THE SELECTIVE ACTION OF PYRAZON (5 AMINO - 4 - CHLORO - 2 - PHENYL - 3(2H) - PYRIDAZINONE) IN PLANTS

Thesis for the Degree of Ph. D.

MICHIGAN STATE UNIVERSITY
GERALD ROBERT STEPHENSON

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THE SELECTIVE ACTION OF PYRAZON
(5-AMINO-4-CHLORO-2-PHENYL-3(2H)-PYRIDAZINONE)
IN PLANTS

presented by

Gerald Robert Stephenson

has been accepted towards fulfillment
of the requirements for

Ph. D. degree in Horticulture

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Major professor

Date Oct. 23, 1968

O-169

ABSTRACT

THE SELECTIVE ACTION OF PYRAZON

(5-AMINO-4-CHLORO-2-PHENYL-3-(2H)-PYRIDAZINONE)

IN PLANTS

by Gerald Robert Stephenson

The selective toxicity of pyrazon (5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone) was examined by treating seedlings of tomato (Lycopersicon esculentum Mill., cv Heinz 1350), German millet (Setaria italica L.) and red beet (Beta vulgaris L. cv Detroit Dark Red) with pyrazon dissolved in the nutrient solutions. Pyrazon, at a concentration of 4.5 μM for 4 weeks, caused complete death of the tomato seedlings, severely inhibited the growth of German millet, but had no effect on red beet. Lower concentrations of pyrazon (0.045 and 0.45 μM) inhibited the growth of millet and tomato and stimulated the growth of red beet.

When the roots were exposed to a 5.1 μM concentration of ^{3}H-pyrazon in the nutrient solution, the greatest root absorption and translocation to the shoot was observed in the susceptible tomato and least in the tolerant red beet, while millet was intermediate.

Applications to the first true leaf indicated that ^{3}H-pyrazon was absorbed by the leaves of all species in the study but that it was not transported basipetally. The toxicity of foliar pyrazon applications to tomato and millet was increased with no injury to red beet by adding the surfactant X-77

(alkylaryl polyoxyethylene) to the spray solutions. The foliar absorption of ^{3}H-pyrazon increased with increasing concentrations of X-77 (0.3-1.3% v/v) and was greatest for the susceptible tomato. Red beet was more tolerant than German millet to foliar applications of pyrazon, even though absorption was similar.

Thin-layer chromatography of root and shoot extracts of tolerant and susceptible plants treated with ^{3}H-pyrazon, revealed that the concentration of pyrazon in the leaves of the tolerant red beet was further reduced by conversion to N-glucosyl pyrazon [N-(2-chloro-4-phenyl-3(2H)-pyridazinone)-glucosamine]. Twelve hr after the infiltration of beet leaf discs with 9.0 µM ^{3}H-pyrazon (in 0.35 M mannitol), over 70% of the pyrazon had been converted to N-glucosyl pyrazon. The conversion of pyrazon to N-glucosyl pyrazon was dependent on the concentration of photosynthate in the leaf tissue, since it was greater in light than in dark and the dark inhibition was overcome by infiltrating the leaf tissue with 0.05 or 0.10 M sucrose.

There was no evidence of the conversion of pyrazon to N-glucosyl pyrazon in eight of nine susceptible species under conditions that red beet converted over 50% of the absorbed pyrazon to the metabolite. Furthermore, in two different inbred lines of red beet, tolerance to pyrazon was greatest for that line which most readily converted pyrazon to N-glucosyl pyrazon.

The presence of pyrazon and pyrazon derivatives in the soil and in sugar beets was examined from one to 16 weeks after the application of pyrazon (phenyl-^{3}H) and pyrazon (4,5-^{14}C) to the soil. This study revealed that

pyrazon was taken up by the beet plant, translocated to the shoot and quickly converted to N-glucosyl pyrazon. This mechanism prevented the accumulation of high concentrations of unaltered pyrazon in the shoot. Two weeks after treatment, ACP (5-amino-4-chloro-3(2H)-pyridazinone) was detected in the soil. ACP was present in the plant from 4 weeks on, apparently as a result of root uptake, and was in turn converted to an ACP-complex with some natural plant compound.

These studies established that the phytotoxic effect of pyrazon is directly related to the quantity of the unaltered herbicide which accumulates in the foliar portion of the plant. This is dependent on the rates of the root uptake, translocation, foliar absorption, and conversion of pyrazon to N-glucosyl pyrazon. Differences in these processes between different plant species are responsible for the selective toxicity of pyrazon.

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By

Gerald Robert Stephenson

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Horticulture

1968

ACKNOWLEDGMENTS

The author wishes to express his sincere thanks to Dr. S. K. Ries for his guidance and assistance with this study. Appreciation is also expressed to Dr. D. R. Dilley, Dr. C. J. Pollard, Dr. A. R. Putnam, and Dr. M. J. Zabik for serving on the guidance committee and for advice during the course of this study.

This research was supported by Public Health Research Grant CC00246 from the National Communicable Disease Center, Atlanta, Georgia. Appreciation is also expressed to Badische Anilin-and Soda-Fabrik AG, Germany, and Amchem Products, Inc., U.S.A. for their financial assistance and for supplying the radio-labeled pyrazon and other chemicals used in this research.

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INTRODUCTION

The selective control of biological pests has concerned man since he began to value one biological organism over another. The continuous refinement and perfection of this process is essential for the efficient production of food and fiber. This is especially critical today due to the problems and needs of a rapidly increasing population.

The development of organic chemicals with selective biological toxicity has been one of man's most remarkable advances for the intelligent control of his environment. Ideally, selective toxicity is used to control undesirable organisms without injuring man, domestic animals, desirable plants or wild life. Selective control of insect pests without injury to important plants is easily obtained because of the great differences between plants and animals. The requirements for selective herbicides are much more critical because the weed to be controlled may be closely related to the crop plant. Thus, selective herbicidal action is often based on critical differences in the growth habit, morphology, or physiology of the weeds or crop plants involved.

Pyrazon (5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone) has been an effective herbicide for beets (Beta vulgaris L.) because of its remarkable selectivity. However, there have been few studies which have contributed to an understanding of its differential effect on tolerant and susceptible species.

In this study, the differences in uptake, translocation, accumulation and metabolism of pyrazon between tolerant and susceptible species are examined. The metabolic fate of this herbicide is given special consideration to elucidate the basis for its selective action.

LITERATURE REVIEW

A selective herbicide controls certain plant species without injuring others. Obviously, for a chemical to modify the growth of a plant, it must arrive at the physiologically active site(s). When morphology, physiology, or environment result in different concentrations of an herbicide at the site(s) of toxic action in different plants, a differential herbicidal effect usually results.

Physical Selectivity

Physical selectivity is based on a differential separation of the plants from the chemical by distance or time (16, 30). Examples of this type of selectivity are: the control of herbaceous weeds beneath woody crop plants; placement of an herbicide on the soil surface near shallow seeded weeds and sufficiently distant from deep seeded crops; direction of sprays toward weeds and away from crop plants; and treatment of weeds while crop plants are dormant or not yet emerged.

Morphological Selectivity

Morphological differences between weed and crop species can also be used to achieve selective weed control. For example, cuticular, positional, or shape differences of leaves can greatly influence the retention of a herbicide

on the foliage and ultimately, the amount of the herbicide which enters the plant (16, 30, 34, 45, 60). With a soil applied herbicide, differences in the shape, size, distribution and density of the roots between the crop and weed species can influence the amount of the herbicide which eventually comes into contact with the plants (16, 30, 34). Differences in the location of the growing point of plants may also provide selective herbicidal toxicity (34).

