RESIDUE REVIEWS

Residues of Pesticides and Other Contaminants in the Total Environment

Editor
FRANCIS A. GUNTHER

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VOLUME 65

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VOLUME 65

Effects of triazine herbicides on the physiology of plants

SINGLE PESTICIDE VOLUME:

EFFECTS OF TRIAZINE HERBICIDES ON THE PHYSIOLOGY OF PLANTS

Foreword

Worldwide concern in scientific, industrial, and governmental communities over traces of toxic chemicals in foodstuffs and in both abiotic and biotic environments has justified the present triumvirate of specialized publications in this field: comprehensive reviews, rapidly published progress reports, and archival documentations. These three publications are integrated and scheduled to provide in international communication the coherency essential for nonduplicative and current progress in a field as dynamic and complex as environmental contamination and toxicology. Until now there has been no journal or other publication series reserved exclusively for the diversified literature on "toxic" chemicals in our foods, our feeds, our geographical surroundings, our domestic animals, our wildlife, and ourselves. Around the world immense efforts and many talents have been mobilized to technical and other evaluations of natures, locales, magnitudes, fates, and toxicology of the persisting residues of these chemicals loosed upon the world. Among the sequelae of this broad new emphasis has been an inescapable need for an articulated set of authoritative publications where one could expect to find the latest important world literature produced by this emerging area of science together with documentation of pertinent ancillary legislation.

The research director and the legislative or administrative advisor do not have the time even to scan the large number of technical publications that might contain articles important to current responsibility; these individuals need the background provided by detailed reviews plus an assured awareness of newly developing information, all with minimum time for literature searching. Similarly, the scientist assigned or attracted to a new problem has the requirements of gleaning all literature pertinent to his task, publishing quickly new developments or important new experimental details to inform others of findings that might alter their own efforts, and eventually publishing all his supporting data and conclusions for archival purposes.

The end result of this concern over these chores and responsibilities and with uniform, encompassing, and timely publication outlets in the field of environmental contamination and toxicology is the Springer-Verlag (Heidelberg and New York) triumvirate:

Residue Reviews (vol. 1 in 1962) for basically detailed review articles concerned with any aspects of residues of pesticides and other chemical contaminants in the total environment, including toxicological considerations and consequences.

Bulletin of Environmental Contamination and Toxicology (vol. 1 in 1966) for rapid publication of short reports of significant advances and discoveries in the fields of air, soil, water, and food contamination and pollution as well as methodology and other disciplines concerned with the introduction, presence, and effects of toxicants in the total environment.

Archives of Environmental Contamination and Toxicology (vol. 1 in 1973) for important complete articles emphasizing and describing original experimental or theoretical research work pertaining to the scientific aspects of chemical contaminants in the environment.

Manuscripts for *Residue Reviews* and the *Archives* are in identical formats and are subject to review, by workers in the field, for adequacy and value; manuscripts for the *Bulletin* are not reviewed and are published by photo-offset to provide the latest results without delay. The individual editors of these three publications comprise the Joint Coordinating Board of Editors with referral within the Board of manuscripts submitted to one publication but deemed by major emphasis or length more suitable for one of the others.

September 1, 1976 Coordinating Board of Editors

Preface

That residues of pesticide and other contaminants in the total environment are of concern to everyone everywhere is attested by the reception accorded previous volumes of "Residue Reviews" and by the gratifying enthusiasm, sincerity, and efforts shown by all the individuals from whom manuscripts have been solicited. Despite much propaganda to the contrary, there can never be any serious question that pest-control chemicals and food-additive chemicals are essential to adequate food production, manufacture, marketing, and storage, yet without continuing surveillance and intelligent control some of those that persist in our foodstuffs could at times conceivably endanger the public health. Ensuring safety-in-use of these many chemicals is a dynamic challenge, for established ones are continually being displaced by newly developed ones more acceptable to food technologists, pharmacologists, toxicologists, and changing pest-control requirements in progressive food-producing economies.

