THE MODE OF ACTION OF MONURON
(3- (4-chlorophenyl) -1,1- dimethylurea)

by

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ABSTRACT

The herbicidal action of monuron, 3-(4-chlorophenyl)-1, l-dimethylurea, cannot be completely realized until the mode of action is fully understood. It is believed that the primary site of action is in the photosynthetic complex.

The present investigations included: The study of the effects of monuron on some enzyme systems, greenhouse studies on the effects of monuron on the morphology of potato plants, field studies on the yield of barley treated with the substituted urea herbicides, the residual effects of the substituted urea herbicides applied to soil, the effects of monuron and the interaction of monuron and vitamin K on the rate of the photolysis of water by isolated chloroplasts.

The photolysis of water was followed by observing changes in potential of a potasium ferricyanide solution containing isolated chloroplasts.

Monuron inhibited the protease enzyme system and stimulated the lipase enzyme system. This herbicide reduced the total weight of potato tubers per plant, the root to top ratio and the total weight of barley grain per acre. The top growth of potato plants and the bushel weight of barley were increased with monuron applications.

 1×10^{-7} moles of monuron reduced the rate of the Hill reaction by more than 50%. The data presented did not confirm vitamin K as a cofactor in the Hill reaction.

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INTRODUCTION

Monuron has become a widely used herbicide. The mode of action of this herbicide is, at present, only partly understood. Until this is completely understood the potential uses of monuron will not be fully exploited.

An attempt has been made in this essay to clarify some of the aspects of monuron's phototoxicity, particularly the inhibition of photosynthesis caused by low concentrations of monuron.

REVIEW OF THE LITERATURE

Monuron, 3- (4-chlorophenyl) -1,1- dimethylurea, first described in 1951 by Bucha and Todd (8) is one member of the substituted urea herbicides. Other commonly marketed substituted urea herbicides include diuron, fenuron and neburon. These herbicides are products of the E.I. du Pont de Nemours Co.

Monuron is a soil sterilant at high rates of application (10-40 lbs. per acre) and it is used as a selective herbicide in certain crops at lower rates (1.1/2 - 2 lbs. per acre). The selective herbicidal use of monuron is now largely being replaced by diuron, 3-(3,4- dichlorophenyl)-1,1- dimethylurea.

The soil pH has a marked effect on the phytotoxicity of the substituted urea herbicides as it effects adsorption on the soil colloids-(10). In water solutions the substituted urea herbicides have a net positive charge (10). Monuron is lost from the soil by leaching, breakdown by soil microorganisms and by photodecomposition. Photodecomposition is especially prevalent in areas of limited rainfall. (15,29,33,37).

Monuron causes one or more of the following symptoms to appear on leaves of susceptible plants: water soak blotch, wilt, petiole and/or stem collapse, indeterminated grey blotch, abscission, rapid yellowing and partial chlorosis. (25). The margins of the older leaves are the first to show chlorosis, - the chlorosis then proceeds towards the centre until the entire leaf is chlorotic.

Barley treated with monuron at 2 lbs. per acre had a deeper green colour than did the controls. (9). Maturation of oats was delayed by several days by the application of monuron at the 2 lb. rate. The same result was noted although less pronounced with barley and wheat. (32). Monuron appears to have some effect on tillering in some species. This has been found in sugar cane and to a small degree in barley. (32).

The 1000 kernel weight of winter barley was increased and the production of anthocyanins was reduced following applications of monuron.

(32).

Excised roots may be grown in nutrient solutions containing lethal quantities of monuron. (26). This suggests that this herbicide is phytotoxic primarily to the aerial portions of the plant.

Monuron enters the plant system most rapidly through the roots, although it appears possible to kill plants by leaf applications and in some cases through stem applications. (12,19,26). Fang and his associates, (12) have found that when monuron, labelled with C_{14} is applied to a leaf 10% of the C_{14} to be translocated throughout the plant very rapidly. The rate of monuron entry into the foliage depends on the thickness of the cuticle. (26). Following the application of C_{14} labelled monuron to the foliage, two compounds containing C_{14} occur. One of these compounds is monuron the second has not yet been identified. It is believed that the

unidentified compound containing C_{14} is the result of the plant incorporating monuron into its metabolism. This belief is supported by the fact that the concentration of the unidentified C_{14} labelled product increases with time, while the concentration of the C_{14} labelled monuron decreases. (12).

Translocation of monuron takes place largely through the xylem tissue, although as indicated above some translocation may occur in the phloem. (26).

Plants grown in monuron treated soil are more subject to injury if post treatment conditions favour rapid transpiration. (23,26). This indeed would be expected as the main site of monuron entry is through the roots and monuron is translocated primarily via the xylem.

Monuron causes no gross morphological changes but does cause some abnormalities on the cellular level. (17). There is no cell proliferation (26), but there is a collapse of the cambium, a disorganization of the phloem, mesophyll and pallisade cells and a decrease in the number of xylem cells. (9). The last mentioned phenomena may be the result of the disorganized cambium or a result of some change in the biochemical constituents of the cell. It has been suggested that the pH within the plant may be altered by the presence of monuron. (32). Many workers believe that the pH determines the production of xylem and phloem from the cambium layer. It is interesting to note that anthocyanin production within some species is altered in the presence of monuron, and that anthocyanin production is also believed to be related to the internal pH of the plant.

Mitosis is retarded by monuron in barley and onion meristems (9) and is arrested at the resting stage in the onion root tip. (16). A break-

down of the nucleus has been observed in onion roots following treatment.

(9).

Minshall and McLarty (21) found that low concentrations of monuron stimulated root growth in several species, while higher concentrations retarded root growth. It was also noted by these workers that the surface cells in the region of elongation were disrupted.

The metabolic pathways within monuron treated plants are somewhat altered. The nitrogen and protein balance is disturbed. The nitrate and the ammonia nitrogen content is lower and the protein content higher in treated plants as compared to non treated plants, (13,30) while the carbohydrate content is reduced. (13).

The phosphorous content has been observed to be increased in some species (36) and the percentage ash appears to be increased in barley following monuron treatments. (32).

At the time the first monuron symptoms become visible, growth has completely stopped. (27). In treated velvet beans, the dry weight of plants was at a maximum four days following treatment; thereafter the dry weight diminished until the eighteenth day when the plants were dead. (28). During this experiment it was found that the monuron content of the leaves increased to a maximum at seven days after treatment and thereafter decreased. The decrease may be due to the breakdown of the herbicide by the plant as suggested earlier.

Using excised bean leaves, Minshall found that from fifteen to twenty micrograms per gram of leaf material reduced the dry weight production by 90%. (24). Less monuron was required to inhibit the dry weight production in young leaves as compared to the mature leaves.

Earlier Minshall found that 17, 30, 48 & 95 micrograms of monuron per gram

of leaf material inhibited the production of dry matter by 89, 100, 100 & 100% respectively and that transpiration was inhibited by 38, 57, 67 & 70% respectively. (22). From this work he suggested that monuron may act as a photosynthetic poison.

In the same year Cooke (11) found that low concentrations $(1 \times 10^{-7} \text{ moles})$ of monuron inhibited the Hill reaction, ie. the photolysis of water, and, Wessels and van der Veen (41) likewise found that this low concentration of monuron inhibited photosynthesis. The latter workers also reported that the inhibition of photosynthesis was localized, indicating that there was little movement of the herbicide within the leaf once the site of action was reached. This does not agree with the results of other workers discussed earlier. Wessels and van der Veen also found that the photosynthetic activity could be retained by washing away the monuron and they assume that the monuron is adsorbed to the cyclopentanone ring of the chlorophyll molecule. The cyclopentanone ring is referred to as the active site.

Abel (1) as well as Coggins and Crafts (10) refer to the work of Wessels and van der Veen and support the hypothesis that monuron is adsorbed to the active site of the chlorophyll molecule. It is believed that all of the substituted urea herbicides behave in this manner - diuron being the most effective, followed by monuron and fenuron. Wessels and van der Veen assign a relative activity for these herbicides of diuron 2500, monuron 125, and fenuron 12.5. (41). The comparative effectiveness of neburon is not known.

Neburon is adsorbed most strongly to cellulose particles, followed by diuron, monuron and fenuron in that order. (10).

Wessels and van der Veen have postulated that vitamin K is the

energy acceptor of the light excited chlorophyll molecules. These workers have found that diuron, in a concentration just sufficient to inhibit photosynthesis and vitamin K occur in nearly the same concentration - that is, about one hundredth of the concentration of chlorophyll molecules. They postulate further that the remaining chlorophyll molecules are either enolized or chelated and thus prevented from being biologically active.

As the major part of the experimental work which follows involves photosynthetic responses it was thought advisable to review briefly the present theory concerning photosynthesis.