Physiological and Biochemical Selectivity

The most interesting type of herbicidal selectivity is biochemical selectivity or selectivity based on characteristics of the protoplasm itself. This type of differential herbicidal toxicity results when different plants are exposed to the same concentration of the toxic chemical. Physiological differences in plants which result in differential uptake, translocation, adsorption to cell constituents, or differential alteration of the chemical before it reaches the site(s) of action are responsible for this type of selective toxicity (30, 42, 59).

Differential uptake and translocation: Radiolabeled herbicides have made it possible to determine the importance of differential uptake and translocation for the selective action of many phytotoxic chemicals. The correlation of plant susceptibility with the absorbtion and translocation of phenoxy herbicides has been established by many investigators (18, 26, 33, 41, 62). Gallup and Gustafson (26) suggested that the limited absorption and translocation of

2,4-D* in tolerant monocotyledonous species was partly due to a block in the intercalary meristem of the leaves. Colby (8), Baker and Warren (4) and Ashton (2) have also shown that tolerance to amiben is in part dependent on the capacity of the plant to bind the amiben in the roots, thus, inhibiting translocation to the shoots. A similar explanation has been advanced for the selective action of linuron on tomato (Lycopersicon esculentum Mill.) and parsnips (Pastinaca sativa L.) (29). Considerably less linuron was translocated from the root to shoot in tolerant parsnip compared to the susceptible tomato. In the above mentioned examples, the investigators correlated differential uptake, translocation and accumulation of the herbicides involved, with a differential toxicity in various plant species. The role of these processes in determining selective toxicity is dependent on the location of the site(s) of action, the location and morphology of the point(s) of entry, and the physiological processes influencing movement of the compound between these points. It is also dependent on structural changes in the herbicide molecule which occur after uptake and before arrival at the site(s) of action.

Differential alteration of herbicides in plants: The selective action of most of the newer and more effective herbicides is based on differences in the alteration of the chemical by different plants. This type of herbicidal action has been extensively reviewed (6, 25, 55, 57, 59). Plants are known to alter

*The chemical names for all herbicides mentioned are given in the Appendix.

herbicides by (1) oxidation, (2) hydroxylation, (3) decarboxylation, (4) demethylation, (5) deesterification, and (6) conjugation.

A well known example of oxidation is the conversion of inactive phenoxy alkane carboxylic acids to the active phenoxyacetic acids by beta oxidation in susceptible species (60).

The alteration of a herbicidal molecule by certain plants often results in an apparent inactivation. This process may be non-enzymatic as is the case for the rapid conversion of simazine by hydroxylation to hydroxy simazine in Zea mays L. (57). Evidence that the above degradation is, in fact, related to selective action is quite adequately supplied by VanOorschot (59). He demonstrated a rapid recovery in the rate of CO_2 fixation by simazine treated corn plants after the simazine treatment had been removed.

Selectivity can also be related to a metabolic deactivation of the herbicide molecule. Some of the more clearly understood examples of this type are the differential decarboxylation of phenoxyacetic acid compounds (15, 38), the differential demethylation of monuron (49, 54) and atrazine (47), and the degradation of propanil to 3,4-dichloro aniline and propionic acid (51). In the case of monuron, it is quite apparent that demethylation is the process responsible for the recovery in the rate of CO_2 fixation by tolerant species after treatment with these herbicides is discontinued (59).

In recent years, it has been established that herbicides may be complexed with natural plant metabolites. Complexes, conjugates, adducts or other combinations are now known in plants treated with amiben, amitrole, maleic

hydrazide (MH), dalapon, symmetrical triazines, phenylureas, phenoxy acids (55) and, according to results to be discussed later in this text, pyrazon (44, 50). These compounds are complexed directly with natural plant substances. However, there are also several cases where the herbicidal molecule first undergoes alteration within the plant before being complexed with some natural compound. For instance, dinoben (9) and propanil (53) are first converted to arylamines and then complexed with glucose. Conjugation is suggested as the subsequent fate for a number of other herbicides which are known to be metabolized to arylamines. However, their respective complexes have not been isolated and identified. These herbicides include the carbamates, CIPC (32) and barban (43) and the ureas, chloroxuron (27) and monuron (54). This phenomenon is not limited to herbicidal chemicals. Evidence for this is provided by Kuhr and Casida (35). They isolated glucosides of at least 10 different methyl carbamate insecticides and their derivatives. Kaslander et. al. (31) also isolated the glucoside of a fungicide, sodium dimethyl dithio carbamate, from potato tubers.

The compounds which are known to make up the natural moiety of herbicide complexes include various amino acids, proteins, glucose, other carbohydrates, lignin, flavins and polyphenols (55).

This nearly ubiquitous occurrence of herbicide complexes, especially glucosides, has led some investigators to suggest that this may be a general detoxication process in plants (24, 55), similar to the detoxication of foreign chemicals in the animal body by glucuronide formation (62). The complexing

of a herbicide molecule with some natural plant constituent could inactivate the herbicide molecule by altering those components of its structure responsible for toxicity (55). It could also facilitate or inhibit the movement of the herbicidal molecule toward or away from the site(s) of action (55).

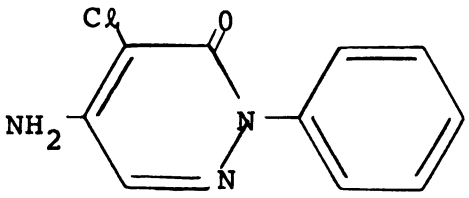
However, it is impossible to make any firm conclusions regarding the influence of complex formation on either the selective action or the mode of action of any herbicide. The occurrence of complexes in both tolerant and susceptible species (8, 53, 57), the difficulty in directly estimating the phytotoxicity of the complexes (52, 53), and the interaction of complex formation with other processes affecting selective action and mode of action (8, 52, 55, 57) seem to be the major reasons for this lack of understanding.

Physical Properties of Pyrazon

Pyrazon, first described by Fischer (21) in 1962, has a molecular wt of 221.6 and the following structural name, 5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone. Pyrazon is highly soluble in benzene and its solubility in water and methanol at 20 C is 0.03 and 3.54 g/L, respectively. Its melting point and decomposition temperature is 207 C (1).

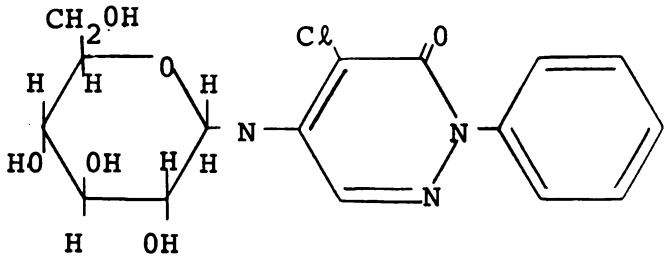
Structures and Names of Pyridazinone Compounds

The chemical structures for all pyridazinone compounds discussed are given on page 9. When referring to any of these compounds, the common name rather than the chemical name will be used.