These matters are of genuine concern to increasing numbers of governmental agencies and legislative bodies around the world, for some of these chemicals have resulted in a few mishaps from improper use. Adequate safety-in-use evaluations of any of these chemicals persisting into our foodstuffs are not simple matters, and they incorporate the considered judgments of many individuals highly trained in a variety of complex biological, chemical, food technological, medical, pharmacological, and toxicological disciplines.

It is hoped that "Residue Reviews" will continue to serve as an integrating factor both in focusing attention upon those many residue matters requiring further attention and in collating for variously trained readers present knowledge in specific important areas of residue and related endeavors involved with other chemical contaminants in the total environment. The contents of this and previous volumes of "Residue Reviews" illustrate these objectives. Since manuscripts are published in the order in which they are received in final form, it may seem that some important aspects of residue analytical chemistry, biochemistry, human and animal medicine, legislation, pharmacology, physiology, regulation, and toxicology are being neglected; to the contrary, these apparent omissions are recognized, and some pertinent manuscripts are in preparation. However, the field is so large and the interests in it are so varied that the editors and the Advisory Board earnestly solicit suggestions of topics and authors to help make this international book-series even more useful and informative.

"Residue Reviews" attempts to provide concise, critical reviews of timely advances, philosophy, and significant areas of accomplished or needed endeavor in the total field of residues of these and other foreign chemicals in any segment of the environment. These reviews are either general or specific, but properly they may lie in the domains of analytical chemistry and its methodology, biochemistry, human and animal medicine, legislation, pharmacology, physiology, regulation, and toxicology; certain affairs in the realm of food technology concerned specifically with pesticide and other food-additive problems are also appropriate subject matter. The justification for the preparation of any review for this book-series is that it deals with some aspect of the many real problems arising from the presence of any "foreign" chemicals in our surroundings. Thus, manuscripts may encompass those matters, in any country, which are involved in allowing pesticide and other plant-protecting chemicals to be used safely in producing, storing, and shipping crops. Added plant or animal pest-control chemicals or their metabolites that may persist into meat and other edible animal products (milk and milk products, eggs, etc.) are also residues and are within this scope. The so-called food additives (substances deliberately added to foods for flavor, odor, appearance, etc., as well as those inadvertently added during manufacture, packaging, distribution, storage, etc.) are also considered suitable review material. In addition, contaminant chemicals added in any manner to air, water, soil or plant or animal life are within this purview and these objectives.

Manuscripts are normally contributed by invitation but suggested topics are welcome. Preliminary communication with the editors is necessary before volunteered reviews are submitted in manuscript form.

Department of Entomology
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September 1, 1976

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J.D.G.

Effects of triazine herbicides on the physiology of plants

By

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Contents

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Foreword

Soon after the discovery of the potent herbicidal activity of certain classes of *N*-alkylated diamino-*s*-triazines, it was recognized that they act through interference with photosynthesis. Photosynthesis has been and is still the center of the interest of plant physiologists, biochemists, and agronomists. It is not surprising, therefore, that *s*-triazines met quickly the attention of a large number of scientists engaged in this argument. Broad practical use of the compounds, moreover, stimulated work on their influence on plant respiration and transpiration, on the plant constituents, on plant morphology and growth, on the seed, and on the reasons for plant variability in resistance.

Some of the aspects mentioned have been reviewed already; however, no effort has been made up to now to gather together the results from the entity of the worldwide research effort documented in a great number of reports, publications, and theses. The authors of this volume merit, therefore, to be acknowledged for filling this gap. Their reviewing and their comments benefit from the fact that their active involvement in basic studies had kept them in touch with the respective scientific community for a long time. The dimension of their undertaking is illustrated by more than 700 references quoted herein.

The picture of the acquired knowledge shows in an impressive way the high degree of cross-fertilization between fundamental studies and the close observation of the phenomena occuring in the field.

