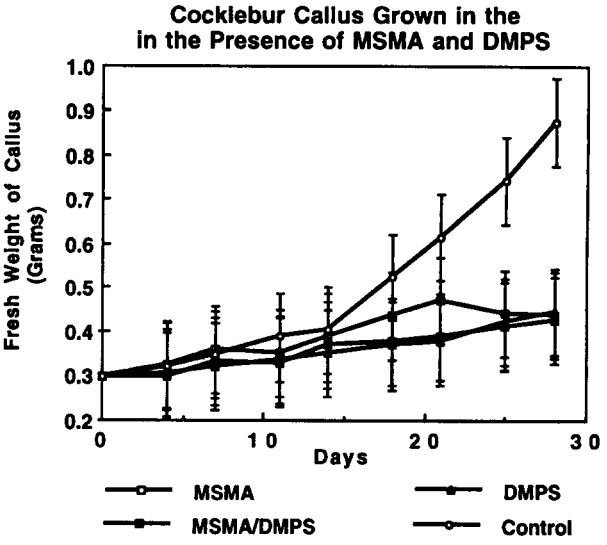

Fig. 5. Susceptible cocklebur callus growth (fresh weight) in the presence of MSMA and DMPS for 4 weeks. Treatments: control [MSMA and DMPS at 0 mg L^{-1}]; MSMA [6.25 mg L^{-1} (0.0428 mM) MSMA]; MSMA/DMPS [6.25 mg L^{-1} (0.0428 mM) MSMA and 9.00 mg L^{-1} (0.0428 mM) DMPS]; DMPS [9.00 mg L^{-1} (0.0428 mM) DMPS]. *Bars* indicate ±SE.

Differential absorption and conjugation could be the basis of arsenical herbicide toxicity (Duke 1992). However, differential uptake and translocation were not observed with [^{14}C]MSMA-treated susceptible and resistant cocklebur and treated cotton (Keese and Camper 1994a). Knowles and Benson (1983) contend that the herbicidal activity of methanearsonate is due to its photochemical reduction to form arsenomethane, a trivalent arsenic containing compound. However, arsenic speciation analysis failed to detect any trivalent arsenic in treated resistant and susceptible cocklebur or in treated cotton (a tolerant plant) (Keese and Camper 1994b). Results of cocklebur shoot and callus studies reported herein show that DMPS does not counteract the inhibitor action of MSMA. If DMPS is a strong chelator of the trivalent form of arsenic, then our study indicates that the pentavalent arsenic in MSMA apparently is not reduced to the trivalent form in the plant, and hence, is not involved in the mechanism of action.

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References

Aposhian HV (1983) DMSA and DMPS-water soluble antidotes for heavy metal poisoning. Annu Rev Pharmacol Toxicol 23:193–215

Aposhian HV, Tadlock CH, Moon TE (1981) Protection of mice against the lethal effects of sodium arsenite: A quantitative comparison of a number of chelating agents. Toxicol Appl Pharmacol 61:385–392

Aposhian HV, Hsu C, Hoover TD (1983) DL- and *meso*-dimercaptosuccinic acid: In vitro and in vivo studies with sodium arsenite. Toxicol Appl Pharmacol 69:206–213

Camper ND, McDonald SK (1989) Tissue and cell cultures as model systems in herbicide research. Rev Weed Sci 4: 169–190

Duke SO (1992) Modes of action of herbicides used in cotton. In: McWhorter CG, Abernathy JR (eds) Weeds of cotton and control. The Cotton Foundation, Memphis, Tennessee, pp 403–437

Ellis JP, Camper ND (1994) In vitro culture of *Xanthium strumarium* (cocklebur). Plant Cell Tissue Organ Cult 36:369–372

Haigler WE, Gossett BJ, Harris JR, Toler JE (1988) Resistance of common cocklebur (*Xanthium strumarium*) to the organic arsenical herbicides. Weed Sci 36:24–27

Hatch RC (1988) Poisons causing abdominal distress or liver or kidney damage. In: Booth NH, McDonald LE (eds) Veterinary pharmacology and therapeutics. 6th ed. Iowa State University Press. Ames, pp 1102–1125

Keese RJ, Camper ND (1994a) Uptake and translocation of [^{14}C]MSMA in cotton and MSMA-resistant and -susceptible cocklebur. Pestic Biochem Physiol 49:138–145

Keese RJ, Camper ND (1994b) MSMA affects amino acid and glutathione content in MSMA-resistant and -susceptible common cocklebur (*Xanthium strumarium* L.) and cotton (*Gossypium hirsutum* L.). WSSA Abstr 34:54

Knowles FC, Benson AA (1983) Mode of action of a herbicide: Johnsongrass and methanearsonic acid. Plant Physiol 71: 235–240

Maiorino RM, Aposhian HV (1985) Dimercaptan metal-binding agents influence the biotransformation of arsenite in the rabbit. Toxicol Appl Pharmacol 77:240–250

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497

Petrunkin VE (1956) Synthesis and properties of dimercapto derivatives of alkylsulfonic acids. Ukr Khim Zh 22:603–607

Sachs RM, Michael JL (1971) Comparative phytotoxicity among four arsenical herbicides. Weed Sci 19:558–564

Tadlock CH, Aposhian HV (1980) Protection of mice against the lethal effects of sodium arsenite by 2,3-dimercapto-1-propanesulfonic acid and dimercaptosuccinic acid. Biochem Biophys Res Commun 94:501–507

Wauchope RD (1983) Uptake, translocation and phytotoxicity of arsenic in plants. In: Lederer WH, Fensterheim RJ (eds) Arsenic: Industrial, biomedical and environmental prospective. Van Nostrand Reinhold, New York, pp 348–375

Wildenauer DB, Reuther H, Weger N (1982) Interactions of the chelating agent 2,3-dimercaptopropane-1-sulfonate with red blood cells in vitro. I. Evidence for carrier-mediated transport. Chem Biol Interactions 42:165–177

Zilkah S, Gressel J (1977) Cell cultures vs. whole plants for measuring phytotoxicity. III. Correlations between phytotoxicities in cell suspension cultures, calli, and seedlings. Plant Cell Physiol 18:815–820

Zilkah S, Bocion PF, Gressel J (1977) Cell cultures vs. whole plants for measuring phytotoxicity. II. Correlations between phytotoxicity in seedlings and calli. Plant Cell Physiol 18:657–670