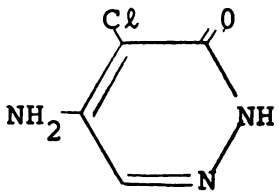
Pyrazon

5-amino-4-chloro-2-phenyl -3(2H)-pyridazinone



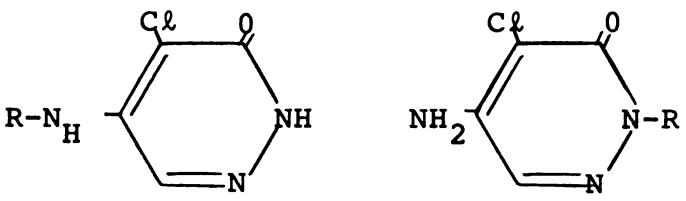
N-glucosyl pyrazon

N-(2-chloro-4-phenyl-3(2H)-pyridazinone) glucosamine



ACP

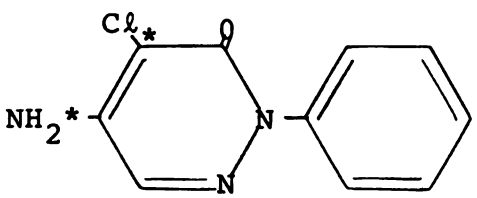
5-amino-4-chloro-3(2H)-pyridazinone



or

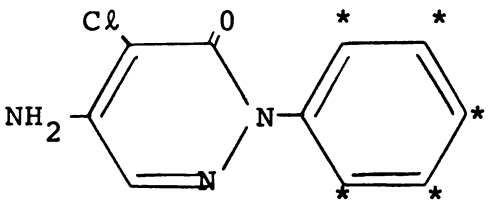
ACP-Complex

R-may be glucose (point of attachment not yet known)



^{14}C-pyrazon

5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone (4, 5 ^{14}C)



^{3}H-pyrazon

5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone (phenyl-2,3,4,5,6 ^{3}H

*Indicates positions of labeling.

Herbicidal Effectiveness

The importance of pyrazon as a herbicide is due to a high level of toxicity to several species of annual broadleafed weeds and to a high tolerance of red beets and sugar beets to the chemical (28). It most effectively controls weeds in beet plantings when applied preemergence to low organic mineral soils, under conditions of adequate moisture, and when the weed spectrum consists primarily of annual broadleafed weeds (1, 5, 40).

The problem of poor annual grass control can be partially overcome by increasing the rate of application or by applying the pyrazon in combination with herbicides more active on grasses, such as trichloroacetic acid (40). The effectiveness of postemergence pyrazon treatments has been increased by the addition of various oils or adjuvants to the spray solution (23). Both red beets and sugar beets generally possess outstanding physiological tolerance to pyrazon throughout all stages of growth except during the "early cotyledon stage" (1, 20, 36).

Fate of Pyrazon in the Soil

Volatility: It is improbable that volatilization results in significant loss of pyrazon from the soil, since the vapor pressure is so low at temperatures usually encountered (20). At 40 C the vapor pressure is 0.074 mm of Hg.

Photodecomposition: After subjecting a pyrazon solution to u.v. irradiation (above 3000A) from a mercury arc lamp, Zabik (64) detected at least twenty different breakdown products. The fragments have not been positively identified, but pyrazon was not recovered. These results indicate that the photodecomposition of pyrazon could occur after exposure to sunlight under field conditions.

Adsorption and movement: In a study of the adsorption of pyrazon by five pure clays, Cooke (12) found that the degree of adsorption was in the order of hectorite > illite and attapulite> halloysite and montmorillonite. In another study, Doherty (13) examined the adsorption of pyrazon by organic matter. He mixed various percentages of the adsorbent with quartz sand and determined the degree of adsorption by a bioassay. Adsorption was related to the stage of decomposition and to the percentage organic matter, and was in the order of fibrous peat > muck soil > sphagnum moss > bentonite clay. Smith (48), in an adsorption study which involved both clay and organic matter, concluded that adsorption of pyrazon in soils was primarily dependent on organic matter content and unrelated to clay content. Phytotoxicity and leaching were inversely related to the amount of adsorption.

Persistence: Pyrazon has a much shorter persistence in soils when compared to other herbicides like the triazines (20). Using a bioassay, Fischer (20) found that there was insufficient pyrazon residue to injure small grains planted 8-10 weeks after the application of 3 Kg/ha. In laboratory experiments,

Drescher (14) found that 40 weeks were required before a soil application of 12 ppm was no longer detectable. His analytical method was sensitive to 0.1 ppm. In outdoor experiments, with both potted soil and field plots, less than 0.2 ppm was detectable 12 weeks after the application of 20 ppm and 4 Kg/ha, respectively (14). Smith (48) found that pyrazon was highly persistent in soils with organic matter in excess of 10% and that disappearance was dependent on the amount and distribution of rainfall.

Fischer was the first to isolate a degradation product of pyrazon from the soil (20). He identified this compound as ACP (5-amino-4-chloro-3(2H)-pyridazinone). In more recent experiments, Burger (7) and Smith (48) have also isolated this compound from pyrazon treated soils. This compound can be first detected 2-4 weeks after pyrazon application (7, 48) and steadily increases in concentration from that point on. According to Fischer (20) ACP is not phytotoxic. All of the investigators refer to this compound as a microbial degradation product, but there are no experiments which specifically substantiate this.

Mode of Action and Selectivity in Plants

Herbicidal activity of pyridazinone compounds: Fischer (20), after measuring the phytotoxicity of over 800 pyridazinone derivatives, found that the most herbicidally active, 4-chloropyridazinone, which did not injure beets possessed a phenyl group at "position 2" and a free amino group at "position 5" on the pyridazinone ring. Substitution or removal of either the free amino

group or the phenyl ring resulted in a general decrease in phytotoxicity. Exchange of the chlorine for a bromine resulted in slightly increased toxicity to grasses but the tolerance in beets was retained. Replacing the free amino group with a methoxy group did not alter general toxicity, however, beet tolerance was destroyed and tolerance in cotton substantially increased. These observations indicated that the presence of a free amino group was essential for general toxicity to be accompanied by tolerance in beets.

Uptake: Field observations have led most investigators (20, 22, 36, 58) to conclude that root uptake is more important for the action of pyrazon than foliar absorption. Postemergence applications of pyrazon alone have given poor results. However, the spectrum of weeds controlled has been increased by the addition of adjuvants to the spray solution without causing injury to beets, as long as application during the "susceptible cotyledon stage" of the beets is avoided (23, 36).

Metabolism: After treating seedlings of tolerant sugar beets and several other susceptible species with 903 μM pyrazon solutions for 2 weeks, Fischer (19) analyzed both the nutrient solution and the plants for metabolites. He detected the presence of ACP in the solution and in all of the species in the test. Since this compound is not phytotoxic and since he found much more of it to be present in the tolerant beets compared to the other susceptible species, he concluded that beets were tolerant because of the capacity to inactivate pyrazon by dephenylation. However, the possibility that this metabolite was made exclusively in the

nutrient solution and then taken up by the plants cannot be excluded on the basis of the evidence provided by Fischer.

More recently, Stephenson and Ries (50) reported the presence of a ^{3}H-labeled metabolite in red beets treated with pyrazon (phenyl-^{3}H). Since the metabolite was labeled with ^{3}H which was contained in the phenyl ring of the pyrazon molecule, it was established that this metabolite was not ACP, the metabolite reported by Fischer (19). Furthermore, the metabolite appeared to be a complex containing intact pyrazon and involving the free amino group on the pyridazinone ring.

Burger (7) studied the appearance of pyrazon metabolites in sugar beets growing in soil treated with ^{14}C-pyrazon. After first detecting pyrazon in the plant, he was able to show the appearance of 3 major ^{14}C-labeled metabolites which he called A, B, and C in order of their appearance. Only metabolite B was conclusively identified as ACP (Fischer's metabolite). Examination of Burger's data indicates that ACP occurred in the plant after it was found in the soil. Metabolite A appeared to be the complex previously reported (50) and metabolite C was strongly hydrophilic. Burger has postulated the following pathway for the degradation of pyrazon in beet plants.

$$\text{Pyrazon} \longrightarrow \text{A} \longrightarrow \text{B} \longrightarrow \text{C}$$

A = "Pyrazon complex ?"