Although much is known on the triazine activity now, more is still open for exploration, so especially the mode of action on the molecular level. This review will be welcome to all those who are and will be challenged by the fascinating objective of better understanding. Future research based on the knowlege of the past experience will allow them to reach new horizons.

Enrico Knuesli

Ciba-Geigy Ltd.
Basle, Switzerland May, 1975

I. Photosynthesis

Many chemicals, some having herbicidal properties, have been shown to interfere with at least one of the steps of photosynthesis. Alterations of this vital and unique process in green organisms result in injury or death of plants. Among the different reactions involved in the photosynthetic process, the Hill reaction is inhibited by many herbicides. The Hill reaction is defined as photosynthetic oxygen evolution and reduction of an electron acceptor by the photosynthetic transport chain, *i.e.*, as the light-induced electron transfer in chloroplasts from water (resulting in its oxidation to oxygen) to an added, artificial or physiological, electron acceptor (TREBST 1972). This reaction is inhibited by phenylureas, *N*-phenylcarbamates, *s*-triazines, acylanilides, uracils, some halogenated benzonitriles, imidazoles and polycyclic ureas, trifluoromethyl-benzimidazoles, pyridazinones, and aminotriazinones. Other herbicides or potential herbicides, like benziminazoles, carbonylcyanidphenyl-hydrazones, and anilinothiophenes are inhibitors or uncouplers of the photophosphorylation coupled to electron transport in the Hill reaction. The action of these or other inhibitors and the necessary general background of photosynthesis was reviewed by GOOD (1961), GOOD and IZAWA (1964), GOOD *et al.* (1966), AVRON (1966), BARTH and MICHEL (1969), BUECHEL (1972), DRABER *et al.* (1974), and TREBST (1970 and 1974), with special attention paid to the Hill reaction (MORELAND 1967 and 1969).

a) Triazine action in photosynthesis

From the early work of MORELAND and co-workers (MORELAND and HILL 1962, MORELAND *et al.* 1959, EXER 1958 and 1961), it is known that the Hill reaction with ferricyanide, Janus green, or other artificial electron acceptors by isolated chloroplasts of barley or spinach was drastically reduced by simazine. Many researchers have shown this inhibition with several triazines (EXER 1958 and 1961, GOOD 1961, GYSIN and KNUESLI 1960 a and b, MORELAND *et al.* 1959, MORELAND and HILL 1962) in isolated chloroplasts or in algae (BRAGINSKI *et al.* 1963, HOLLISTER and WALSH 1973, ZWEIG *et al.* 1963, WALSH 1972), as well as with the other herbicides mentioned above (see reviews there). In studies with intact plants, uptake of CO_2 was reduced by simazine, atrazine, simetone, ametryn, atraton, and metoprotryn in a wide series of terrestrial (ASHTON *et al.* 1960; BOLHAR-NORDENKAMPF 1970; COUCH 1966; COUCH and DAVIS 1966; FUNDERBURK and CARTER 1965; GYSIN and KNUESLI 1960; HARTISCH 1967; IMBAMBA 1970; IMBAMBA and MOSS 1971; MASHTAKOV and PROHORCIK 1966; OLECH 1968; VAN OORSCHOT 1964, 1965, 1968 and 1970 a and b; ROTH 1959; SASAKI and KOZLOWSKI 1965 and 1967 b; SIKKA and DAVIS 1969; SMITH and ILNICKI 1972 a and b; SPESIVTSEV *et al.* 1969 a and b; TIESZEN 1970; ZWEIG and ASHTON 1962) and aquatic plants (RAU and GRIMME 1971, SUTTON *et al.* 1969) which were crops and weeds either tolerant or susceptible to triazine herbicides. This inhibition of CO_2-uptake was followed by a sharp decrease of the total sugar content in the plant (KUZNETSOV 1966, LIEPINA 1969, MASHTAKOV and PROKHORCIK 1962 a and 1966, SWIETOCHOWSKI *et al.* 1966, TIMOFEVA 1967). Starch disappeared from the leaves of *Coleus* after triazine treatment as demonstrated by GAST (1958). Phytotoxicity symptoms from triazine application resulting from depletion of carbohydrates were overcome by glucose in simazine-treated barley plants (**MORELAND** *et al.* 1959). Also sucrose suppressed the disappearance of starch in *Coleus* leaves treated with triazines (GAST 1958). In wheat seedlings, the effects of atrazine, simazine, and prometryn on the enzyme activity of ribulose diphosphate carboxylase were investigated, which is considered to be a key enzyme during CO_2-assimilation. This enzyme was not influenced by the triazines (DECLEIRE and BASTIN 1973).

