Some Physiological Responses of *Sorghum bicolor* to Benzene Hexachloride

Aurora G. Salazar[1] and Gary M. Paulsen*

Benzene hexachloride (BHC) supplied to *Sorghum bicolor* seedlings grown in nutrient solutions enhanced growth at intermediate concentrations and inhibited growth at high concentrations. Weights of all plant parts were greater at intermediate BHC concentrations than at low or high BHC concentrations. Photosynthesis rates were not affected except at the highest BHC concentration, but respiration rates decreased successively as BHC concentrations increased. Chlorophyll *a* and *b* concentrations decreased with higher BHC levels but were otherwise unaffected. Reducing sugars, sucrose, and starch concentrations generally paralleled growth pattern responses to increasing BHC concentrations. Phytotoxicity of BHC to seedlings was from inhibition of respiratory processes; growth stimulation was a direct response to BHC independent of benefits of insect control.

Chlorinated hydrocarbon insecticides frequently affect crop plants and soil microorganisms as well as their intended objectives. Benzene hexachloride (BHC) can affect plants directly during application or, because of its extreme persistency (Alexander, 1965), by accumulating in soil residues. Many studies have emphasized adverse effects of BHC on plant growth. Reported adverse effects include inhibited seed germination (Horiguchi, 1952), chromosomal aberrations (Kostoff, 1948), retarded cell division and accelerated cell enlargement (Sass, 1951), and decreased plant growth rates (Brass and Ware, 1960; Gould, 1956). Some phytotoxic effects of BHC probably occur at the chromosomal level. Simkover and Shenefelt (1952), for example, reported that BHC apparently destroyed DNA and had its greatest effect on meristematic areas. Hopkins (1952), however, concluded BHC functioned by inhibiting enzymes involved in biosynthesis after initially activating hydrolytic enzymes.

Reports on growth-stimulating effects of BHC contrast with those on phytotoxic effects. BHC stimulated germination and growth of clover [Randall, A. P. (cited by Unraw and Harris, 1961)], enhanced root growth of conifers (Simkover and Shenefelt, 1952), increased yields of carrots and onions (Boswell *et al.*, 1955), and increased dry matter accumulation in corn (Hanower *et al.*, 1960). The most striking growth stimulation was reported by Ruge (1952). BHC applied to blossoms increased bean yields markedly by increasing seed size (weight) without significantly affecting seed numbers. All four BHC isomers used were nearly equally effective. Growth stimulation caused by BHC apparently is not associated with the compound's insecticidal effects. Boswell *et al.* (1955), for instance, suggested BHC exerted a secondary or indirect effect on plants.

Few effects of BHC on plant chemical composition have been reported. BHC had no effect on protein, oil, or iodine number in soybeans (Probst and Everly, 1957) or on N and P concentrations in clover foliage [Randall, A. P. (cited by Unraw and Harris, 1961)]. However, BHC destroyed DNA in conifers (Simkover and Shenefelt, 1952) and decreased protein concentration in corn (Hanower *et al.*, 1960).

Reasons for the differential phytotoxic and growth-stimulating effects of BHC are not clear. However, the protective action of glucose against BHC toxicity, and the ability of high BHC concentrations to overcome that protective effect (Hopkins, 1952) suggest that photosynthesis is involved directly in plants' responses to BHC. Investigations reported here determined phytotoxic and growth-stimulating effects of BHC on plant constituents and processes associated with photosynthesis.

MATERIALS AND METHODS

One-week-old *Sorghum bicolor* (L.) Moench. seedlings germinated in vermiculite were transplanted to nutrient solutions in 2-l. styrene containers. Each container held six seedlings. Macronutrients and micronutrients were supplied at levels recommended by Hoagland and Arnon (1950) and Johnson *et al.* (1957), respectively. Technical γ BHC (99+% γ isomer) dissolved in 5.0 ml of acetone was added to each container to obtain 0, 0.01, 0.1, 1.0, and 10.0 μM concentrations. An additional control (0 μM BHC) treatment without acetone was included but had no effect on the results. The solutions were aerated 24 hr before transplanting seedlings to remove the acetone. All treatments were replicated three times. After transplanting, the seedlings were grown 2 weeks in environmental chambers maintained at 30° C day and 20° C night temperatures with a 16-hr light period. Light intensity at plant height was 32,000 lux and relative humidity was 40%. The solutions were aerated continuously and adjusted to pH 5.0 periodically with H_2SO_4.