B = ACP (5-amino-4-chloro-3(2H)-pyridazinone)

C = Strong hydrophilic substance?

This postulated pathway also appears to reject the possibility that ACP is taken

up from the soil, in favor of the hypothesis that it is synthesized in the plant.

Ries, Zabik, Stephenson and Chen (44) have since isolated milligram quantities of the "pyrazon complex." Using thin-layer chromatography, gas liquid chromatography, and infrared spectrophotometric procedures, they were able to identify this metabolite as N-(2-chloro-4-phenyl-3(2H)-pyridazinone)-glucosamine, or N-glucosyl pyrazon.

Selective Action

Several investigators have reported the outstanding selective action of pyrazon (1, 22, 57). Even Chenopodium album L. and Amaranthus retroflexus L., weed species which are closely related to beets botanically and in growth habit, are highly susceptible to pyrazon (1, 22).

Possibly the most dramatic demonstration that beets are physiologically tolerant to pyrazon is that given by VanOorschot (59). He treated tolerant sugar beets and susceptible oats (Avena fatua L.), with pyrazon dissolved in the root nutrient solution until the rate of CO_2 fixation by sugar beets had been inhibited by 50%. He then removed the pyrazon treatment and observed that the rate of CO_2 fixation by beet recovered completely within 12 hr. There was no recovery observed for the susceptible oats. His interpretation of these results was that beets can take up sufficient pyrazon to be injured, however, there must be a means for either rapidly deactivating pyrazon or for removing it from the site of action.

Mode of Action

Frank (22) found that the inhibition of the Hill reaction by pyrazon was the same for chloroplasts isolated from tolerant beets or from susceptible Chenopodium album L. The concentration which caused 50% inhibition was 6.5 μM. However, when leaf discs of these two species were cultured in logarithmic concentrations of pyrazon, oxygen evolution by C. album discs was suppressed to a greater extent than beet. These results seem to be in agreement with VanOorschot's work which suggests that pyrazon is a photosynthetic inhibitor and that there is a capacity for the inactivation of pyrazon in leaves of beet (59). In contrast to this, Eshel (17) found no significant difference in the oxygen evolution from leaf discs of tolerant beets and susceptible tomato which had been shaken for 30 min in pyrazon solutions. The concentration required for 50% inhibition was comparable to that observed by Frank (22) for isolated chloroplasts. Using tomato leaf discs, Eshel (17) observed a greater phytotoxic effect in the light than in the dark.

The respiration of leaf discs and root tissues from both C. album and beets was not affected by 24 hr treatment with pyrazon concentrations up to 4.5 μM. It should be noted that this concentration exceeded the maximum solubility in water.

Zemanek et. al. (65) found that pyrazon inhibited catalase activity in mustard (Sinapis alba L.) but not in sugar beet. The enzyme assay was made 1, 4 and 7 days after treatment with a 0.4% pyrazon solution (solubility in

water is 0.003%) at the "2 true leaf" stage.

Makovcova et. al. (39) showed that pyrazon increased succinic dehydrogenase activity in sugar beet and inhibited it in Sinapis alba L. (mustard). This was true for both tissue homogenates and intact plants. They concluded that this differential effect on SDH activity explained the difference in the phytotoxicity of pyrazon to these species.

MATERIALS AND METHODS

Growth Studies

The effect of pyrazon on the growth of red beet (cv. Detroit Dark Red), German millet (Setaria italica L.) and tomato (cv. Heinz 1350) was determined using pyrazon applied to the roots in nutrient solutions. The experiment was conducted in controlled environment chambers at 26 C during the 16 hr day (2,000 ft-c) and 13 C for the 8 hr night. Plants were obtained from seed germinated in individual 10 cm clay pots filled with quartz sand. Moisture and nutrients were applied with a half-strength Hoagland's nutrient solution. The moisture level was maintained 2 cm below the seed until germination. Subsequently, equal amounts of nutrient solution were added every other day. After the first true leaf appeared and the plants had been thinned to five per pot, pyrazon was incorporated into the nutrient solution at logarithmic concentrations from 0 to 4.5 μM. The pots were removed from the chamber each week and flushed with distilled water to prevent increases in concentration of the nutrient elements and pyrazon due to evaporation, and to maintain a pH of about 6.5. Four weeks after treatment the plants were removed from the pots, washed with water, oven dried and weighed.

Additional studies were conducted in which the influence of pyrazon on the growth of the tolerant red beet was examined. The conditions in these experiments were the same as above. The concentrations of pyrazon utilized were 0, 0.045,

0.45 and 4.5 μM.

Procedures for Experiments Utilizing Radioactively Labeled Pyrazon

The ^{3}H-labeled pyrazon used in the uptake, translocation, and metabolism experiments and the ^{14}C-labeled pyrazon used in the long term metabolism study were synthesized and provided by Badische Anilin and Soda Fabrik AG, Germany.

The ^{3}H-pyrazon as prepared had a specific activity of 174.41 μc/mg and the phenyl ring was uniformly labeled with tritium. The ^{14}C-pyrazon had a specific activity of 21.21 μc/mg. The ^{14}C atoms were located at the 4 and 5 positions in the pyridazinone ring. All stock solutions were prepared by dissolving known amounts of the compound in absolute ethanol.

A Packard 3003 Liquid Scintillation Spectrometer was used to determine radioactivity. The scintillation spectrometer was equipped with automatic external standardization which made it possible to correct for quenching due to the presence of plant tissue or solvents in the counting solution. Suitable quench curves were prepared (46) and counts per minute (cpm) were converted to disintegrations per minute (dpm). The only exception to this was the assay of radioactivity in chromatographic fractions since the counting conditions were essentially identical for all samples and relative differences in the cpm were suitable for the intentions of the experiments.

The scintillation cocktail used for the assay of radioactivity contained toluene as the solvent and 4g/L of BBOT (2,5-Bis[5-tert-Butylbenzoxazolyl)]-thiophene) as the solute fluor. High activity ethanol soluble samples were

counted using a toluene-BBOT/ethanol 15:1 or 15:0.5 (v/v) cocktail. Low activity aqueous and ethanol-ethanolamine 2:1 (v/v) samples were counted using a toluene-BBOT/triton X-100 10:4 (v/v) cocktail.

Procedures for the Freeze-drying of Plant Tissue

After harvest, all plant tissue from experiments utilizing ^{14}C or ^{3}H-pyrazon was washed with water or ethanol and placed in glass containers. The containers were then placed in a slurry of dry-ice and Dowanol (1-methoxy-2-propanol; propylene glycolmethyl ether) at -78 C until the tissue was frozen (30 to 60 min).

The containers of frozen tissue were placed on a heat rack (26 C) inside the vacuum chamber of a Virtis Unitrap Model 10-1-2 freeze dryer. A vacuum (less than 10μ Hg) was applied until the tissue was dry (at least 48 hr).

The dry wt of all experimental samples was obtained immediately after removal from the freeze dryer.

Uptake and Translocation of ^{3}H-Pyrazon by Tolerant and Susceptible Species

Root uptake and translocation: The seedlings of the three species, (beet, German millet, and tomato) were grown in quartz sand until the cotyledons expanded. Three seedlings, one of each species, were then transferred to aerated nutrient solutions in 50 ml beakers. The seedlings were suspended in the nutrient solution through perforations in a foil covering.

The plants were grown with a day length of 16 hr (1500 ft-c) and a constant temperature of 22 C. Ten μc (57.4 μg) of the ^{3}H-pyrazon was added to each beaker. The resulting concentration of pyrazon was 5.18 μM.