Photosynthesis is the conversion of radiant energy to chemical energy. No life on earth would be possible without this energy conversion group of reactions.

Photosynthesis, until quite recently, was thought of as a simple, yet unresolved reaction or series of reactions that were represented by the following equation: $6 \text{ CO}_2 + 6\text{H}_2\text{O} \longrightarrow \text{C6H}_{12} \text{ O}_6 + 6\text{O}_2$. It is assumed that by some mechanism six atoms of carbon from atmospheric carbon dioxide were attached to six molecules of water. At the same time the six molecules of the newly formed carbohydrate fused to form a hexose sugar.

It was sometime later that the workers queried the presence of the 3, 4, 5 and 7 carbon sugars that were found in plant material. It was then thought that photosynthesis may be best represented by the equation: $nCO_2 + 2nH_2O + 1$ ight energy \longrightarrow $(CH_2O)n + nO_2 + nH_2O$

This reaction, referred to as the Hill reaction, or the photolysis of water, may be followed by using an artificial hydrogen acceptor in place of carbon dioxide. Frequently used hydrogen acceptors are ferricyanide and benzoquinone. These reactions are:

(1)
$$2H_2O + 4K_3 \text{ Fe(CN)}_6 + 4K^+ \frac{\text{light}}{\text{chlorophyll}}$$
 $4K_4 \text{ Fe(CN)}_6 + 4H^+ + O_2$

potassium ferricyanide potassium ferricyanide

It is evident that the K⁺ ions replace the H⁺ ions leaving the H⁺ free in solution.

(2)
$$2 = 0 + 2H_20$$
 $\frac{1ight}{chlorophyll}$ $\frac{2HO}{chlorophyll}$ Hydroquinone

In may species the photolysis of water does not occur. These species have a different donor. Such is the case of the green sulfur bacteria. The Hill reaction of the green sulfur bacteria is represented: $\begin{array}{c} \text{light} \\ \text{CO}_2 + 2\text{H}_2\text{S} & \\ \hline \text{chlorophyll} & \text{CH}_2\text{O} + \text{H}_2\text{O} + 2\text{S}. \end{array} \text{ It is apparent that} \\ \text{the same general scheme takes place as in the photolysis of water, thus} \\ \text{a general equation for the Hill reaction may be represented as (38):} \\ \text{CO}_2 & 2\text{H}_2\text{A} & \\ \hline \text{chlorophyll} & \\ \hline \text{CH}_2\text{O} + \text{H}_2\text{O} + 2\text{A}. & \\ \hline \text{It has been recognized} \\ \text{for some time that photosynthesis consists of a light and a dark reaction.} \\ \text{The photolysis of water is a light reaction and the reduction of carbon} \\ \text{dioxide is a dark, or enzymic reaction.} \end{array}$

If photosynthesis is considered to terminate at the synthesis of the hexose sugars, then other dark reactions include the incorporation of the reduced carbon dioxide into ribulose 1,5- diphosphate to form two molecules of phosphoglyceric acid, as well as all the other enzymic reactions which take place in the synthesis of hexose sugars.

The light reaction consists of many more reactions than the photolysis of water - a number of which have not yet been resolved.

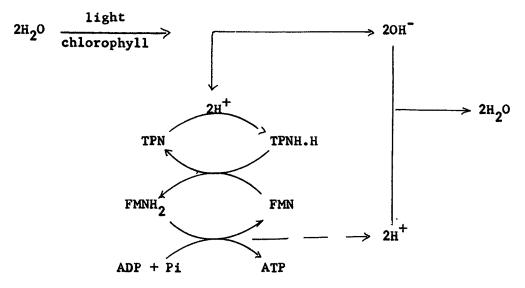
Photosynthetic phosphorylation is the synthesis of energy rich adenosine
triphosphate (ATP) utilizing light energy and adenosine diphosphate (ADP).

This may be contrasted to mitochondrial phosphorylation in which the

formation of ATP is at the expense of energy rich compounds within the cell and ADP.

The ATP required for the metabolic processes within the plant may be supplied, during daylight, by photosynthetic phosphorylation, even though CO₂ and/or water are not available. (2,5). It has been postulated that early forms of life were more dependent on photosynthetic phosphorylation than on carbon assimilation.

There are two types of photosynthetic phosphorylation: cyclic phosphorylation and terminal phosphorylation. (4,39,40). Cyclic phosphorylation is that which does not require water or carbon dioxide. This may be represented by the following scheme. (39).



Modified from Whatley et al. (39). FMN is the abbreviation for flavomononucleotide and TPN the abbreviation for triphosphopyridine nucleotide. These workers suggest that TPN and FMN are the energy transfer agents. Terminal transpiration requires the presence of water and follows a different scheme. (40).

 to carbohydrate.

The Hill reaction and phosphorylation are linked reactions.

The rate of the Hill reaction may be increased by three and one half times by having conditions present which allow the formation of ATP. (6).

Arnon (4) has prepared the following two systems of cyclic phosphorylation; one scheme requires vitamin K as an energy transfer, the other system requires FMN. The vitamin K pathway may be represented by the following equations. (4):

2 chlorophyll molecules _______ 2 chlorophyll + + 2e

vitamin K + 2e _______ reduced vitamin K

reduced vitamin K + 2Fe⁺⁺⁺ cytochrome I ______ vitamin K + 2 Fe⁺⁺

cytochrome I

2 Fe⁺⁺ cytochrome I + 2 chlorophyll⁺ + ADP + Pi _____ 2 Fe⁺⁺ cytochrome I + 2 chlorophyll + ATP.

The FMN pathway is represented by the following equations. (4):

2 chlorophyll — light 2 chlorophyll + 2e

TPN + 2e reduced TPN

reduced FMN + 2Fe⁺⁺⁺ cytochrome II _______ 2 FMN + 2 Fe⁺⁺ + cytochrome II

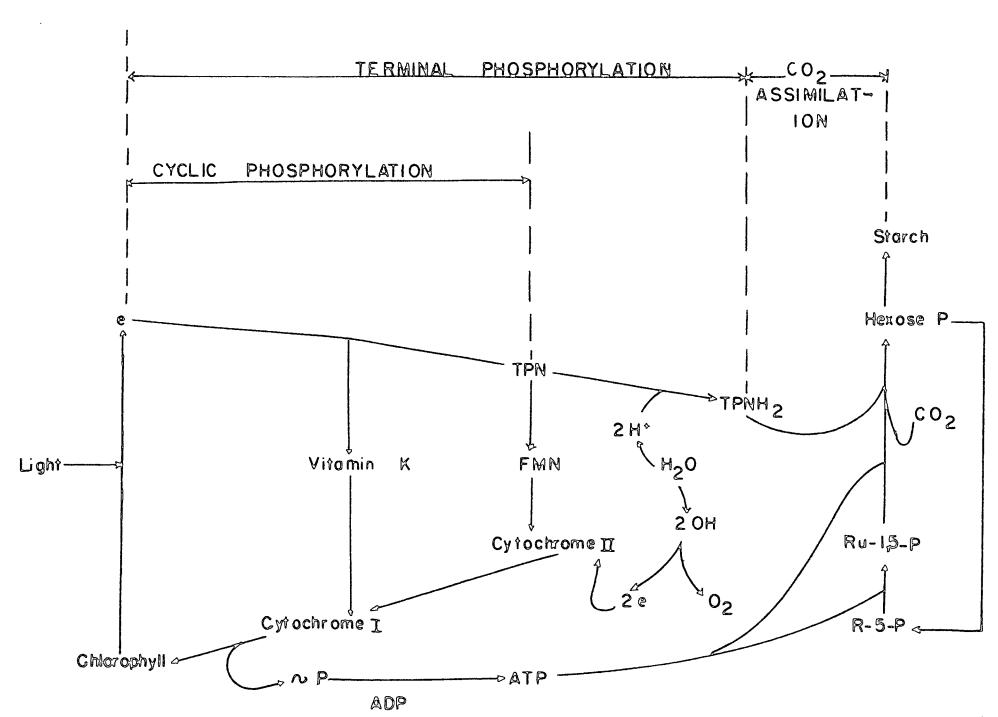
2 Fe⁺⁺ cytochrome II + 2 Fe⁺⁺⁺ cytochrome I _______ 2 Fe⁺⁺⁺ cytochrome

II + 2 Fe⁺⁺ cytochrome I

2 Fe⁺⁺⁺ cytochrome I + 2 chlorophyll⁺ + ADP + Pi ______ 2 Fe⁺⁺⁺ cytochrome I + 2 chlorophyll + ATP.

The complete system is represented on page 10.