In a recent study, VAN OORSCHOT (1974) investigated the effect of CO_2-free air and CO_2-free nitrogen on the initiation of phytotoxicity symptoms in *Phaseolus vulgaris*. He found that CO_2-free air increased the degree of photosynthetic inhibition in the plants with exposure time and with light intensity. CO_2-free nitrogen delayed the beginning of injury symptoms. From his results he concluded that a lack of carbohydrates is not the only cause of the herbicide action.

These results together with the inhibition of the Hill reaction by *s*-triazines suggested that the action of the herbicide is located at the early steps in the photochemical conversion of energy during the photosynthetic process but before the biosynthesis of saccharides. The exact location of the inhibitory site of most of the herbicides has developed from studies with the herbicide DCMU which have been extended to triazines, namely:

—photoreduction and CO_2-reduction of H_2-adapted algae were not inhibited by amino-triazines (BISHOP 1962),

—O_2-evolution, photoreduction and photophosphorylation were inhibited by *s*-triazines with ferricyanide or FMN as electron acceptors (GOOD 1961),

—PMS-mediated cyclic phosphorylation was not sensitive to triazines (GOOD 1961), and

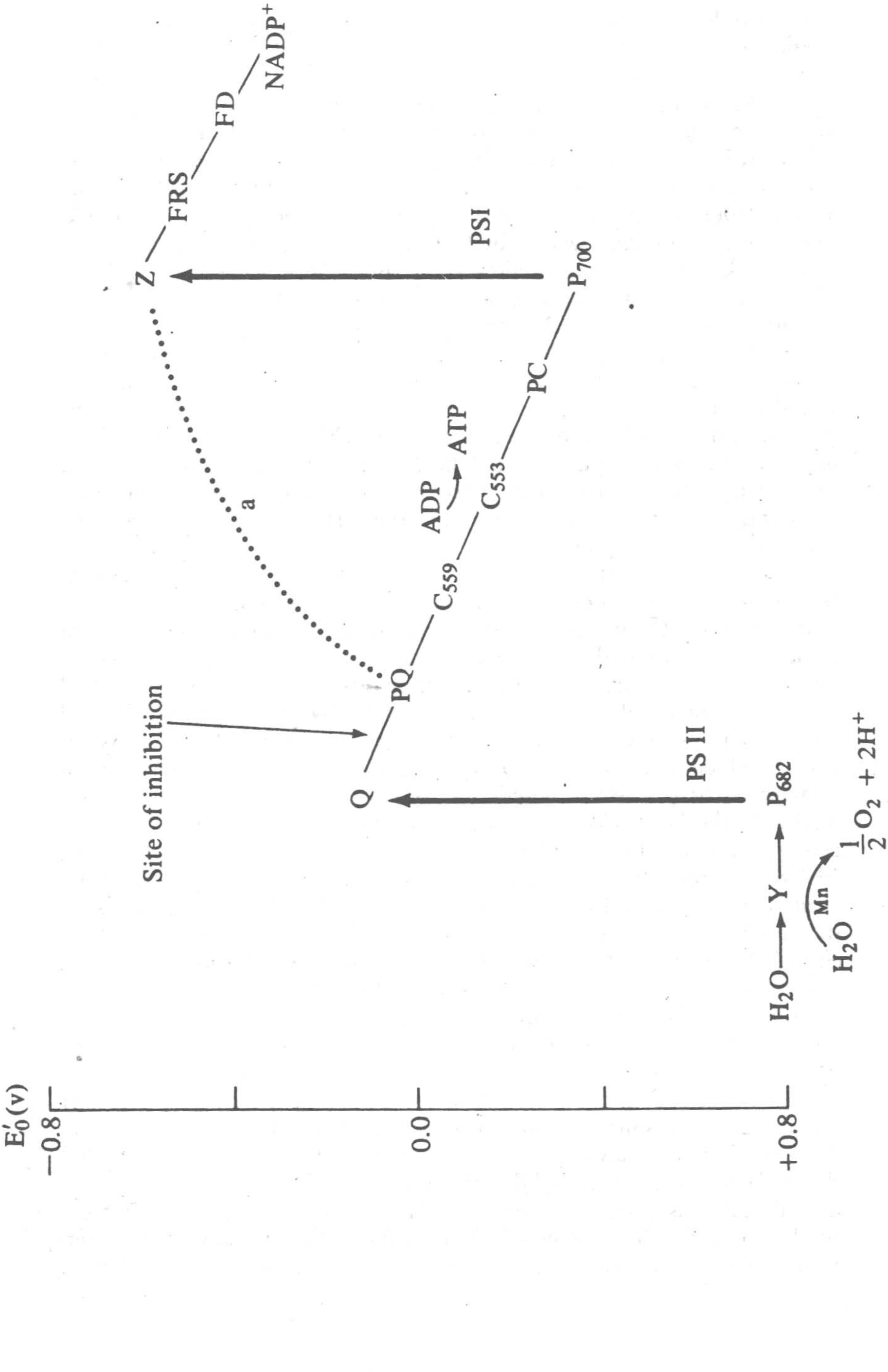
—noncyclic photophosphorylation can be restored by an electron donor for photosystem I.

