*Mode of action of simazine [2-Chloro-4, 6-bis (ethylamino) S-triazine] in relation to Phytotoxicity.

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Many investigations have been conducted on hormonal herbicides like 2, 4-Dichlorophenoxy acetic acid and similar compounds but relatively few studies have been made on 2-chloro 4, 6-bis (ethylamino)-S-triazine (simazine). The general processes of absorption, translocation, growth regulation, and mechanism of herbicidal action of a broad spectrum of herbicides have been reviewed recently (9, 10, 22, 25, 30, 33, 37). Most of the investigations with simazine have dealt primarily with corn and have been largely concerned with symptomatology and toxicology. The chemistry, absorption, translocation, metabolism and toxicity of triazine herbicides have been summarised (17, 26). A few investigations have dealt wholly with the absorption and translocation aspects of simazine (6, 11, 31). It was established by Exer (12) and Moreland *et al.* (27) that simazine acts as a strong inhibitor of the Hill reaction (Photolysis of water). A series of studies by Gysin and associates (16 & 17) have given Impetus to relating chemical structure to phytotoxicity.

MATERIALS AND METHODS

These studies were conducted in the Botany and Plant Pathology Department of Kansas State University at Manhattan (Kans.) U.S.A. Plants were grown in green houses of the department under controlled light and temperatures.

Species used were wheat (*Triticum aestivum*, Linn Var. Large grey). Both represent relatively susceptible species compared with corn (*Zea mays*, Linn.). Their morphology and physiology have been studied (21, 24, 28).

Chemicals : The two labelled atoms C and Cl and the molecular position of labelling in the simazine molecule were the determining factors in selecting

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isotopes employed. The three radio active isotopes used in the tracer studies were (a) ^{14}C-ring-labeled simazine (b) ^{36}Cl-labelled simazine and (c) ^{14}C-ring labeled 2-hydroxy simazine. All the above isotopes are long life beta emitters. Solutions of the compounds were prepared using deionized distilled water. Because simazine is only slightly protractedly soluble in water, it was first dissolved in redistilled chloroform which was later evaporated off the aqueous solution with an air stream.

Treatments of plants : All studies involved root absorption only. Stock solutions of the three labelled compounds were prepared to give a concentration of 5 mg/litre. Twenty ml. of a stock solution (having a level of 1 2 μc. radioactivity per jar in the ^{14}C-ring-labelled simazine, and the ^{14}C-ring-labelled hydroxy simazine forms, and 0.6 μc per jar in Cl-labelled form were introduced into half strength Hoagland No. 2 solution. Low levels of radio activity were used to avoid radiation damage to the test species Thus twenty ml of aqueous solution of the labeled form was added to 480 ml. The ten day old wheat or sun flower seedlings were transferred to each of the radio active solutions for study. Harvested plants were used either for auto radiography or for counting and chromatography.

The distribution of the isotopic material in the treated plant was determined by auto-radiography; the quantitative data, by counting with a liquid scintillation spectrometer, and metabolites were identified by Co-Chromatography. The autoradiogrophic technique used was that of Crafts and Yamaguchi (9) except that plant materials were exposed to X-ray film at low temperatures (-2°C in a refrigerator without freeze drying). At harvesf plants were blotted free of excess solution and covered with a saranwrap sheet. They were then placed in contact with Kodak-No-screen-X-ray-film, and exposed for three weeks in the dark.

A tricarb liquid scintillation spectrometer, model 314-DC was used to obtain quantitative determinations of radioactive simazine. The designation sample, as used here denotes the vial+the solvent+the scintillation+the tagged activity. All chemicals (Scintillation grade) and the low potassium glass vial (20 ml capacity) were obtained from the Packard Instruments Company For non-aqueous extractions the solvent solution was dioxine (spectro) one litre, PPO 7g, POPOP 50 mg. and naphthalene 100 gms). Material preparation for such countings involved methanol extraction and centrifugation. At designated times, plants were removed, roots blotted dry, fresh weights of roots and shoots were taken and these plant parts extracted separately by boiling 5 minutes in

methanol. These plant parts were then homogenised for 10 minutes. The homogenate was obtained by straining through cheese cloth followed by centrifugation for 10 minutes at 10,000 G. The supernatent of the homogenate was further reduced under vacuum to a ratio of 1 gm fresh weight to 4 ml of methanol by means of a centrifugal biodryer These reduced extracts were used for all quantitative determinations of both 14 C-ring and 36 Cl-labelled forms of radio active simazine and also for spotting on chromatographic paper to identify metabolites.