There is good evidence that vitamin K type compounds play a role in the Hill reaction (7). Chloroplasts which have been extracted with cold petroleum ether lose the faculty to carry on the Hill reaction. This faculty may be returned by the addition of the petroleum ether extract,



menadione (vitamin K_3) or to a lesser degree by the addition of carotenoids. (7,20). Other evidence now indicates that the carotenoids do not play a role in the Hill reaction (31) but may have a function in phototropisms, as in some of the light stimuli experienced in the animal kingdom. (38). It must be pointed out that vitamin K type compounds occur associated with the chloroplasts of all green plants.

Thus the role of the various plant pigments associated with photochemistry is not yet fully understood. Chlorophyll a is associated with the photolysis of water and with the evolution of oxygen. Chlorophyll b is said to transfer the excitation energy of the fucoxanthins and the phycobilins is transferred to chlorophyll a with an efficiency of 100%. The carotenoids transfer the excitation energy with an efficiency of 20-40%. (35). These, as mentioned above, are also believed to play a role in phototropisms. (38).

MATERIALS AND METHODS

A. The effect of monuron on various enzyme systems within the plant.

(i) Catalase System

The procedure followed for this and the other enzyme systems tested are essentially those described in Loomis and Shull. (18).

The material used for the catalase system was fresh leaves and buds of <u>Spiraea</u>. The time required to liberate 5.0 ml. of oxygen was recorded. 20.0 ml. of monuron in concentrations of 100, 10, 1.0, 0.1, 0.01, 0.001 and 0.000 ppm. were used to make up the total volume as described.

(ii) Oxidase System

The plant material used was freshly picked <u>Forsythia</u> flowers.

1.0 gram of the material was ground with mortar and pestle in 10 ml. of
a monuron solution, in one of the following concentrations: 100, 10, 1,
0.1, 0.01 and 0.00 ppm., then 5 drops of 1% guaiacum solution were added.

In the case of one run 1.0 gram of the material was ground, 10.0 ml. of distilled water were added, the suspension was boiled, allowed to cool and 5 drops of 5% guaiacum added.

(iii) Peroxidase System

The procedure employed here was essentially the same as that employed to follow the oxidase system, however, the material used was sweet potato. 1.0 gram portions of the material were ground in a mortar and 10.0 ml. of monuron added in the same concentrations as used in the oxidase system. 5 drops of 1% guaiacum solution were then added followed by 5 drops of 4% hydrogen peroxide.

(iv) Lipase System

A solution of monuron in varying concentrations, replaced the 10.0 ml. of water described in the procedure. The concentrations of monuron used were: 10, 1.0, 0.1, 0.01 and 0.00 ppm. of monuron. The control contained no monuron and boiled chloroplasts. These were used on husked castor beans.

(v) Protease System

The plant material used was Austrian Winter peas.

B. Some effects of Monuron on potatoes.

Whole seed potatoes were sown in six inch pots containing washed sand. Monuron was applied to the various pots at the following rates: 41b. per acre, 21b. per acre, 11b. per acre, 1/21b. per acre and 0 1b. per acre. There were 10 pots per treatment. The pots were set in a randomized block pattern in a green house, and watered regularly every six days with 200 ml. of Hoagland's complete solution. After a growing period of 108 days, observations were made.

C. Some effects of the Substituted Urea Herbicides in Barley.

Monuron, diuron, fenuron and neburon were applied to 30 sq. foot plots which had been sown to barley. The herbicides were applied at two rates, 2 and 1 lbs. per acre and were applied either as an emergence or post-emergence treatments. There were four replicates of each treatment. The plots were randomized. Fenuron was ground to fine powder and the required weight was mixed with soil and applied to the required plots. A polyethylene screen, enclosing 30 square feet, was used to prevent drift during application. A hand sprayer was used for all treatments, except the fenuron treatments described above. Observations were taken five times during the growing season.

D. The residual effect of four Substituted Urea Herbicides.

Agronomy Field, University of British Columbia, were each replicated four times. The herbicides used were monuron, fenuron, diuron and neburon at 4 and 2 lbs. per acre. The herbicides were applied to fallow ground. The residual effects of the herbicides were assessed 7 times by counting quadrats of seedling weeds on the treated plots as compared to the non-treated plots. Fenuron was applied by mixing the required weight of powdered herbicide with sand and applying it to the plot. Monuron was applied with a small, wheeled pressure sprayer with a six foot boom. This apparatus was not satisfactory for herbicides formulated as wettable powders, and for diuron and neburon a small hand sprayer was used.

E. Effect of monuron on the Hill reaction and the interaction of vitamin K type compounds.

The chloroplasts were prepared by taking 100 grams of fresh, washed mature market spinach without the midrib, and blending for thirty seconds in a Waring blender with cold 0.5 M. sucrose. The homogenate was strained through several layers of cheesecloth into a 250 ml. erlenmeyer flask in an ice bath. The erlenmeyer flask was covered with towelling to exclude as much light as possible.

The crude chloroplast suspension was then placed in two 50 ml. tubes and centrifuged in a Servall refrigerated centrifuge, at 1000 X gravity for five minutes. The supernatant was discarded and the "pellet" in each tube was resuspended in 10 ml. of 0.5 M sucrose. The chloroplasts were washed three times in this manner. Following the washing all the chloroplasts were suspended in 80 ml. of 0.5 M. sucrose

and placed in an ice water bath. An ether-dry ice bath was prepared and 36 - 15 ml. plastic test tubes were placed in the ether-dry ice bath to cool, then 2.0 ml. of the chloroplast suspension was added to each of the 36 tubes. After 5 minutes in the ether-dry ice bath the frozen chloroplast suspensions were stored under dry ice in a deep freeze. The temperature of storage was approximately - 25°C. This procedure was modified from Gorham and Clendenning (14). The chloroplasts as required were thawed at room temperature by stirring with a glass stirring rod. As soon as it was evident that there were no frozen particles in the suspension, they were added to the reaction mixture. This was done at time zero.

The electron acceptor, or Hill solution, used was 0.001 M. $K_3Fe(CN)_6$ in 0.1 M phosphate buffer at the pH of 6.85. The above procedure was modified from the method of Spikes et al. (34).

Alkaline pyrogallol purified commercial nitrogen was bubbled into the Hill solution in order to prevent the chloroplast material from settling.

It was found that the Hill solution, with purified nitrogen being bubbled through it, and without chloroplasts, maintained a constant potential reading for one hour with the lamp on. However, it was necessary to maintain a constant temperature.

The apparatus used to measure the potential consisted of a Beckman model H-2 pH meter equipped with a standard calomel electrode, a platinum electrode, and a glass bottomed water bath standing on an open ended box in which a 1000 watt light bulb was situated.

The water bath was filled with cold water and enough ice to cover the water surface. A small stirring propeller was emmersed in

in the ice water mix to keep the temperature constant throughout.

A stand with a "snap" clamp was placed in the water bath in order to keep the 50 ml. beaker containing the reaction mixture a constant distance from the bottom of the water bath, thus the light in each experiment was filtered through the same amount of water, which was three inches.

The Hill solution was placed in a 50 ml. beaker which had previously been scrupulously cleaned. The beaker was then placed in the "snap" clamp in the ice water bath. The ice water reached to within 3/8 of an inch from the top of the beaker. When the thermometer placed in the beaker registered 1.5°C. the nozzle attached to the nitrogen supply, and the electrodes were emersed in the Hill solution.

The electrodes were left in the Hill solution for at least five minutes to equilibrate the temperature. At this time, a frozen chloroplast sample was taken from the deep freeze and thawed. When the chloroplast suspension reached the slurrey state the pH meter was standardized and the lamp turned on. As soon as the chloroplast suspension was in the fluid state it was poured into the beaker, the stop watch started and the zero time potential reading taken. Potential readings were taken at one minute intervals for five minutes. The system used to calculate the millivolt readings was that suggested in the book of instructions that is supplied with the pH meter. The formula is:

(7.0 - pH reading) X 60 = millivolts.

The net millivolt change was recorded. It must be noted that the initial millivolt readings for different samples varied greatly. This is thought to be due to deposits on the platinum electrode. Between readings the platinum electrode was washed in a detergent, scrubbed with

a scouring powder, washed in an acid bath (20 - 40% HCl) and then rinsed in distilled water and dried. The net change in potential appeared to be a satisfactory method of measuring the rate of photolysis.

Monuron (Dupont 80% wettable) was made in solutions which contained concentrations of 1 X 10⁻³ to 1 X 10⁻¹² moles of monuron per ml. The required concentration of monuron was added by placing 1.0 ml. of the solution of the correct molarity in the reaction mixture.

To remove the lipid material the thawed chloroplast suspension was placed in a 250 ml. separatory funnel with 25 ml. of pure petroleum ether and shaken in an ice bath for fifteen minutes. The chloroplast material was drawn off and added to the reaction and the Hill reaction was followed as described above.