Photosynthesis and respiration rates of the seedlings were measured after 2 weeks' growth. Containers holding the seedlings were placed in an air-tight plexiglas chamber under three 300-W cool-beam spot lamps and four 25-W "Grow Lux" fluorescent lamps. Temperature in the chamber was maintained at 30° C by passing the inside air at 10 km per hr velocity through a heat exchanger. Carbon dioxide concentration in the chamber was maintained at 300 ppm by adding outside air of known CO_2 level into the system through rotometers. Carbon dioxide levels in the chamber and outside air were monitored and recorded continuously with a Beckman 215 ir gas analyzer coupled with a strip chart recorder. Respiration was determined by the rate CO_2 accumulated in the chamber in darkness with no outside air added. Photosynthesis and respiration rates were calculated per dm^2 of plant leaf area (Hesketh and Musgrave, 1962).

Seedlings were sectioned into roots, stems plus leaf sheaths, and leaf blades by separating them at the mesocotyl and leaf collar after the previous measurements were made. A 1-g

Department of Agronomy, Kansas State University, Manhattan, Kansas 66502
[1] Department of Plant Science, South Dakota State University, Brookings, South Dakota 57006

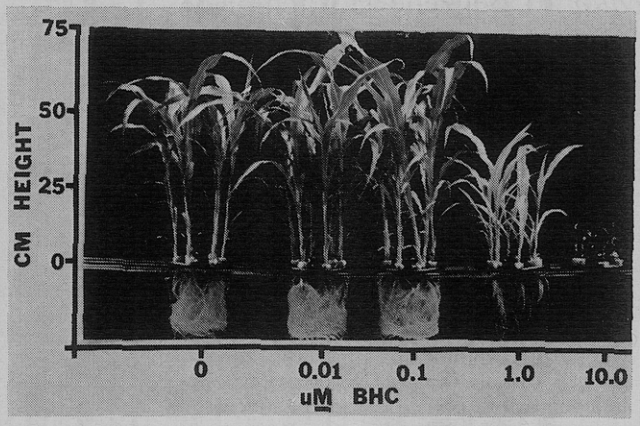

Figure 1. Appearance of *Sorghum bicolor* seedlings grown in nutrient solutions containing benzene hexachloride (BHC)

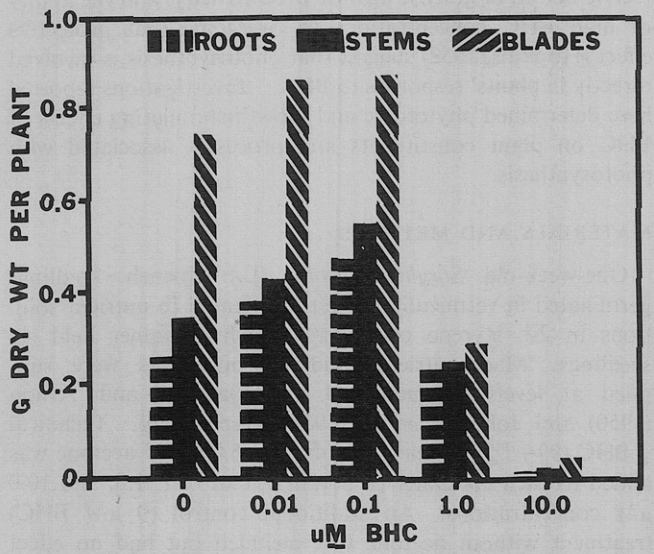

Figure 2. Dry weights of roots, stems, and leaf blades of *Sorghum bicolor* seedlings grown in nutrient solutions containing benzene hexachloride (BHC). LSD (0.05) is 0.06, 0.07, and 0.12 for differences in root, stem, and blade weights, respectively, among BHC concentrations