Therefore, inhibition of CO_2-evolution, noncyclic electron flow, and phosphorylation occur only when water is the electron donor. Consequently, the mechanism of inhibition involved should be the one responsible for the oxidation of water to molecular oxygen (BISHOP 1962, GOOD 1961, HILTON *et al.* 1963). Reviews and detailed discussion of primary processes and energy transfer have been presented by FORK and AMESZ (1969), HOCH and KNOX (1968), KOK and CHENIAE (1966), and more recently by CHENIAE (1970) and TREBST (1974).

The physiological and biochemical studies on the location of the inhibitory site of the herbicides in the photosynthetic water-splitting reaction suggested an inhibition of photosynthetic electron transport close to photosystem II.

Biophysical studies on fluorescence emission, action spectra, intensity yield, life time and polarization, and fast oxygen evolution measurements gave detailed information on the reaction mechanism and sequence of carriers around photosystem II including information on energy transfer, distribution of pigments within the photochemical centers, and action of added chemicals. In the presence of an artificial electron acceptor, fluoresence of algae at a wavelength above 650 nm under incident light ($\lambda = 475$ nm) presents the following pattern: first, fluorescence rises rapidly after the light is turned on, then increases to a maximum, and finally drops slowly to reach a steady state (LAVOREL 1959 and 1962). Simazine and atrazine were found to increase the fluorescence intensity of *Chlorella*, and no decline followed the rise of fluorescence (ZWEIG *et al.* 1963). The magnitude of increase of fluorescence intensity paralleled the inhibitor potencies of the 2 chemicals on oxygen evolution, *i.e.*, the pI_{50} correspond to the pF_{50} (quantitative evaluation of 50% fluorescence increase). In spinach chloroplasts, MURATA *et al.* (1966 a and b) reported a slightly different situation: no maximum of fluorescence appeared in the transient phase, and fluorescence intensity reached a steady state without any previous rise; simazine had different effects on the time course of fluorescence, *i.e.*, prolongation of the transient phase at low concentrations and its shortening at high concentrations. However, no interpretation was given of this last point.