Vitamin K₃ (menadione) was added to the chloroplast material in pure petroleum ether. One ml. aliquots were pipetted into a separatory funnel containing the chloroplast suspension. The separatory funnel was kept in an ice water bath. Purified commercial nitrogen was passed through the stem of the separatory funnel at a rate which was just sufficient to prevent the chloroplast material from falling down the stem. At this rate of nitrogen flow it required five minutes to evaporate all of the petroleum ether. The nitrogen flow was left on for six minutes to ensure that all the petroleum ether had been evaporated.

Vitamin K_5 (synkamin) was added directly to the reaction mixture as it is a water soluble analogue of vitamin K. The literature referring to this analogue states that each ampule contains 4.0 mg. of active material. This was assumed to be correct, as the percentage of active material was not known. In any case, the same solution of vitamin K_5 was used for any one group of experiments so that any error

introduced would be constant.

During the spring of 1960 chloroplasts were used that were prepared from spinach grown in California. In May, 1960 spinach was no longer imported from California and locally produced spinach was on the market.

It was found that chloroplasts prepared from the locally grown spinach were inactive during May and the first two weeks of June. It is believed that the inactive state of the chloroplasts is a result of the unusually cold and wet weather experienced in the Vancouver area at that time.

RESULTS

A. The Effect of Monuron on Various Enzyme Systems

(i) Catalase System

The evidence of this exploratory experiment (Appendix Table I) indicates that if there is any influence shown on the catalase enzyme system by monuron it is masked by time. It appears that there may be a natural inhibitor to the catalase system which breaks down after the leaves and buds have been severed from the plant.

(ii) Oxidase System

The results indicate that no influence is exerted by monuron on the oxidase system (Appendix Table II).

It must be pointed out that these tests were not quantitative.

(iii) Peroxidase System

The results obtained in these tests indicate that there is little or no effect on the peroxidase system shown by monuron (Appendix Table III).

As in the tests with the oxidase system, the tests with the peroxidase system were not quantitative.

(iv) Lipase System

Some activation of this system is apparent in this preliminary trial (Appendix Table IV).

Before any firm conclusions can be drawn more investigation will be required.

(v) Protease System

The evidence gathered in these experiments suggest that monuron inhibits this enzyme system (Appendix Table V).

B. Some Effects of Monuron on Potatoes

Monuron shows little effect on the length of the root systems of

Fig. 1

curve # 1; Weight of the root system.

curve # 2; Weight of tubers.

curve # 3; Weight of tops.

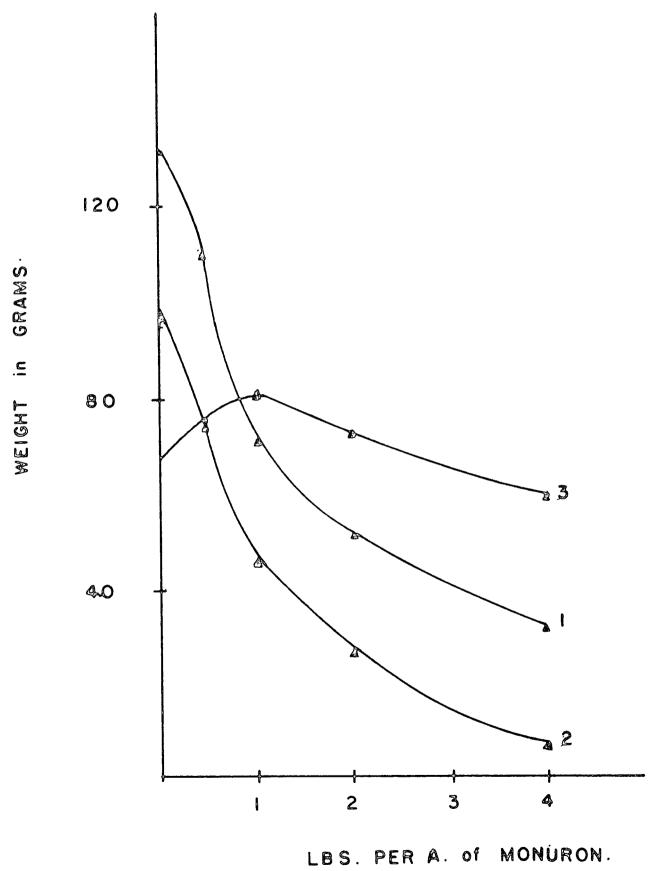


fig. 1

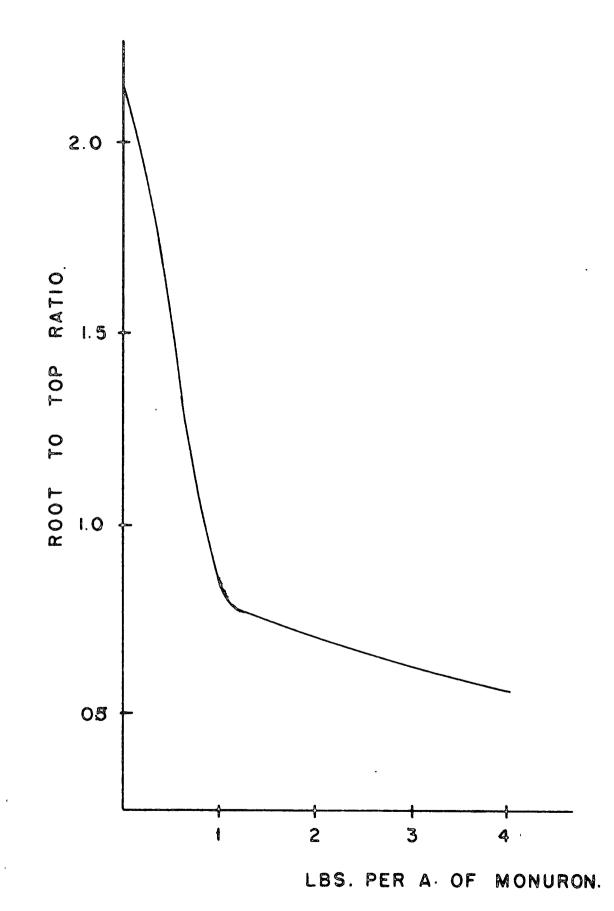


fig. 2.

potato plants, however the length of the tops was increased with all applications of monuron. There was an increase in the weight of the tops found in the plants treated up to two lbs. per acre. The weight of the root system and the total weight of tubers were reduced with increased rates of monuron applied. The total weight of tubers was reduced by 54% with the application of one lb. per acre and by 93% with the application of four lbs. per acre of monuron. (fig. 1, Appendix Table VI).

The number of tubers per plant was reduced as the rate of monuron increased. There was a 64% reduction in the number of tubers per plant with the application of four lbs. per acre of monuron. The average weight per tuber was greatly reduced with monuron applications. For example there was a 52% reduction of tuber weight with the application of one lb. of monuron, and a reduction of 77% with the four lb. rate.

The evidence does not suggest that monuron has any effect on the number of stems per plant.

The root to top ratio was greatly reduced by monuron treatments.

There is a 64% reduction in this ratio with one lb. per acre applied, a

74% reduction with four lbs. per acre applied. (fig. 2).

There appears to be a tendency for monuron to cause swelling at the nodes. However, this tendency does not appear to increase uniformly with increased rates of monuron. The maximum swelling was observed at the one lb. rate.

The size of the first leaf was reduced with monuron at rates which exceed 1/2 lb. per acre. The greatest reduction in leaf size was noticed between those treated at the 1/2 lb. rate and those treated at

the one lb. rate. The degree of development of the potato plants appeared to be arrested with monuron applications.

There is an increase in the leaf colour intensity of plants treated with monuron at one half and one lb. rates. At rates exceeding one lb. per acre, there is a decrease in green colour intensity and the leaves become yellow. Plants treated with monuron at rates exceeding one half lb. per acre tended to have chlorotic margins. Chlorosis of leaf margins is a recognized symptom of monuron damage. In all treatments there were some plants with dead leaves present. The two and four lbs. per acre treatments had a higher frequency of plants with dead leaves present (50 and 70% respectively). All the treatments had plants with dead spots on some of the leaves. This may have been due to a nutrient deficiency and thus may not be attributable to monuron damage directly.

Many of the plants treated with monuron at the four lb. rate tended to have the main stem dying back. In some cases the lateral branches became dominant. Similar, but less obvious dying at the stem apices was observed in the plants treated at the two lb. rate.

C. Some Effects of the Substituted Urea Herbicides on Barley

All data may be seen on Table VII, VIII & IX of the Appendix.

The four substituted urea herbicides used in this experiment were most effective in controlling weed when applied as pre emergence herbicides rather than post emergence herbicides.