The plants were harvested after 15 min, 1, 24, 48 and 72 hr, separated into root and shoot, and washed thoroughly with water to remove the unabsorbed chemical. The plant parts were then freeze-dried and weighed.

The plant parts were extracted by grinding in ethanol in a Kontes tissue grinder. A portion of this extract was counted to determine the total amount of radioactivity in the sample. This was done separately for the roots and shoots to determine that retained in the root versus the amount translocated to the shoot.

Foliar absorption and translocation: The cultural procedures in this experiment were identical to those in the root uptake experiment. A treatment solution was prepared by adding 25 μl (5 μc) of the stock ^{3}H-pyrazon solution to 225 μl of distilled water. Five μl of this solution (0.1 μc) were applied with a Hamilton syringe to the center of the first true leaf.

Three replicates of each of the three species were harvested 1, 24, 48, and 120 hr after treatment. The treated leaves were washed by dipping five times in separate vials containing 1 ml of absolute ethanol to remove unabsorbed ^{3}H-pyrazon. The leaves and non-treated portions of the shoots and roots were freeze-dried, weighed, and extracted as in the root uptake experiment. Portions of the washings and plant extracts were counted to determine the percentage of ^{3}H-pyrazon absorbed and translocated by the three species.

Influence of surfactant X-77 on the phytotoxicity of pyrazon: For these studies, the same three species (beet, German millet and tomato) were grown in the greenhouse in a sand-soil-peat mixture (1:1:1). Supplementary nutrients were added every two weeks via a Hoagland's nutrient solution. Average day and night temperatures were 32 C and 24 C, respectively. The treatments were applied when all of the species had one or two true leaves. Pyrazon (80% wp formulation) was applied at the rate of 3.0 lb/A in a 40 gpa volume and the surfactants were tested at various percentages of this spray volume. The treatments were applied exclusively to the foliage by covering the soil surface with vermiculite during treatment.

The surfactant X-77 (alkylaryl polyoxyethylene) was included in a preliminary experiment, in which several surfactants were tested at the rate of 0.67% (v/v) for enhancing the action of pyrazon on German millet and tomato without injuring beet.

In a subsequent study, the optimum rate for X-77 was determined by applying pyrazon in solutions containing 0, 0.33, 0.67, 1.0 and 1.33% (v/v) surfactant. Red beet and German millet were the species used in this study.

In all of these tests, injury was rated visually on a 1 to 9 scale three weeks after treatment (1 indicating no injury, 9 indicating death of the plant).

Influence of X-77 on the foliar absorption of pyrazon: In this study, seedlings of red beet, German millet, tomato and turnip (Brassica rapa L., cv Just Right) were grown as previously described for the root uptake experiment. A

16 hr, 24 C day (2,000 ft-c) and an 8 hr, 16 C night were maintained throughout the experiment.

The plants were treated by placing a 10 µl droplet of the appropriate solution on the center of the first true leaf of each plant. Each droplet contained 149 µg pyrazon (80% wp) plus 0.2 µc ^{3}H-pyrazon (1.2 µg) and either 0, 0.5, or 1.0% (v/v) surfactant X-77. The concentration of pyrazon in the droplets was comparable to the concentration used in normal field applications.

The plants were all harvested 120 hr after treatment. The harvesting, extracting and counting procedures were identical to those described previously for the foliar absorption study.

Metabolism of Pyrazon in Tolerant and Susceptible Species

Thin-layer chromatography procedures for detecting ^{3}H-labeled pyrazon and metabolites: The relative amounts of ^{3}H-labeled metabolites (mainly N-glucosyl pyrazon) were determined by thin-layer chromatography of the extracts of plant tissue. In all cases the plant tissue was frozen, freeze-dried, and weighed before extraction.

Thin-layer chromatography was performed using Eastman prepared silica gel Chromagrams with two solvent systems. Solvent system I was benzene-ethanol 75:25 (v/v) and solvent system II was chloroform-ethanol 95:5 (v/v). The plant extracts were applied to the chromatograms with a Hamilton syringe using the streaking method of application. The extracts were co-chromatographed with ^{3}H-pyrazon, technical non-labeled pyrazon and technical ACP.

The thin-layer chromatogram sheet was divided into vertical sections 2-4 cm in width by scraping narrow portions of the silica gel adsorbent from the plastic support. This prevented lateral contamination between adjacent extracts on the chromatogram. The chromatogram was removed from the developing chamber when the solvent front had moved 10 cm above the point of application. The 2 x 10 cm 'sub-chromatograms' corresponding to each of the plant extracts were cut out from the thin-layer sheet. Each of these sections was then divided into 20 (0.5 x 2 cm) or 10 (1 x 2 cm) segments along the line in which the solvent had moved. The radioactivity in each section was determined by liquid scintillation spectrometry. All data were converted to percent of total cpm on the chromatogram by dividing the cpm in each section by the total cpm for all sections. The Rf values of the ^{3}H-labeled compounds as well as the percent conversion of pyrazon to metabolite(s) were determined in this manner. Acceptable chromatograms had a total of at least 300 cpm.

The Rf values were also determined by a diazotization reaction which resulted in a red-violet color indicating the presence of a substance with a 'free amino group' on an aromatic ring (62). In this reaction, the amino group is diazotized by exposure to fumes of nitrous acid followed by coupling with beta-napthol in fumes of NH_3 to produce the highly colored 'Azo-compound'. This procedure was effective for determining the presence of technical pyrazon and ACP.

Metabolism of pyrazon applied to the roots: Red beet, German millet, and tomato seedlings were grown and treated with ^{3}H-pyrazon in the root medium, using the procedures of the root uptake study. The only exception was that each 50 ml beaker contained five seedlings of one species instead of one seedling of each species. The plants were harvested after 72 hr and the presence of ^{3}H-labeled metabolites in the roots and shoots determined by thin-layer chromatography.

Metabolism of pyrazon applied to the foliage: The treated leaf extracts from the surfactant study were also examined for ^{3}H-labeled metabolites. The species in this study included red beet, German millet, turnip and tomato. Due to the low concentration of radioactivity in the extracts, only the extracts of the leaves treated with ^{3}H-pyrazon and 1.0% (v/v) X-77 were examined.

Metabolism studies with species susceptible to pyrazon: To further determine the relevance of pyrazon metabolism to the selective action of this herbicide in plants, a metabolism study with several susceptible species was conducted. The species included in the study, in addition to the tolerant red beet, were soybean (Glycine max (L.) Merr. cv Chippewa 64), squash (Cucurbita pepo cv Early Summer Yellow Crookneck), cucumber (Cucumis sativus L. cv SMR 18), pea (Pisum sativum L. cv Red Jade), and corn (Zea mays L. cv Michigan 400).

All of these species are susceptible to pyrazon (1) and furthermore, all except corn (8) metabolize amiben, a herbicide very similar in structure to

pyrazon.

The seed was germinated in moist vermiculite and the seedlings treated with ^{3}H-pyrazon by vacuum infiltration to minimize differences in uptake. This was done by submerging the seedlings in a 0.35 M mannitol solution containing ^{3}H-pyrazon at a concentration of 9.0 μM. The solution was evacuated for 30 min with a water aspirator. The seedlings were kept submerged for 15 min following the return of the solution to normal atmospheric pressure. After infiltration, the seedlings were placed in petri dishes and were kept moist by placing a folded tissue soaked with distilled water in each dish. The seedlings were incubated with constant light (1,500 ft-c) and temperature (26 C). After 20 hr, the seedlings were harvested, freeze-dried, extracted and the percent pyrazon metabolism determined.