Aliquots (100 μl) of the methanol extract were used for radioassay by placing in 10 ml of the proper scintillating solution in a low potassium glass vial. Calibration checks using standard samples, as well as back ground counts using blanks scintillator solutions were made prior to each counting period. Net radio activitv was plotted against time and the curves obtained were used to interpret accumulation as related to metabolic changes. The autoradiograms were obtained by exposing a chromatogram to Kodak-No-Screen-X-Ray film for four weeks.

Limited studies were directed towards alleviating simazine toxicity in wheat plants by supplying a 6% aqueous solution of glucose. The solution was supplied to the wheat plant through a mature leaf (the leaf of which had been excised) by immersing it in the solution.

Because simazine is considered to inhibit the Hill reaction by blocking oxygen evolution, the effect of spraying a 0 5% aqueous solution of riboflavin 5 phosphate to simazine treated wheat seedlings was observed. The spray application, which covers the entire shoot of the seedling, was repeated for four consecutive days. In a few cases the 6% glucose feeding and the riboflavin 5 phosphate spraying were combined.

RESULTS AND DISCUSSION

Toxicity in relation to light and darkness : Corn, wheat sunfiower and cotton seedlings were grown in darkness for seven days in 5 mg/litre unlabeled simazine solution showed no visible signs of simazine toxicity. In light (summer green house conditions) several toxic symptoms developed in the seedlings after seven days and eventuallv the seedlings died. The inability of simazine to induce toxicity and death in darkness, indicated that it acted as a photosynthetic poision which confirms reports by Gast (14) and Gysin and Knusli (17).

Symptoms of simazine toxicity in wheat seedlings : There were no differences between unlabelled and labled forms of simazine at the same concentration applied through a solution culture either in appearance, time, symptoms developed or time of ultimate death. No visible symptoms in either control or experimental plants were observed in the first seven days. On the eighth day leaf tip of treated plants turned brownish and appeared slightly burned. Such plants were also some what stunted as the emergence of the third leaf was delayed. The discolouration accompanied by dehydration slowly progressed from tip to base and death occured on the 21st day. The nature and sequence of symptoms were those of wilted plants. Roots of both control and experimental plants were normal, the first seven days afrer being treated but were then inhibited in treated plants.

Symptoms of simazine toxicity in sunflower seedlings : Sunflower exhibited symptoms earlier and death resulted sooner. Visible symptoms appeared on the fourth day after treatment as white necrotic areas along leaf margins. Those areas dried slowly and became brittle and slowly progressed inward Such areas were originally deep green but soon turned a brownish hue. Older leaves exhibited both symptoms first as they died Younger leaves and bud scales developed similar symptoms. No visible effect was observed on roots, the first four days after treatment after which all root development was inhibited in treated plants.

Three hours after being treated the labeled isotope had moved in limited quantities to plant shoots and had begun to accumulate at tips of wheat and margins of sunflower leaves. Marked accumulation in those areas began three days following treatment and progressively continued until death.

Distribution patterns with ^{36}Cl-labeled simazine in wheat and sunflower seedlings : Gross distribution patterns of ^{36}Cl were identical with those of the ^{14}C-ring labeled from in wheat but not in sunflower. In sunflower interveinal and marginai accumulation occured simulataneously. Consequently wheat seedlings lived 28 days and the sun flower 35 days so life spans were extended seven days in wheat and 22 days in sun flower by winter conditions. The autoradiogram made from a sun flower plant 30 days after simazine application showed low accumulation of the chemical even at leaf margins and plant attained considerable size. Both findings indicate slow absorption of simazine. Under growth chamber conditions absorption rate in both treated and control plants was quite rapid.