In field observations the better crop growth was generally observed in the post emergence treatments. Neburon was the exception here, there being little difference between crops in the pre and post emergence treated plots.

- #1 Control.
- #2 monuron at 2 lbs./A., as a pre emergent.
- #3 monuron at 2 lbs./A., as a post emergent.
- #4 monuron at 1 lb./A., as a pre emergent.
- #5 monuron at 1 lb./A., as a post emergent.
- #6 diuron at 21b./A., as a pre emergent.
- #7 diuron at 2 lbs./A., as a post emergent.
- #8 diuron at 1 lb./A., as a pre emergent.
- #9 diuron at 1 lb./A., as a post emergent.
- #10 neburon at 2 lbs. /A., as a pre emergent.
- #11 neburon at 2 lbs./A., as a post emergent.
- #12 neburon at 1 lb./A., as a pre emergent.
- #13 neburon at 1 lb./A., as a post emergent.
- #14 fenuron at 2 lbs./A., as a pre emergent.
- #15 fenuron at 2 lbs./A., as a post emergent.
- #16 fenuron at 1 lb./A., as a pre emergent.
- #17 fenuron at 1 lb./A., as a post emergent.

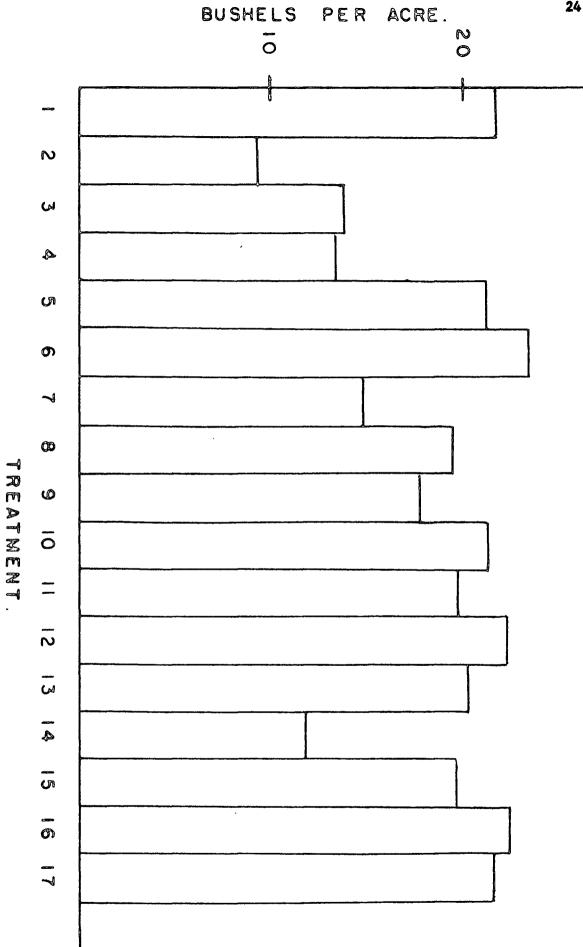


fig. 3.

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Fig. 4
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- #1 Control.
- #2 monuron at 2 lbs./A., as a pre emergent.
- #3 monuron at 2 lbs./A., as a post emergent.
- #4 monuron at 1 lb./A., as a pre emergent.
- #5 monuron at 1 lb./A., as a post emergent.
- #6 diuron at 2 lbs/A., as a pre emergent.
- #7 diuron at 2 lbs./A., as a post emergent.
- #8 diuron at 1 lb./A., as a pre emergent.
- #9 diuron at 1 1b./A., as a post emergent.
- #10 neburon at 2 lbs./A., as a pre emergent.
- #11 neburon at 2 lbs./A., as a post emergent.
- #12 neburon at 1 lb./A., as a pre emergent.
- #13 neburon at 1 lb./A., as a post emergent.
- #14 fenuron at 2 lbs./A., as a pre emergent.
- #15 fenuron at 2 lbs./A., as a post emergent.
- #16 fenuron at 1 lb./A., as a pre emergent.
- #17 fenuron at 1 lb./A., as a post emergent.

Monuron and diuron at both the one and two 1b. per acre treatments, applied both as pre and post emergence herbicides were the most effective weed control agents. Neburon at the two 1b. per acre, applied as a pre emergent, gave only fair weed control.

In general, the yield as measured by weight of grain was reduced by applications of the substituted urea herbicides. Diuron at the two lb. per acre, as a pre emergence herbicide, did not reduce the total weight of grain per acre, nor did neburon at one lb. per acre, fenuron at one lb., or monuron at one lb., as a post emergent.

The number of bushels per acre was generally reduced by the application of these substituted urea herbicides. The noticeable exception was diuron at two lbs. per acre as a pre emergence herbicide. (fig, 3.). The bushel weight was found to be increased by all applications of these herbicides except for diuron at two lbs. per acre, as a pre emergent. (fig. 4).

Diuron, and possibly neburon, may offer promise as effective herbicides in cereal crops. From the evidence gathered in this experiment, diuron appears to hold the greatest promise.

- D. The Residual Effect of Four Substituted Urea Herbicides

 All data may be seen in Table IX of the Appendix.
 - (i) Fenuron:

Fenuron is the most water soluble of these four herbicides,

3,500 ppm. at room temperature. It would be expected that this herbicide
would leach from the soil most readily.

The degree of weed control of this herbicide increased until the twentieth day, thereafter the herbicidal action appeared to dissipate. After the twentieth day there was a rainfall of more than 0.7 inches.

This rain probably leached a large percentage of the fenuron from the rhizophere.

At the four lb. rate, the degree of weed control at 89 days was 3. (The weed control was measured on an arbitrary scale of 0 to 5, with 0 representing no weed control in the plots.) At the two lb. rate, the degree of control decreased after twenty days, and at the end of 89 days the degree of control in the plots was less than two.

(ii) Monuron:

Monuron is water soluble to the extent of 230 ppm. This herbicide exhibited its maximum degree of control within ten days after application, under the conditions present for this experiment. The degree of control decreased for the next twenty days, thereafter remaining fairly constant.

At the four 1b. rate, monuron gave a maximum degree of control of 4.8, and at the end of 89 days the degree of control was four. The two 1b. rate gave slightly less control than did the four 1b. rate.

The two rates appear to dissipate at nearly the same rate.

(iii) Diuron:

Diuron is water soluble to the extent of 42 ppm. and appeared to be the most residual of the herbicides tested here. It controlled all vegetation for ten days at the four lb. rate and for twenty days at the two lb. rate. (The greater control shown at the two lb. rate is undoubtedly due to the weed populations within the plots.)

The degree of control at the end of 89 days was 4.5 at the four 1b. rate, and 4.0 at the two 1b. rate.

(iv) Neburon:

Neburon, with a solubility of 4.8 ppm., is the least soluble of

Fig. 5

Curve #1 No monuron added.

Curve #2 No monuron added, chloroplasts boiled.

Curve #3 1 X 10⁻³ moles of monuron added.

Curve #4 1 X 10⁻⁵ moles of monuron added.

Curve #5 1 X 10⁻⁷ moles of monuron added.

Curve #6 1 X 10⁻⁹ moles of monuron added.

Curve #7 1 X 10⁻¹⁰ moles of monuron added.