Since atrazine and simazine change the time-course of fluorescence, this would mean either a direct interaction with chlorophyll in the reaction center II, presumably at the acceptor site of photosystem II, or some interference with the donor site. In the first case triazines would interfere with the flow of electrons from water to excited chlorophyll molecules which will then remain with their electron holes unfilled and will bleach (VAN OVERBEEK 1962). This would also explain the increase of toxicity which parallels the increase of light intensity as experienced with atrazine (ASHTON 1962) showing an action spectrum similar to chlorophylls. In the latter case, triazines would interfere with the electron acceptor of photosystem II. This acceptor termed *Q* (see Fig. 1) (DUYSENS 1963) could react by quenching the fluorescence of chlorophyll (KAUTSKY *et al.* 1960, LAVOREL 1959 and 1962) and would be converted to a substance which may reduce in turn another component of the "cytochrom" chain. By using plastids isolated from the red alga *Porphyricium* sp., NISHIMURA and TAKAMIYA (1966) showed that reduction of cytochrom$_{553}$ by light absorbed by phycobilins was inhibited by simazine. Applying the interpretation of 2 photochemical systems proposed by DUYSENS and AMESZ (1962) and DUYSENS *et al.* (1961) to this type of red algae, the authors suggested that the reduction of cytochrom$_{553}$ by photosystem II was inhibited by simazine, but not its oxidation by photosystem I. From these results and analysis of fluorescence data of chlorophyll in isolated chloroplasts, it was suggested that the simazine as DCMU inhibited one or several reactions close to photosystem II (MURATA *et al.* 1966 a and b). As shown in the "Z"-scheme of electron flow (Fig. 1) developed later, we can now locate the photosystem II further back in the oxidation-


E'_0 (v)
−0.8
0.0
+0.8
Z
FRS
FD
$NADP^+$
PSI
P_{700}
PC
C_{553}
ADP
ATP
C_{559}
PQ
a
Site of inhibition
Q
PS II
P_{682}
H_2O
Y
Mn
H_2O
$\frac{1}{2}O_2 + 2H^+$

reduction process. The site of action of *s*-triazines could be between water and cytochrom$_{553}$.

From all the experimental data discussed, it is derived that the *s*-triazines, like DCMU, are considered as inhibitors of the acceptor site of photosystem II and would inhibit the reduction of the acceptor of photosystem II at the expense of water oxidation as well as at the expense of oxidation of artificial electron donors for photosystem II (Duysens 1963). The exact location of the inhibiting site would be between the primary quencher *Q* of photosystem II and plastoquinone pool.

Van Rensen (1971) suggested also a competition between simetone and a substance close to *Q* which accepts the electron back from cyclic electron flow. He proposed a competition between simetone and the oxidized form of this "substance" and *Q*. This was evidenced by a decrease of simetone toxicity with light intensity, some consistent inhibition of cyclic photophosphorylation, a requirement of two electron donors (*Q* and the "substance") during cyclic phosphorylation under white light, and an increase of simetone toxicity in red light compared to that under white light conditions. The substance in question was tentatively identified as plastoquinone, which could react in its oxidized form with simetone by forming hydrogen bonds. However, no direct experimental evidence supported such a hydrogen bonding hypothesis. Other experiments seem to suggest that inhibition or an additional inhibition takes place before photosystem II (Etienne 1974, Renger 1973).