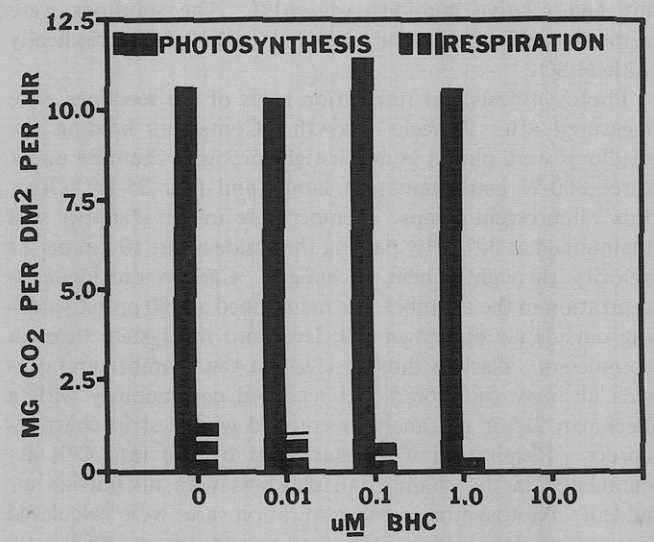

Figure 3. Photosynthesis and respiration rates in *Sorghum bicolor* seedlings grown in nutrient solutions containing benzene hexachloride (BHC). LSD (0.05) is 1.9 and 0.2 for differences in photosynthesis and respiration rates, respectively, among BHC concentrations

sample of the leaf blades from each treatment was analyzed for chlorophyll *a* and *b* by the method of Arnon (1949). The remaining samples were dried at 70° C to constant weight.

Dried leaf-blade samples were ground to 40-mesh size. Reducing sugars and sucrose were extracted by shaking 1-g tissue samples in 50 ml of warm 80% (v/v) ethanol for 2 hr and filtering the extracts. The residues were held for starch analysis, and ethanol was evaporated from the filtrates and replaced with water. Reducing sugars and sucrose, respectively, were measured by Shaffer–Somogyi copper–iodometric titration (Smith *et al.*, 1964) before and after hydrolyzing aliquots of the filtrate in 1.0 N H_2SO_4. The residue remaining after ethanol extraction was air-dried, refluxed to gelatinize starch, and incubated with takadiastase enzyme at 38° C for 48 hr (Smith *et al.*, 1964). The solution was deproteinated with $Pb(CH_3COO^-)_2$, filtered, and hydrolyzed in 1.0 N H_2SO_4. After the filtrate was neutralized, reducing sugars were analyzed as described previously. Glucose standards and appropriate conversion factors were used to calculate carbohydrate concentrations.

RESULTS AND DISCUSSION

Appearance of sorghum seedlings after receiving various BHC concentrations is shown in Figure 1. Seedlings treated with the intermediate BHC concentration were tallest and had the most extensive root systems. Control seedlings that received no BHC were shorter and had less extensive root systems. At higher BHC levels, growth of shoots and roots was retarded. Seedlings were stunted and chlorotic when treated with 1.0 μM BHC. At the highest BHC level, 10.0 μM, seedlings were severely stunted and necrotic, and extensive breakdown of root tissue was evident. The growth pattern of shoots and roots over all BHC levels was similar to that observed for conifer roots by Simkover and Shenefelt (1952). The response of plant weights to BHC paralleled closely their appearance (Figure 2). Most dry matter accumulated at the intermediate BHC concentration; accumulation was intermediate at the lowest BHC concentrations and least at highest BHC concentrations. All plant parts responded proportionately to BHC. Apparently, differences in BHC content among plant parts (Haines, 1956) had little effect on their relative growth responses.