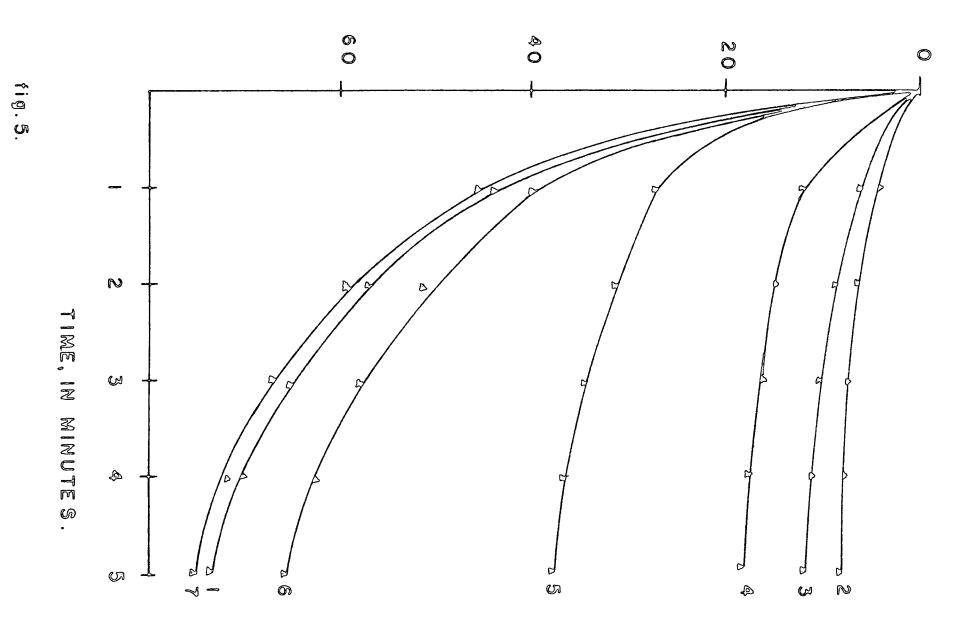


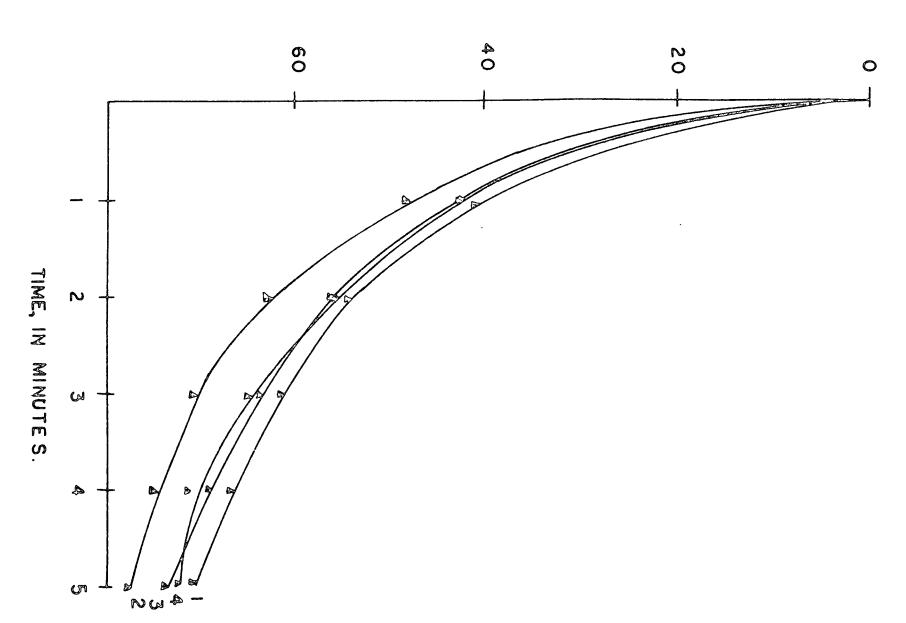
Fig. 6

Curve #1 No monuron added.

Curve #2 1 X 10⁻¹⁰ moles of monuron added.

Curve #3 1 X 10⁻¹¹ moles of monuron added.

Curve #4 1 X 10⁻¹² moles of monuron added.



the four herbicides studied here. It would be expected that the maximum degree of control would be reached at a later time than that of the other three herbicides. This, however, was not observed.

The maximum degree of control at the four 1b. rate was found to be at ten days after treatment, thereafter decreasing rapidly until thirty days, then increasing for the next ten days and remaining fairly constant until the end of the test period. The degree of control at 89 days was 3.0.

The maximum degree of control at the two 1b. rate was also reached within ten days. Thereafter the degree of control decreased until the end of the test period, 89 days, when the degree of control was 2.2.

The most common weeds found on the majority of plots were:

Lady's thumb Polygonum persicaria L., rib grass Plantago, lanceolata L.,

broadleaf plantain Plantago major L., meadow foxtail Alopecurus

pratensis L., and orchard grass Dactylis glomerata L

E. The Effect of Monuron on the Photolysis of Water and the

Interaction of Vitamin K Type Compounds on the Photolysis

Reaction

Monuron in low concentrations inhibits the Hill reaction of the photolysis of water. It was found that 1×10^{-7} moles of monuron in the reaction mixture will inhibit the Hill reaction by more than 50% (see Table I and fig.5). There appears, from this data, that there may be a slight stimulus given to this reaction by very low concentrations of monuron. (see Table II and fig. 6).

Table I

THE EFFECT OF VARIOUS CONCENTRATIONS OF MONURON ON THE RATE OF THE HILL REACTION

Time in minutes	Char Control	nge in potent control, boiled	ial, measured 1 X 10 ⁻³ M. monuron	in millivol 1 X 10 ⁻⁵ M. monuron	1 X 10 ⁻⁷ M. monuron
1	43.75	4.00	5.75	11.50	27.00
2	57.50	6.75	8.25	14.50	31.50
3	66.50	7.00	10.00	15.75	34.75
4	70.2 5	7.50	10.50	17.25	36.50
5	74.00	8 .2 5	12.00	19.00	38.00
	•				

Table I (con'	t.)
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Time in minutes	1 X 10 ⁻⁹ M. monuron	1 X 10 ⁻¹⁰ M. monuron
1	39.50	45.25
2	51.25	59.50
3	58.00	66.75
4	62.25	71.75
5	66.00	75.75

Table II

THE EFFECT OF EXTREMELY LOW CONCENTRATIONS OF MONURON ON THE RATE OF

THE HILL REACTIONS

Time, in minutes	control	1 X 10 ⁻¹⁰ M. monuron	1 X 10 ⁻¹¹ M. monuron	1 X 10 ⁻¹² M. monuron
1	41.50	48.00	43.00	40.75
2	53.75	62.50	56.00	55.75
3	61.50	70.50	63.75	64.75
4	67.00	74.50	69.00	71.25
5	70.75	78.00	73.75	73.00

The addition of vitamin K type compounds resulted in a slight inhibition of the Hill reaction. (see Tables III & IV).

Time, in minutes	Potential c	hange, measured l ml. of pet. ether added, no vit. K ₃	in millivolts l mg. of vit. K ₃	0.1 mg. of vit. K ₃
1	48.75	38.75	33.75	37.00
2	64.25	55.25	47.75	52.00
3	70.50	62.25	56.00	59.00
4	76.25	67.75	61.25	63.75
5	80.25	71.50	65.50	70.00

Table IV THE EFFECT OF THE ADDITION OF VITAMIN K_5 TO THE RATE OF THE HILL REACTION

Time, in minutes	Control	0.1 mg. of Vitamin K ₅
1	44.75	30.75
2	59.25	43.25
3	66.50	51.75
4	72.50	57.00
5	76.50	61.50

The relative inhibition of the Hill reaction was greater with the addition of vitamin K_5 than it was with the addition of vitamin K_3 , indeed there was very little inhibition shown by 0.1 mg. of vitamin K_3 . Much of the inhibition shown with the addition of vitamin K_3 is due to denaturation of the chloroplasts by light and warmth. This is evident when compared to the tests where petroleum ether was added without vitamin K_3 . (see Tables III & IV).

Vitamin K_5 is formulated as the hydrochloride of 4- amino - 2-methyl - 1- napthol. It is possible that the chloride ions are responsible for the inhibition.

Removal of the petroleum ether soluble fraction resulted in a slight stimulation of the Hill reaction (Table V).

Table V

THE REMOVAL OF THE PETROLEUM ETHER SOLUBLE FRACTION

Time, in minutes	Control	Shaken, with no petroleum ether	Shaken with petroleum ether
1	44.75	31.00	35.25
2	59 .2 5	44.00	48.25
3	66.50	51.25	5 6.2 5
4	72.50	56.75	61.50
5	76.50	60.25	64.75

The addition of 0.1 mg. of vitamin K_5 to the reaction mixture, containing 1 X 10^{-7} moles of monuron almost completely inhibited the Hill reaction, whereas the addition of vitamin K_3 showed no inhibition, other than that due to the denaturation of the chloroplasts by light and warmth. (Table VI).

Table VI

THE INTERACTION OF MONURON AND ANALOGUES OF VITAMIN K.

Time, in minutes	Control	_		_	1 X 10 ⁻⁷ M. monuron
1	56.75	34.50	4.50	24.25	24.00
2	70.75	43.00	7.00	27.75	28.75
3	79.2 5	45.50	7.00	30.75	31.25
4	85.7 5	48.50	9.00	31.75	33.75
5	89.00	49.50	10.00	34.00	34.75

Each figure in Tables I to VI is the average of four trials.

DISCUSSION

The mode of action of the substituted urea herbicides in all probability involves alterations in the biochemistry of the plant.

It has been shown in the literature that many deviations from the normal take place in plants subjected to the urea type herbicides. Many of these deviations are difficult to discern, such as the onion root tip growth being arrested at the resting stage of mitosis. (9). Other deviations are more pronounced, such as the recognized leaf symptoms of monuron injury.

The present work confirms that monuron may alter the protein balance within the plant system, e.g. the protease system of enzymes may be inhibited. There also seems to be a stimulation to the lipase system of enzymes.