Present findings on possible interactions of atrazine with manganese emphasizes this hypothesis from another point of view. It was shown in physiological experiments that atrazine phytotoxicity was enhanced by phosphorus in cotton and soybeans (Adams and Espinoza 1969, Adams *et al.* 1971). Increase of P-uptake did not increase accumulation of atrazine in soybean green tissues, but it induced a manganese (Mn) deficiency (Adams *et al.* 1971, Sun and Adams 1971). By increasing the Mn level in the nutrient solution, inhibition of photosynthesis by atrazine could be delayed (Sun and Adams 1971). Therefore, it was suggested

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Fig. 1. Photosynthetic electron flow from water to NADP:
PS I and PS II = photosystems I and II, respectively
Y = natural electron donor of PS II
P_{682} = trap pigment of PS II, a form of chlorophyll absorbing at 682 nm
P_{700} = trap pigment of PS I, a form of chlorophyll absorbing at 700 nm
Q = natural quencher of fluorescence from PS II, primary acceptor of electrons coming from PS II
PQ = plastoquinone
PC = plastocyanin
Z = primary acceptor of PS I, possibly non-heme iron
FRS = ferredoxin reducing substance, function not completely clear
FD = ferredoxin
C_{559} = cytochrom b_{559}, exact location unclear
C_{553} = cytochrom$_{553}$ or cytochrom f
a = cyclic electron flow *in vivo*

that atrazine may block photosynthesis by interacting with the level of chloroplast Mn. The role of Mn in the oxygen evolution system has been discussed and reviewed by GAVALAS and CLARK (1971), CHENIAE and MARTIN (1968), and CHENIAE (1970). A definition of the exact size of the photochemical complex *E* in connection with triazine-inhibited site would be valuable for the understanding of the target of action at the molecular level. RENGER (1970) proposed a model for the natural donor of photosystem II termed *D* or *D II* in which 6 Mn ions were bound. The theoretical value would be 8 Mn/unit of photosystem II, which has been estimated to be around one/250 to 500 chlorophyll molecules (CHENIAE and MARTIN 1968). The site of triazine inhibition would be in one equivalent of an oxygen-evolving unit composed of 250 to 500 chlorophylls and 8 Mn ions. These values are in the same order as the quantasome (BRANTON 1968, PARK and BIGGINS 1964) as suggested by MORELAND (1965) in an oxygen-unit concept, except that the number of Mn ions seems to be different. Triazines may not act only at the Mn level of Y because O_2 evolution was reduced in Mn-deficient chloroplasts or algae but not photoreduction (SPENCER and POSSINGHAM 1961). This fact stresses the possible plurality of sites of actions for triazine herbicides.

Also, attempts have been made from physiological studies to explain the phytotoxic action of the triazine herbicides from interference with photosynthesis. The photosynthetic inhibition in *Phaseolus vulgaris* following low and high pre- and posttreatment levels of illumination was followed by VAN OORSCHOT and VAN LEEUWEN (1974). They noted an increased inhibition of net photosynthesis at the lower pretreatment and the higher posttreatment levels of illumination, which the authors ascribe to an enhanced photo-oxidation. ASHTON (1962 and 1965) reported a direct relationship between light intensity and the degree of injury which atrazine and monuron produced in oats and red kidney beans. With increasing amounts of light the toxicity of the herbicide increased. The action spectrum of the phytotoxicity by atrazine fits with the action spectrum of the chlorophylls in photosynthesis, showing a maximum of injury occurring at 428 nm and 658 nm. These results have been interpreted to mean that triazines involve chlorophylls a and b in forming phytotoxic substances which are formed upon illumination. This hypothesis had already been proposed by SWEETSER *et al.* (1961) investigating the mode of action of the phenylureas. However, the phytotoxic intermediates in question have not been characterized.

One of the consequences of the destruction of the photosynthetic apparatus which is visualized by electron microscopy studies (ASHTON *et al.* 1963 b and 1966, HILL *et al.* 1968) is the decrease of the chlorophyll content (BRUINSMA 1965, KALININ and PONOMAREV 1963, MASHTAKOV and PROHORCIK 1962 b, SABUROVA and PETUNOVA 1965, SIVTSEV and KUZNETSOVA 1973, SWIETOCHOWSKI *et al.* 1966) as evidenced by a chlorosis of leaves of triazine-treated plants. Atrazine reduces both the chlorophyll a and the chlorophyll b contents (FREEMAN and RENNEY 1966). No direct