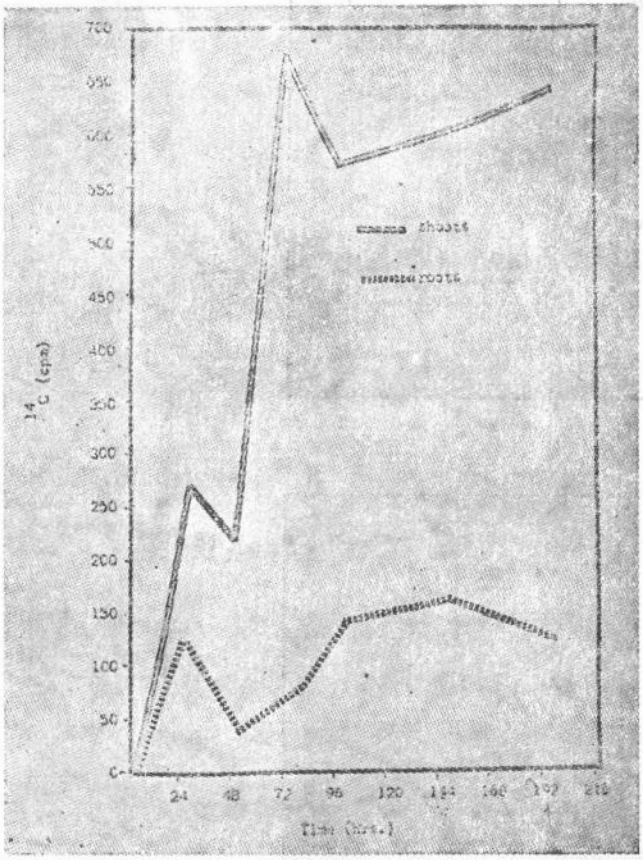

Fig. 1
Curve showing the uptake of ^{14}C-ring labeled simazine by wheat plant.

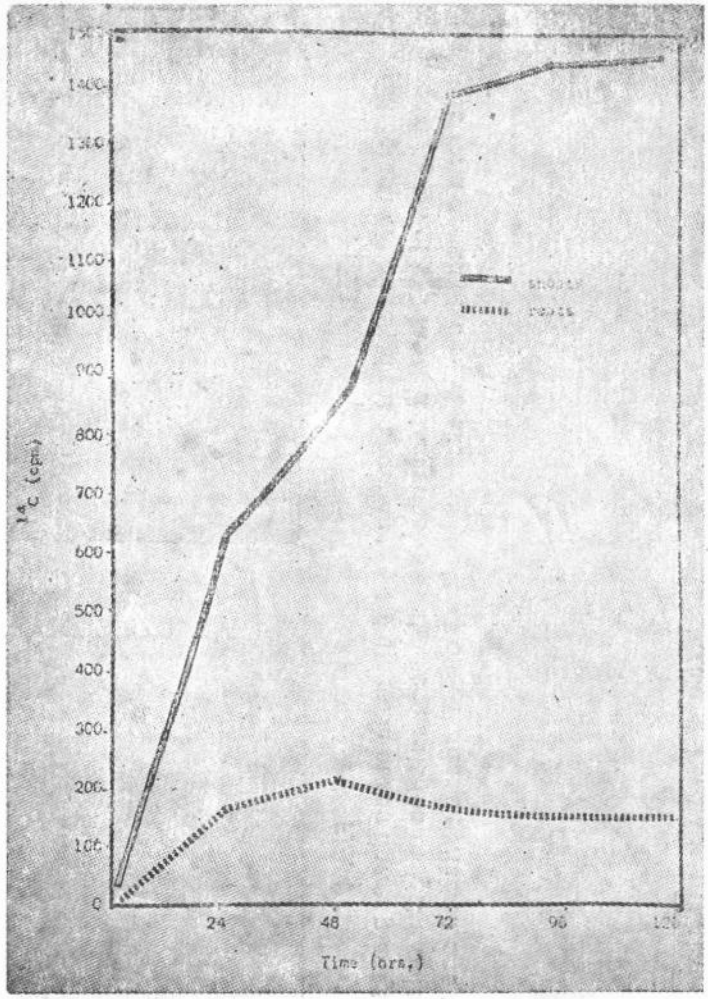

Fig. 2
Curve showing the uptake of ^{14}C-ring labeled simazine by the sun flower plant.