Metabolism of pyrazon by inbred lines of red beets: The seeds from 24 different inbred lines of red beets were germinated in 10 cm clay pots filled with soil under greenhouse conditions during June. Day temperatures averaged 32 C and night temperatures averaged 24 C. One of the second pair of true leaves was removed from a plant of each line and placed in a labeled tube in an ice bath at 0 C. Leaf discs were obtained with an 8 mm cork borer.

The leaf discs from each line were enclosed in a moistened cheese cloth bag and labeled. The discs were then vacuum infiltrated with the 9.0 μM ^{3}H-pyrazon and 0.35 M mannitol solution and placed in separate petri dishes (10/dish) for the 10 hr incubation with constant light at 26 C. One ml extracts (50% ethanol) were prepared and the percent metabolism of pyrazon determined.

Since this was a preliminary experiment only one observation per line was made.

From these 24 inbred lines, two lines which showed a considerable difference in the capacity to metabolize pyrazon were chosen for a replicated time course metabolism study. The replicate leaves were obtained from three different plants of each line. The procedure and conditions for the experiment were exactly the same as previously described. Incubation times of 0, 4 and 8 hr were employed.

Influence of pyrazon on the growth of two inbred lines of red beet: In order to determine the relationship between the capacity to metabolize pyrazon and tolerance to this chemical, the effect of pyrazon on the growth of the above inbred lines of red beet was examined.

Pre-germinated seed of the two lines was transferred to quartz sand nutrient culture in 6 cm styrofoam cups (three seeds/cup). When the first true leaves formed, pyrazon was incorporated into the nutrient solution at concentrations of 0, 11, and 22 μM. The conditions during growth were a 16 hr day (1,500 ft-c), 8 hr night, and a constant temperature of 26 C.

The seedlings were harvested 10 days after treatment and dry wts were obtained as a measure of growth.

The Role of Light in the Metabolism of Pyrazon by Red Beet

Study with intact plants: Seeds of red beet were germinated in moist quartz sand. The seedlings were transferred to aerated nutrient culture in

50 ml beakers (five seedlings/beaker) when the first true leaves were formed. The seedlings were placed in two growth chambers at a constant temperature of 26 C. The lights were turned off in both chambers for 24 hr before the incorporation of ^{3}H-pyrazon (4.5 µM) in the nutrient solutions. At this time, the lights in one chamber were turned on (1,500 ft-c). Five replicate harvests were made from both the light and dark after 24, 48, and 72 hr. During the harvesting process, the room was kept dark except for a small green "safe light." The plants were freeze-dried, extracted, and the percent conversion of pyrazon to metabolite determined by thin-layer chromatography.

Studies with infiltrated beet leaf discs: In order to minimize differences in uptake due to variation in plants and to the effect of light on the rate of pyrazon uptake, subsequent studies utilized beet leaf discs infiltrated with pyrazon rather than intact plants.

The leaf discs were infiltrated with 9.0 µM ^{3}H-pyrazon in 0.45 M mannitol as previously described. The infiltration was performed in a dark room, with only a green "safe light" to allow visibility. For dark incubations, the petri dishes were covered with aluminum foil from the beginning of the incubation until after the leaf discs had been frozen with dry ice and Dowanol. The light intensity for light treatments was 1,500 ft-c. All experiments were conducted at a constant temperature of 26 C. The incubation periods were terminated at the desired times by the freeze-drying process. The discs were then weighed, extracted and the percent metabolism determined.

In the initial experiment, the percent conversion of pyrazon to metabolite

was determined under conditions of a normal photoperiod consisting of a 16 hr day (1, 500 ft-c) and an 8 hr night. The discs were infiltrated at the beginning of the 16 hr day and harvests were made after 0, 6, 12, 24, 36, and 48 hr.

In subsequent experiments, leaf discs were obtained from beet plants which had received either 12 hr light or 48 hr dark prior to treatment. The leaf discs from these two sources were then infiltrated with pyrazon and the percent conversion of pyrazon to metabolite determined after 0, 6, 12, and 24 hr incubation in the light and dark.

Influence of supplemental sucrose on pyrazon metabolism: Leaf discs from plants which had received a 48 hr dark treatment prior to infiltration were used to study the influence of supplemental sucrose on pyrazon metabolism in the dark. Sucrose was dissolved in the infiltration solution at concentrations of 0, 0.05 and 0.10 M. The concentration of mannitol was correspondingly reduced to maintain the nearly isotonic conditions of the 0.35 M solution. The metabolism of pyrazon in light without sucrose compared to metabolism in the dark with 0, 0.05 and 0.10 M sucrose was examined after 4 hr.

Enzymatic Studies

The enzymatic conversion of pyrazon to N-glucosyl pyrazon was attempted, using a modification of the procedures of Frear (46). He isolated and partially purified the enzyme UDP-Glucose:Arylamine N-Glucosyltransferase, which converts amiben to N-(3-carboxy-2, 5-dichlorophenyl)-glucosylamine.

The shoots (32 g fresh wt) of 4 week old red beet seedlings were frozen in liquid nitrogen. The frozen tissue was then homogenized for 5 min in a dry ice-acetone slurry (-80 C), and allowed to stand at -25 C for 2 hr. The acetone was replaced and the homogenate was maintained for 15 hr at -25 C before removing the acetone.

The powder was washed three times with 10 volumes of acetone (-30 C) and three times with 10 volumes acetone-ether 1:1 (v/v) (-30 C) and then dried by vacuum (less than 100 μHg) for 8 hr and the dry wt obtained.

The total nitrogen, alcohol soluble and insoluble nitrogen, and TCA precipitable nitrogen per 100 mg powder were determined by micro-Kjeldahl procedures.

The dried acetone powder (100 mg) was extracted by shaking with 5 ml 0.05 M phosphate buffer (pH 7.5) for 2 hr at 0 C followed by centrifugation at 2,000 x g for 10 min. A 2 ml portion of the supernatant at this stage was retained for enzymatic assay. The remaining 2.5 ml was eluted through a Sephadex G-25 column with 0.05 M phosphate buffer (pH 7.5) at the rate of 2 ml/min. The elution volume (20-28 ml) for the protein in the extract was determined by measuring the absorbence of the fractions at 260 and 280 mμ with a Beckman DU spectrophotometer and by measuring the protein content of the fractions using the Lowry method for protein determination (37). The void volume of the column was found to be 21 ml by eluting blue dextran.

A crude extract of fresh beet leaf tissue was also prepared by homogenizing 5g tissue in 10 ml of 0.05 M phosphate buffer, pH 7.5, at 0 C followed by

centrifugation at 1000 x g for 10 min. The enzyme activity of the crude extract was determined before and after partial cleanup with a Sephadex G-25 column at 0 C.

The reaction mixture was essentially the same as that used by Frear (24) and consisted of the following: 2 μM UDPG, ADPG or TDPG, 100 μM phosphate buffer (pH 7.5), 0.10 μM ^{3}H-pyrazon, deionized water, and enzyme extract. The reaction volume was 1.0 ml and incubation was at 25 C. The percent conversion of pyrazon to N-glucosyl pyrazon was determined after 30 min and 1 hr.

Long Term Metabolism Study with ^{3}H and ^{14}C-Pyrazon in Sugar Beets and Soil

Treatment: Sugar beets (cv MSU 126 x 5460) were planted in the greenhouse in clay pots containing a mixture of sand, soil, and peat (1:1:1). Adequate moisture was maintained by watering daily with tap water until emergence and alternately with tap water and half-strength Hoagland's solution thereafter. The experiment was conducted from February until July. Average day and night temperatures were 32 C and 24 C, respectively. The plants were not injured by the pyrazon, however, due to restricted root growth, these greenhouse grown plants were much smaller than normal field grown plants of the same age.