Monuron reduced the root growth of potato plants. This may be due to a shortage of photosynthetic products. In the literature it has been reported that excised roots may be grown in nutrient solutions containing lethal quantities of monuron. (26). However, if there was a shortage of photosynthetic products one would expect a similar reduction in top growth, unless food transport is being restricted. The evidence gathered here shows that there is an increase in top growth in plants that have been treated with up to two lbs. per acre of monuron.

It is apparent that the inhibition of photosynthesis is not the complete answer here. It is possible for example that an imbalance with one of the growth hormones is created by monuron.

The substituted urea herbicides enter the plant mainly through the root system. It is not surprising therefore, that the best weed control was found in the plots where the herbicides were applied as pre

emergents. When applied as a post emergent much of the herbicide would remain on the foliage and would not be as free to enter the plant system. In pre emergence applications all of the herbicide would reach the soil surface and, following precipitation, would be leached to a rhizophere which would be much shallower than would be the case in later applications.

Diuron, applied as a pre emergence herbicide, at two lbs. per acre, did not reduce the yield in barley, and at the same time provided good weed control As mentioned earlier diuron may offer good weed control in cereal crops. If this is the case some of the disadvantages of using hormone type herbicides would be overcome. It is felt that this is an avenue of research that should be investigated thoroughly.

Coggins and Crafts (10) have reported that the substituted urea herbicides have a not positive charge in aqueous solutions. It is reasonable to assume that the herbicide molecule retains this positive charge within the plant. The positively charged herbicide molecule would then be in the position to change the configuration of many enzyme molecules by the disruption of hydrogen bonds, or other electrostatic forces, which are required to maintain enzyme configuration.

The specificity of enzymes is believed to be largely dependent upon the configuration of the enzyme molecule, as well as the configuration of the substrate molecule.

The photolysis of water appears to be stimulated by very dilute concentrations of monuron (1 X 10⁻¹⁰ moles added to the reaction mixture)—this stimulation is lost at lower and higher concentrations. It is not known if the overall rate of photosynthesis is increased with these low concentrations of monuron. However it was apparent that the potato plants

treated with monuron up to one lb. per acre had a more intense green colour, and presumably these plants had a greater concentration of chlorophyll. It may be that the whole photosynthetic mechanism becomes more active with low concentrations of monuron. Whether the net assimilation is increased is still lacking proof.

Monuron at higher concentrations (1 X 10⁻⁷moles added to the reaction mixture) inhibits the photolysis of water by more than 50%. If the photolysis of water is inhibited the whole photosynthetic series of reactions will be inhibited. These reactions are dependent on the photolysis of water to provide hydrogen ions to reduce the carbon dioxide to carbohydrate and possibly to reduce TPN, which may be required in the synthesis of ATP. It is not known whether or not monuron will inhibit the reduction of carbon dioxide or the synthesis of ATP per se, however as pointed out above these reactions will be inhibited, even though the influence is indirect.

It has been reported in the literature that vitamin K type compounds are required in the photolysis of water reactions. (7). The evidence gathered here does not support this report. Natural occurring vitamin K type compounds are petroleum ether soluble, but after shaking chloroplasts in cold petroleum ether, and separating, the rate of the Hill reaction was found to increase. The procedure in the literature (7) for extracting the petroleum ether fraction from chloroplasts could not be repeated, as it was not possible to recover the chloroplasts intact from the fritted glass. However shaking the chloroplasts in 25 ml. of cold petroleum ether should have given similar results. One possible explanation for the contrary results would be that the petroleum ether was not able to penetrate the lipo-protein matrix which surrounds the

chloroplasts, and thus was not reaching the vitamin K.

The addition of vitamin K_5 , and to a smaller degree vitamin K_3 , retarded the rate of the Hill reaction. An excess of vitamin K type compounds following the addition of the synthetic annalogues of vitamin K might cause the pathway to become blocked. It is felt however, in the light of the evidence gathered here that the vitamin K type compounds do not play a role in this phase of photosynthesis. These compounds probably do take part in either the cylic phosphorylation or terminal phosphorylation, or both.

Vitamin K_5 , which is prepared as the hydrochloride of 4 - amino-2-methyl-1-napthol, acted synergistically with monuron to inhibit the Hill reaction. This synergistic relation with monuron may have been caused by the chlorine ion combining with the monuron at the meta position thereby forming diuron. In support of this explanation the vitamin K_3 shows no effect, (Table VI), either stimulatory or inhibitory, when in the presence of 1 x 10⁻⁷ moles of monuron. Vitamin K_3 and K_5 have the same physiological properties, at least in animals.

The apparatus used to measure the progress of the Hill reaction was modified from that described in the literature. It was found that by having ice in the water bath the temperature of the reaction mixture, even with the lamp on, could be kept constant for the length of the run. When running water was used it was not possible to keep the temperature from rising at least two degrees C. in the five minute test period. The measurement of potential is very sensitive to temperature fluctuations.

SUMMARY

- (1) Monuron has no evident effects on the catalase, oxidase or peroxidase systems of the test plants. An inhibition of the protease system and a stimulation of the lipase system was evident.
- (2) Monuron modifies growth of the potato plant by decreasing the weight of roots, the number and weight of tubers, and by increasing the weight of the tops.

Low rates of application tend to increase the intensity of leaf colour, while applications greater than one lb. per acre appear to decrease the green colour intensity.

- (3) The weight of barley grain per acre, and the number of bushels per acre tend to be reduced with applications of the substituted urea herbicides. The weight per bushel, however, tends to be increased.
- (4) Diuron appears to be the most residual of the four herbicides tested. Neburon, which is almost ten times less soluble in water than diuron did not give as satisfactory weed control.
- (5) Monuron inhibits the photolysis of water by 50% at concentrations as low as 1 \times 10⁻⁷ moles in the reaction mixture. A concentration of 1 \times 10⁻¹⁰ moles in the reaction mixture increased the rate of the Hill reaction.

These experiments did not show any evidence that vitamin K type compounds play a role in the photolysis of water. The removal of the petroleum ether soluble fraction resulted in an increase in the rate of the Hill reaction.

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APPENDIX

Table I

The Effect of Monuron on the Catalase System

Concentration of monuron, in ppm.	Time, in seconds, required to liberate 5.0 ml. of oxygen
0.000	72.0
100.0	69.5
10.0	93.2
1.00	87.8
0.10	41.6
0.01	36.4
0.001	34.4
100.0	22.9
0.000	21.8

(The length of time between each test was approximately ten minutes. The time lapse between the last test of the 1.00 ppm. series and the first of the 0.10 ppm. series was 90 minutes.)

Table II

The Effect of Monuron on the Oxidase System

Concentration of monuron in ppm.	Immediate colour change with 1% guaiacum	
100.0	Yes	
10.0	Yes	
1.0	Yes	
0.1	Yes	
0.01	Yes	
0.000	Yes	
boiled 0.000	No	

Table III

The Effect of Monuron on the Peroxidase System

Concentration of monuron in ppm.	Immediate colour change with 1% guaiacum.	Immediate colour change with 4%
100.0	No	Yes
10.0	No	Yes
1.0	No	Yes
0.1	No	Yes
0.01	No	Yes
0.000	No	Yes
boiled 0.000	No	No

Table IV

The Effect of Monuron on the Lipase System

Concentration of monuron in ppm.	Mls. of 0.1 N fatty acids present
10.0	0.644
1.0	0.568
0.1	0.533
0.01	0.546
0.000	0.430
boiled 0.000	0.333

Table V

The Effect of Monuron on the Protease System

Concentration of monuron, in ppm.	% Protein
100.0	20.7
10.0	26.0
1.0	30.5
0.1	29.7
0.01	31.2
0.000	32. 5
boiled 0.000	26.5

Table VI

Some Effects of Monuron on Potato Plants (Each figure is an average of 10 pots.)

Rate of monuron, 1bs/A	Length tops	in cm. roots	Weight i tops	n gms. roots	Weight of tubers in gms.	Root to top ratio
4.0	44.7	31.3	59.1	32.1	6.3	0.563
2.0	44.5	33.5	72.3	52.7	27.4	0.715
1.0	48.1	32.4	81.4	70.9	44.8	0.803
0.5	42.3	28.3	74.8	110.4	74.4	1.575
0.0	38.3	33.0	65.4	134.3	98.6	2.198
Rate of monuron, lbs./A	Numbers	er of stems	Swelling at nodes	Relative size of first leaf.	Degree of Develop- ment.	Intensity of colour
4.0	1.5	2.7	2.4	1.6	0.7	2.6
2.0	4.1	2.3	2.1	2.5	1.0	3.2
1.0	5.3	1.8	2.5	2.9	1.5	4.1
0.5	5.1	2.1	2.2	3.8	1.0	3.5
0.0	5.5	2.5	1.2	3.8	1.7	3.4
Rate of monuron in lbs./A		% having bleached margins	% having dead leaves		% havin dead sp in leav	ots
4.0		50	7	70		-
2.0		60	50		100	
1.0		60	2	0	90	
0.5		10	3	0	60	
0.0		0	2	0	70	

Swelling at the nodes, 0 indicates no swelling and 5 indicates maximum swelling.