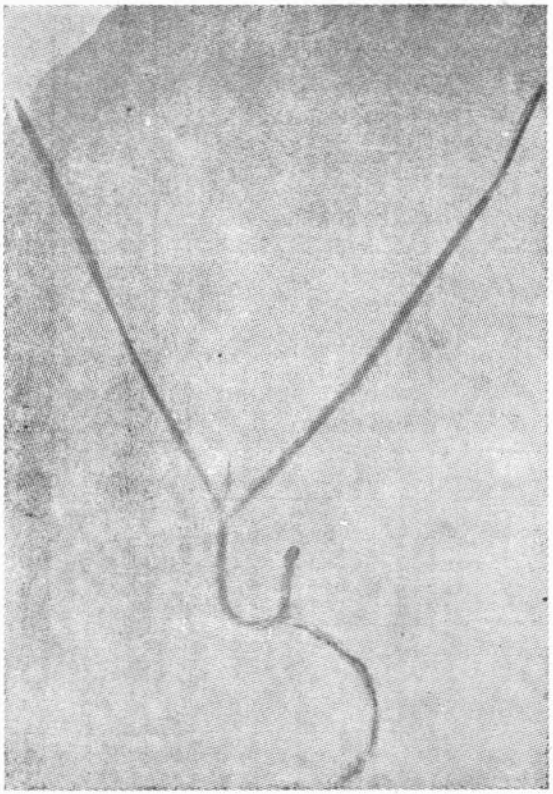

Fig. 3
Auto radiograph of wheat plant showing the accumulation of ^{14}C labeled simazine fed for a period of one week.

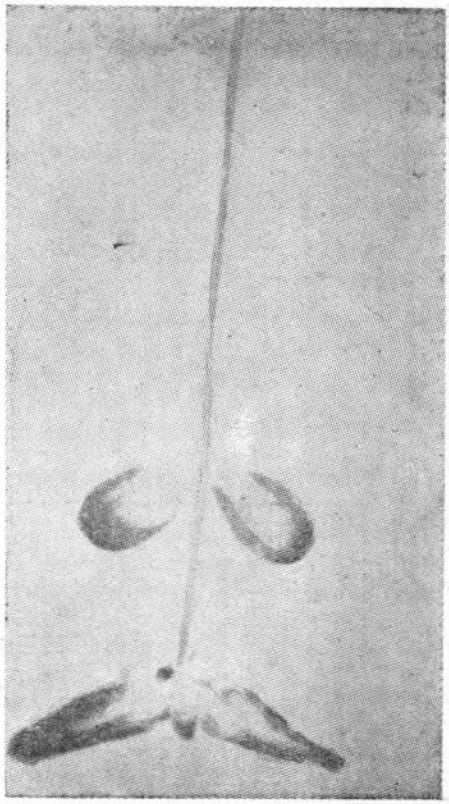

Fig. 4
Autograph of sun flower plant showing the accumulation of ^{14}C labeled simazine fed for a period of one week.

Effect of ^{14}C–ring labeled simazine on *Elodea* : To investigate further the relation of transpirational rate to translatory movement of and hence time and extent of toxicity development of simazine, a subsidiary experiment was conducted using water weed, *Elodea densa* (planch) easpary. One set of terminal stem was submerged in tap water while the second was submerged in tap water containing 1.2 μc. of ^{14}C-ring labeled simazine. After 35 days of such treatment, those in tap water were normal in appearance while treated ones were slightly burned at the tips without degradation of the Chloroplasts. However, inhibition was observed in the terminal bud growth of the treated clones. It can be inferred from our subsidiary study that movement and lethal accumulation of simazine chiefly depended on transpirational rate. In our study all evidence indicated that the upward movement of simazine into and through the shoot dependent on transporation and that the amount translocated is approximately related to transpirational rate. Our findings regarding translocation of and sites of accumulation of, the herbicide completely agree with those reported by Gysin and Knusli (17), Berezoski and Kerakkai (3) and Sheets (31).

Our results with *Elodea*, however, do not support that widely held assumption by herbicidal physiologists that amitrole is a photosynthetic poison, Castelfranco and Bisalputra (5) as are monuron and simazine. In our studies simazine failed to induce chlorosis in newly formed *Elodea* buds.