After development of the first true leaves, the seedlings were thinned to 4, 3, 2, and 1 seedling(s) per pot for the first, second, third and succeeding harvest, respectively. The treatment solutions were prepared by dissolving

17.5 mg ^{14}C-pyrazon and 4.85 mg ^{3}H-pyrazon +12.65 mg technical pyrazon in 2 liters of half-strength Hoagland's solution. The observable activity of the ^{14}C and ^{3}H-pyrazon solutions was 170 cpm/µl and 137 cpm/µl, respectively. One-half of the pots were treated with the ^{3}H-pyrazon solution and one-half with the ^{14}C-pyrazon solution. The treatment solutions were applied to the soil surface at the rate of 1 ml/10g or 0.875 mg/Kg soil.

Harvests: Three replicates of both the ^{3}H-pyrazon and ^{14}C-pyrazon treated plants were harvested 1, 2, 4, 6, 8, 12, and 16 weeks after treatment. The plants were removed from the soil (except for small branch roots and root hairs), separated into root and shoot, weighed, freeze-dried and weighed again. The dried tissue was ground to a powder with a mortar and pestle. The soil was weighed, air dried fot 48 hr, weighed again, and finally sifted to remove small stones and extraneous matter.

Extraction: The entire powdered samples of the roots and shoots were extracted four times by homogenizing in 50% ethanol (2 ml/g dry wt) for 5 min with a Virtis homogenizer. The extraction for each sample was carried out in a single polyethylene bottle, so that the tissue could be precipitated by centrifugation and the supernatant replaced with fresh 50% ethanol after each homogenization.

The extraction procedure for the soil was identical to that used for the tissue samples, except that only 100g portions of each sample were extracted and the soil-50% ethanol suspension was placed on a shaker for 10 hr instead

of homogenizing it for 5 min.

The roots from the last harvest were subjected to a more rigorous extraction procedure to estimate the concentrations of the water soluble and insoluble metabolites. The roots were first extracted as described above with 50% ethanol. The remaining tissue residue was extracted 4 times with 2 volumes of benzene. A portion of the aqueous fraction from above was then extracted three times with 9 volumes of benzene before and after acidification with HCl (0.2N), heating (100 C for 30 min) and neutralization (pH 7.0) by the addition of NaOH. The various benzene fractions were concentrated to 5 ml and the radioactivity determined.

After extraction, 25 mg portions of the ^{14}C soil, root and shoot residues from the 16 week harvest were combusted and the $^{14}CO_2$ counted to estimate non-extractable radioactivity. This was done according to the procedure of Schoniger (62), as modified by Aya (3). The percent recovery of the combustion process was determined by combusting known quantities of ^{14}C-pyrazon added to samples of dry non-treated soil, roots and shoots.

Detection and identification of ^{3}H-labeled and ^{14}C-labeled pyrazon derivatives: The pyrazon and its metabolites present in the soil and shoot extracts were separated by thin-layer chromatography, using 20 x 20 cm glass plates coated with 250μ silca gel G. Solvent system III (benzene-ethanol 60:40 (v/v)) was the primary system used. The chromatograms were developed 16 cm from the origin. The Rf values of the radioactive substances present were determined by separating the chromatogram for each extract into 16 sections. Each 1 cm

section was scraped from the plate into a scintillation vial. One ml of ethanol and 15 ml of the scintillation cocktail was added and the radioactivity determined.

Due to extremely high concentration of sugar and to the low concentration of radioactivity in the root extracts, only pyrazon and its benzene extractable metabolites could be separated by thin-layer chromatography procedures.

The positions of the labeling, ^{14}C at the 4 and 5 positions of the pyridazinone ring and ^{3}H at the 2, 3, 4, 5, and 6 positions in the phenyl ring, were used to determine the presence or absence of these rings in each of the metabolites. The Rf values of the radioactive compounds were also compared to those for pyrazon, and ACP, using solvent system III and IV [aceton-dioxane-NH_4OH (25%) 45:45:10 (v/v/v)].

The interrelationship of two metabolites labeled only with ^{14}C was determined by an infiltration experiment. ACP (4, 5-^{14}C) was obtained from a soil extract by preparative thin-layer chromatography using 20 x 20 cm glass plates coated with 1 mm silica gel G, and solvent system III. ACP was eluted from the gel with absolute ethanol, evaporated to dryness and added to a solution containing 0.10 M β-D(+)-glucose and 0.25 M mannitol. Sugar beet leaf discs were infiltrated with this solution, incubated for 4 hr, freeze-dried, extracted and the radioactive compounds separated by thin-layer chromatography using solvent system III.

The third ^{14}C-labeled metabolite which appeared in the plant was separated from the other two by thin-layer chromatography, scraped from the plate, and eluted from the gel with absolute ethanol. This extract was then rechromatogrammed

before and after acid hydrolysis (0.2N HCl, 100 C for 30 min).

Experimental Design and Statistical Analysis

A randomized complete block design with not less than three replications was employed in all simple and factorial experiments. In experiments involving more than one species and in those with light and dark treatments a split plot design was utilized. The data from all experiments pertaining to differential growth, uptake, translocation, or metabolism were analyzed by analysis of variance and, whenever a significant F value was obtained for main effects or interactions, they were partitioned into single degrees of freedom for comparison of individual means.

Data from thin-layer chromatograms illustrating radioactive pyrazon or metabolite peaks are presented as the mean of three replications but were not subjected to statistical analysis.

RESULTS AND DISCUSSION

Growth Studies

Red Beet, millet, and tomato displayed contrasting degrees of tolerance to pyrazon applied to the roots. Dry wts indicated that red beet, in contrast to millet and tomato, was not injured as the concentration of pyrazon was increased to 4.5 μM (Table 1). Millet was more tolerant than tomato even though the response of the two species was similar on the basis of dry wt. Tomato plants treated with 4.5 μM died approximately 2 weeks before harvest while all of the millet plants survived this treatment for the 4-week period. It was apparent from these results that beets were very tolerant, millet, intermediate, and tomato, very susceptible to pyrazon. This situation was similar to field observations where annual grasses are not effectively controlled and many annual broadleafed weeds are susceptible. These species were chosen as test species for studies to determine the basis for the selective herbicidal action of pyrazon.

There was also an apparent stimulation of the growth of red beet by pyrazon at a concentration of 0.45 μM (Table 1). Additional experiments were conducted in which the effect of pyrazon at low concentrations on the growth of red beet was determined. Of the four concentrations examined, 0.045 and 0.45 μM concentrations of pyrazon appreciably increased the growth of red beet (Figure 1).

Thus, it was apparent that red beets were tolerant to herbicidal concentrations and stimulated by low concentrations of pyrazon.

Table 1. The growth of red beet, millet and tomato exposed to various concentrations of pyrazon in the nutrient solution. [1/]

pyrazon concn (μM)	Dry wt expressed as a % of the control		
	Species		
	red beet	millet	tomato
0	100	100	100
0.045	99	108	105
0.45	111	82	45
4.5	90	18	5

[1/] The F value for the interaction of species x a linear increase in pyrazon concentration was significant at the .01 level.

Uptake and Translocation of ^{3}H-Pyrazon by Tolerant and Susceptible Species

Root uptake and translocation: After an initial period of rapid uptake by all three species (Table 2), the concentration of pyrazon in the roots leveled off and was highest for the susceptible tomato, intermediate for the moderately tolerant millet, and lowest for the tolerant red beet. Thus, a differential in the rate of uptake by the roots could be one of the factors responsible for the selective action of pyrazon.