Table VI CROP GROWTH

The	Eff	ects	of	Monuron	on	Barle	V

Herbicide	Rate, in lbs./ A.	Time applied	27	observ 32	ations, 42	days after 52	applic. 73
monuron	2.0	pre e.	2.0	2.0	2.0	2.0	1.25
monuron	2.0	post e.	3.25	3.5	3.75	3.75	4.0 #
monuron	1.0	pre e.	2.5	2.75	3.0	3.75	2.6 #
monuron	1.0	post e.	3.25	3.75	3.75	4.25	3.0 #
diuron	2.0	pre e.	2.25	2.75	3.0	3.75	2.6 #
diuron	2.0	post e.	3.25	3.75	4.25	3.75	4.0
diuron	1.0	pre e.	2.75	2.75	3.25	3.75	3.25
diuron	1.0	post e.	4.0	4.0	4.0	4.25	4.25
neburon	2.0	pre e.	3.25	3.75	3.75	4.25	4.25
neburon	2.0	post e.	3.25	3.5	3.25	3.75	4.0 #
neburon	1.0	pre e.	3.5	4.25	4.25	4.5	4.25
neburon	1.0	post e.	3.25	4.0	4.75	4.0	4.3 #
fenuron	2.0	pre e.	2.0	2.0	2.0	2.75	2.3 #
fenuron	2.0	post e.	4.0	3.75	3.75	4.0	4.0 ##
fenuron	1.0	pre e.	2.5	2.25	2.5	3.5	3.75
fenuron	1.0	post e.	3.5	4.25	3.0	4.0	4.0 #
control	n/a	n/a	3.5	3.5	3.5	4.0	4.25
control	n/a	n/a	3.25	4.0	4.25	4.25	4.3 #
control	n/a	n/a	3.5	4.25	3.75	4.25	3.5 排
control	n/a	n/a	3.5	3.5	3.5	3.75	4.3 #

An arbitrary scale was used to measure the vegetation on the plots. The scale was from 1 to 5, with 1 representing no plants alive and 5 representing the most active plant growth.

one plot was lost by trampling etc. before the last observation was made.

two plots were lost by trampling etc. before the last observation was made.

Relative size of the first leaf, 0 indicates a small first leaf and 5 indicates a large first leaf.

Degree of Development, 0 indicates the least and 5 indicates the greatest development in the plant.

Intensity of colour, 0 indicates the least intense colour and 5 indicates the most intense colour.

Table VII

Weed Infestation

Herbicide	Rate, in lbs./A.	Time applied	Obsei 27	vation, 32	days aft	er applic	ation. 73
monuron	2.0	pre e.	1.0	1.0	1.0	1.0	1.25
monuron	2.0	post e.	3.0	4.25	4.75	4.5	3.0 #
monuron	1.0	pre e.	1.0	1.25	1.25	2.0	1.6 #
monuron	1.0	post e.	3.25	4.25	3.75	3.75	3.0 #
diuron	2.0	pre e.	1.0	1.0	1.25	1.75	1.3 #
diuron	2.0	post e.	3.25	4.0	4.75	4.5	2.0
diuron	1.0	pre e.	1.0	1.0	1.25	1.5	1.25
diuron	1.0	post e.	4.25	4.25	4.75	4.5	2.5
neburon	2.0	pre e.	1.5	1.25	1.25	1.75	1.75
neburon	2.0	post e.	3.25	3.75	4.0	4.0	2.6 #
neburon	1.0	pre e.	1.5	1.75	1.75	1.75	2.25
neburon	1.0	post e.	4.0	4.25	4.25	4.5	3.3 #
fenuron	2.0	pre e.	1.0	1.5	1.25	1.75	2.0 #
fenuron	2.0	post e.	3.25	4.0	4.0	4.0	2.0 ##
fenuron	1.0	pre e.	1.25	2.5	2.25	3.0	2.75
fenuron	1.0	post e.	4.0	3.75	4.25	4.5	3.0 #
control	n/a	n/a	3.75	4.75	4.75	4.75	4.0
control	n/a	n/a	4.5	4.25	4.5	5.0	3.0
control	n/a	/ n/a	3.75	4.5	4.75	5.0	3.5 ##
control	'n/a	n/a	3.0	4.0	4.25	5.0	3.0 #

[#] one plot was lost by trampling etc. before the last observation was made.

^{##} two plots were lost before the last observation was made.

An arbitrary scale was used to measure the weed cover in the plots.

The scale was from 1 to 5, with 1 representing no weeds present and 5 representing a heavy weed cover.

Table VII

The Effect of the Substituted Urea Herbicides on Barley Yields

<u> </u>	riect or the	C DUDUCECUC		DIGIGES C	d balley	ITCING
Herbicide	Rate, in lbs./A	Time applied	Ave. Weight per plot in lbs.	Weight per A.	Ave. bushel weight	Yield, bu./A.
monuron	2.0	pre e.	0.399	4345	46.75	9.29
monuron	2.0	post e.	0.567	6175	45.25	13.65
monuron	1.0	pre e.	0.577	6284	46.25	13.59
monuron	1.0	post e.	0.678	9540	45.0	21.20
diuron	2.0	pre e.	0.679	10629	45.72	23.25
diuron#	2.0	post e.	0.571	6218	42.7	14.56
diuron	1.0	pre e.	0.811	8832	45.5	19.42
diuron	1.0	post e.	0.743	8091	45.75	17.69
neburon	2.0	pre e.	0.820	8930	42.5	21.01
neburon	2.0	post e.	0.792	8625	44.0	19.6
neburon	1.0	pre e.	0.901	9812	44.25	22.17
neburon#	1.0	post e.	0.890	9692	47.96	20.21
fenuron	2.0	pre e.	0.497	5412	45.25	11.96
fenuron	2.0	post e.	0.811	8832	45.2 5	19.52
fenuron#	1.0	pre e.	0.926	10084	45.25	22.29
fenuron#	1.0	post e.	0.866	9431	43.67	21.60
control	n/a	n/a	0.786	8560	43.25	19.79
control #	n/a	n/a	0.986	10738	44.0	24.40
control#	n/a	n/a	0.819	8920	43.3	20.6
control#	n/a	n/a	0.845	9202	43.3	21.25

[#] one plot was lost and not harvested.

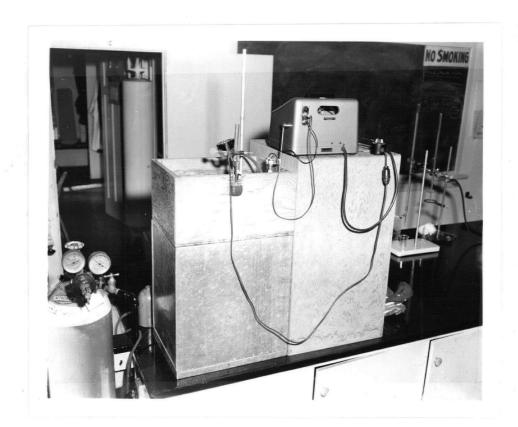
Table IX

The Residual Effects of the Four Substituted Urea Herbicides

Herbicide	Rate, in	C	Observations, days after treatment.					
	lbs./A	4	7	11	20	30	51	89
monuron	4.0	4.0	4.8	4.2	4.7	4.0	4.2	4.0
monuron	2.0	2.8	4.0	4.7	4.2	3.7	4.0	3.7
diuron	4.0	3.2	4.2	5.0	4.7	4.7	4.2	4.5
diuron	2.0	3.0	4.2	5.0	5.0	4.2	4.0	4.0
neburon	4.0	2.5	2.8	4.7	4.0	3.2	3.7	3.5
neburon	2.0	1.7	3.0	3.5	3.5	3.2	2.7	2.2
fenuron	4.0	1.0	2.8	3.2	3.5	2.7	3.0	3.0
fenuron	2.0	0.75	1.7	2.5	2.7	2.7	2.5	1.7
control	n/a	0.5	0.75	0.25	0.5	0.0	0.0	0.0
control	n/a	0.0	0.25	0.5	0.0	0.0	0.0	0.0

The vegetation cover was measured on a scale of 0 to 5, where 0 represented a maximum vegetation cover and 5 represented no plant life on the plot, i.e. maximum residual effect.

During the time of the experiment a total of 4.19 inches of rain was recorded at the University of B.C.meteorological station.



Apparatus used to measure potential change