Photosynthesis rates were not affected significantly by BHC except at the highest concentration (Figure 3). Although the photosynthesis rate was slightly higher at the BHC level that produced most growth, the rate was not significantly different from the other four lower treatment rates. At the highest BHC level, however, photosynthesis had ceased. Unlike photosynthesis rates, respiration rates responded significantly to each BHC increment (Figure 3). Respiration rate was highest in the untreated control, decreased as BHC concentration was increased, and ceased at the highest BHC concentration. Differences in photosynthesis and respiration rates among the four lower BHC concentrations do not support the conclusion of Hopkins (1952) that BHC functioned by inhibiting enzyme systems involved principally in biosynthesis. Instead, respiration was relatively more sensitive than photosynthesis at similar BHC levels. That suggests that the effect of BHC was more intimately associated with mitochondrial functions than with chloroplast functions.

Chlorophyll *a* and *b* concentrations responded to the two highest BHC concentrations but not to the three lowest BHC concentrations (Figure 4). The chlorotic and necrotic

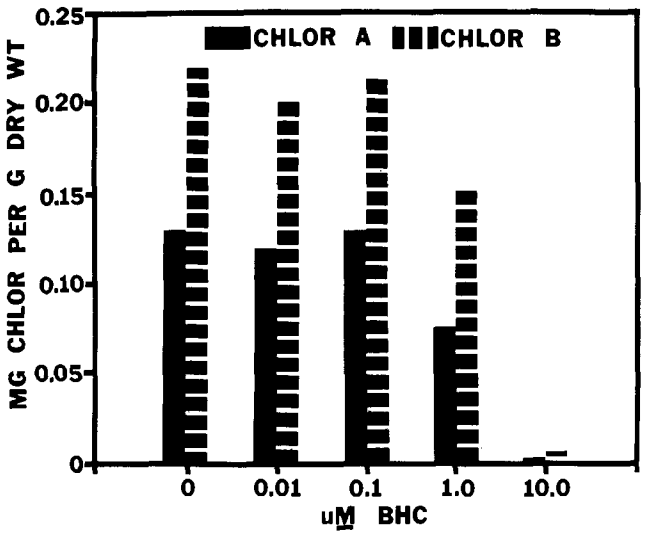

Figure 4. Chlorophyll *a* and *b* concentrations in *Sorghum bicolor* seedlings grown in nutrient solutions containing benzene hexachloride (BHC). LSD (0.05) is 0.03 and 0.04 for differences in chlorophyll *a* and *b* concentrations, respectively, among BHC concentrations

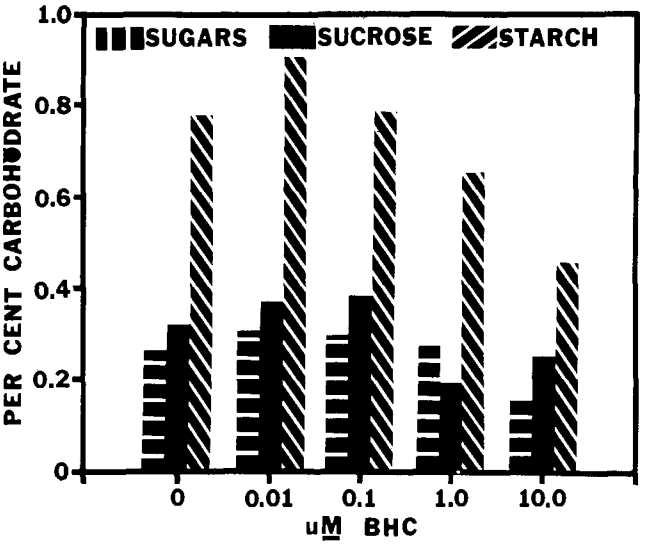

Figure 5. Reducing sugars, sucrose, and starch concentrations in *Sorghum bicolor* seedlings grown in nutrient solutions containing benzene hexachloride (BHC). LSD (0.05) is 0.09, 0.10, and 0.21 for differences in reducing sugar, sucrose, and starch concentrations, respectively, among BHC concentrations

conditions of sorghum seedlings at the 1.0- and 10.0-μM BHC levels, respectively, are indicated by lower chlorophyll concentrations at those levels. Chlorophyll concentration at the 1.0-μM BHC level apparently did not limit photosynthesis rate because, although the chlorophyll concentration decreased, the photosynthesis rate remained constant. Likewise, BHC at growth-stimulating rates did not affect chlorophyll concentrations as it affected foliage color (Ruge, 1952). No differences were observed between the responses of chlorophyll *a* and *b* to BHC treatment.