Distribution patterns with ^{14}C-ring labeled 2-hydroxy simazine in wheat and sunflower seedling : Autoradiographic studies, however, showed that while in sunflower, there was no evidence of absorption of hydroxy simazine for at least 30 days, wheat had considerable uptake with uniform distribution by that time. Wheat's general distribution contrasted with leaf tip (wheat) and leaf margin (sunflower) accumulation of the 2 toxic isotopes of simazine. Results indicate that sun flower seedlings did not absorb hydroxy simazine while. wheat seedlings did absorb atleast some of the compound. It is possible that replacing the chlorine atom of the simazine molecule by a hydroxy group caused less absorption as a result of reduced membrane permeability. Another factor is the non toxic nature of the hydroxy simazine occuring within the living plant as the result of metabolic hydrolysis of simazine which had entered the plant through its roots.

In the sun flower the data showed nearly a linear curve but a plateau developed shortly before death of the plant. The lack of a signoid pattern suggests that degradative process is less marked in this species. The data, indicate,

however, that sunflower's absorption rate is greater than wheat's. The variation in absorption rate may represent a species difference, or it could result from greater leaf area in the comparably aged sunflower seedling causing accelerated transpiration in sunflower plants.

Accumulation studies involving ^{36}Cl-labeled simazine extracted with methanol showed a constant amount of simazine (22 cpm) Autoradiographic evidence, contrariwise showed a progressive increase in the accumulation of this isotopic form with time that ultimately killed seedlings of both species. The crude plant extract however showed a much higher count (480 cpm) than the extract obtained using any of the commonly employed solvents (methanol, chloroform, or 80% ethanol) individually. Failure to obtain an extract of this isotope with any of those solvents (or in water which was used because of the possibility of the isotope being converted to the chloride form) indicated that simazine molecule labeled with ^{36}Cl has either been split or conjugated by metabolic process in the plant. Although the symptomology and gross distributions of the ^{14}C-ring and ^{36}Cl-labeled isotopes are identical, the metabolic fate of the 2 forms is apparently different. Additional critical work with the ^{36}Cl-labeled isotopic form to establish its mode of action is needed. It promises greater return than similar work with the ^{14}C-ring labeled.

Detoxication studies : Experiments were conducted to ascertain potentialities of wheat and sun flower seedlings to detoxify simazine. They were fed varying levels of simazine and then were transferred to half strength nutrient solutions free of simazine. Ten day old seedlings of both wheat and sunflower were grown in a nutrient solution containing ^{14}C-ring labeled simazine removed after 24 or 72 hours the roots thoroughly washed with distilled water and transferred to a nutrient solution simazine free.

Results with wheat and sunflower seedlings : The seedlings of both the species were apparently capable of recovering so long as there was no visible injury to the apical meristem. In wheat seedlings grown in ^{14}C-ring labeled simazine (at a level of 1.2 μc per jar) for either 24 or 72 hours no radioactive materials move for at least 15 days into the new growth formed after transfer to a simazine free nutrient solution. Certain seedlings were studied for 30 days and no visual evidence was obtained of movement of radioactive materials into the tissues formed after transfer. Further more complete metabolic degradation had occured. Rate and extent of recovery of treated plants in simazine free nutrient solution were correlated with concentration of simazine in the wheat seedling as measured by liquid scintillation counting. Thus a wheat seedling

ings dies 21st Day and sunflower seedlings died by 13th day. Both were capable of recovery from simazine toxicity when transfered from simazine containing nutrient solution to simazine free nutrient solution prior to visible injury of their apical meristems. Autoradiographic evidence indicated no recirculation of simazine in toxic concentrations in either species. Feeding of the 6% glucose solution to a simazine treated wheat seedling through its leaf delayed development of toxic symptoms for 4 days. Feeding 6% glucose solution through a leaf combined with spraying the shoot with 0.5% solution with riboflavin 5 phosphate delayed development of toxicity in wheat seedlings for 15 days. Both species completely degraded ^{14}C-ring labeled simazine under conditions of a limited concentration of isotope within the plant as shown by both autoradiography and liquid scintillation. Presence of the chlorine atom in simazine molecule was found to be crucial for the development of toxicity in plants. Replacement of the chlorine atom by the hydroxyl group prevented toxicity development.

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