Changes in the carbohydrate composition of sorghum seedlings among BHC treatments generally followed the growth rate response (Figure 5). Of the carbohydrates analyzed, reducing sugars and starch were affected more than sucrose by BHC. The concentration of each carbohydrate constituent generally increased slightly as BHC was increased from the control to the intermediate level, and then declined. However, the BHC levels where the lowest and highest carbohydrate concentrations occurred were different for each carbohydrate. Thus, the highest reducing sugar and starch concentrations occurred at 0.01 μM BHC, while sucrose continued to increase to 0.1 μM BHC. Similarly, the lowest reducing sugar and starch concentrations occurred at 10.0 μM BHC, while the lowest sucrose concentration occurred at 1.0 μM BHC.

The mode of action of BHC in stimulating and inhibiting sorghum seedling growth at intermediate and high concentrations, respectively, is associated with chromosomal and physiological processes. Chromosomal effects, probably at the level of DNA, and the resulting plant malformations are well documented (Brass and Ware, 1960; Kostoff, 1948; Sass, 1951; Simkover and Shenefelt, 1952). However, those effects have been studied mostly in young seedlings and might not apply to older seedlings and plants. Similarly, inhibition of biosynthetic functions might be involved (Hopkins, 1952). Although one biosynthetic process, photosynthesis, was not inhibited at threshold phytotoxic BHC levels, other biosynthetic processes might respond differently. However, the present investigations indicated that inhibition of respiratory processes was more important than inhibition of biosynthetic processes in BHC phytotoxicity.

Growth stimulation induced by BHC is less documented and studied than the phytotoxicity response. Although BHC might act as a synthetic plant growth regulator, it appears likely that growth stimulation occurs by some mechanism other than alteration of plant morphology. It is clear, however, that growth stimulation is a direct plant response to BHC independent of any benefit imparted by insect control.

LITERATURE CITED

Alexander, M., *Advan. Appl. Microbiol.* **7,** 35 (1965).
Arnon, D. I., *Plant Physiol.* **24,** 1 (1949).
Boswell, V. R., Clore, W. V., Pepper, B. P., Taylor, C. B., Gilmer, P. M., Carter, R. L., *USDA Tech. Bull.* 1121 (1955).
Brass, C. L., Ware, G. W., *J. Econ. Entomol.* **53,** 110 (1960).
Gould, H. J., *Plant Pathol.* **5,** 105 (1956).
Haines, R. G., *J. Econ. Entomol.* **49,** 563 (1956).
Hanower, P., Janicka, I., Brzozowska, J., Hoff, M., *Rocz. Nauk Roln. Ser. A* **80,** 741 (1960).
Hesketh, J. D., Musgrave, R. B., *Crop Sci.* **2,** 311 (1962).
Hoagland, O. R., Arnon, D. I., *Calif. Agr. Exp. Sta. Cir.* 347 (1950).
Hopkins, H. T., *Plant Physiol.* **27,** 526 (1952).
Horiguchi, H., *Ovo Kontyu* **1,** 183 (1952).
Johnson, C. M., Stout, P. R., Boyer, T. C., Carlton, A. B., *Plant Soil* **8,** 337 (1957).
Kostoff, D., *Science* **109,** 467 (1948).
Probst, A. H., Everly, R. T., *Agron. J.* **49,** 577 (1957).
Ruge, U., *Angew Bot.* **26,** 130 (1952).
Sass, J. E., *Science* **114,** 466 (1951).
Simkover, H. G., Shenefelt, R. D., *J. Econ. Entomol.* **45,** 11 (1952).
Smith, D., Paulsen, G. M., Raguse, C. A., *Plant Physiol.* **39,** 960 (1964).
Unraw, A. M., Harris, G. H., *Can. J. Plant Sci.* **41,** 578 (1961).

Received for review November 30, 1970. Accepted April 21, 1971.