Figure 1. Stimulation of the growth of red beet by low concentrations of pyrazon. The graph represents the combined data of two experiments. The F value for the quadratic effect of pyrazon concn was significant at the .01 level.

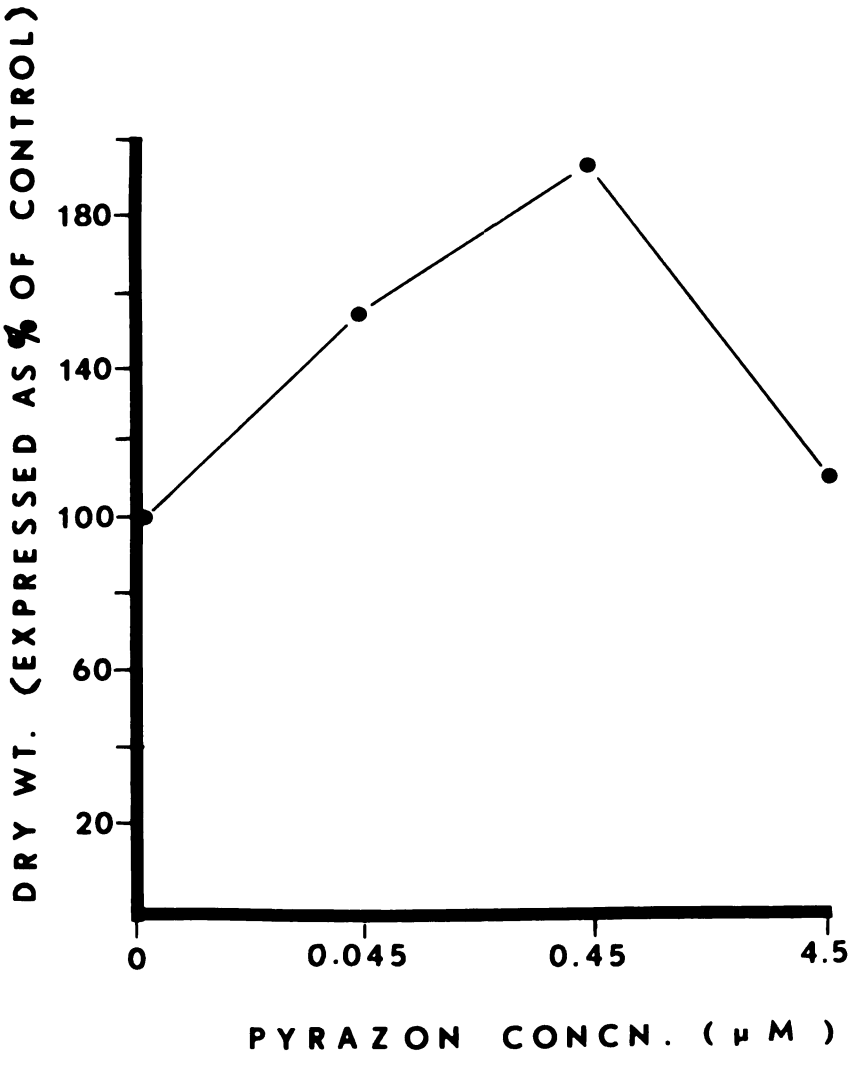

DRY WT. (EXPRESSED AS % OF CONTROL)
180
140
100
60
20
0
0.045
0.45
4.5
PYRAZON CONCN. (μM)

Table 2. Absorption of ^{3}H-pyrazon by the roots of red beet, millet and tomato. [1/]

Species	Concentration of radioactivity in the root (dpm/mg dry wt x 1000) Time (hr)				
	1/4	1	24	48	72
Red beet	2.5	2.7	2.3	2.1	1.7
Millet	1.6	1.9	3.7	4.0	4.0
Tomato	3.0	5.1	4.7	4.5	4.7

[1/] The F value for the interaction, species x treatment time, was significant at the .01 level.

The rate of translocation of ^{3}H-pyrazon from root to shoot in the three species, estimated by measuring the concentration of radioactivity per unit shoot weight at the various harvest times, clearly reflected the difference in root uptake (Table 3). The concentration of radioactivity in the shoot of the susceptible tomato was higher than that in red beet throughout the entire experiment. The moderately tolerant millet was again intermediate. The ratio of the concentration of radioactivity per mg dry wt after 72 hr was approximately 2.5/2.0/1.0 for tomato, millet and beet shoots, respectively, and clearly correlated with the degrees of tolerance of these species to pyrazon. Thus, the rate of translocation from root to shoot could be a second factor responsible for the selective action of pyrazon.

Table 3. Movement of ^{3}H-pyrazon from root to shoot in red beet, millet and tomato. [1/]

Species	Concentration of radioactivity in the shoot (dpm/mg dry wt x 1000) Time (hr)				
	1/4	1	24	48	72
Red beet	0.80	0.40	3.72	10.83	12.62
Millet	0.03	0.22	7.09	13.60	25.27
Tomato	0.87	1.23	16.90	27.68	31.43

[1/] F values for differences between species and harvest times were significant at the .05 level.

Foliar absorption and translocation: In the foliar absorption study, the amount of ^{3}H-pyrazon absorbed, expressed as a percentage of that applied, was greatest for the susceptible tomato, intermediate for red beet, and lowest for the moderately tolerant millet after 120 hr (Table 4). There was no basipetal translocation of the pyrazon from the treated leaf to other parts of the plant in any of the three species.

Influence of surfactant X-77 on the phytotoxicity of pyrazon: The addition of the surfactant X-77 at the rate of 0.67% (v/v) to pyrazon spray solutions applied to foliage, resulted in increased injury to young seedlings of millet and tomato with no effect on red beet (Table 5). A synergistic effect was seen, especially in the case of millet, which was not injured by either pyrazon or X-77 applied separately. The greatest injury to millet without increased toxicity to red beet occurred when pyrazon (3 lb/A) was applied in 0.67 or

1.0% (v/v) X-77 spray solutions (Figure 2).

Table 4. Absorption of ^{3}H-pyrazon by the foliage of red beet, millet and tomato. 1/

Species	Percent absorption Time(hr) 1	24	48	120
Millet	9.4	9.7	9.0	18.5
Red beet	19.9	19.6	29.2	33.9
Tomato	27.8	25.6	29.5	48.0

1/ F value for the interaction of species x treatment time was significant at .01 level.

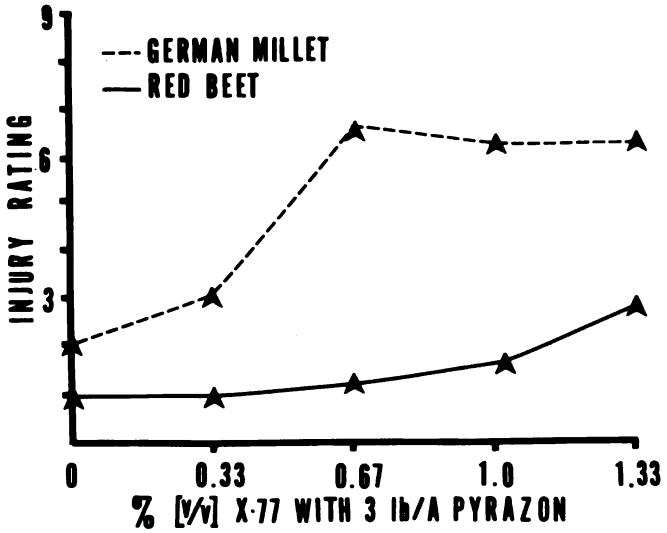
Table 5. Injury ratings 1/ to three plant species 23 days after foliar application of pyrazon and X-77.

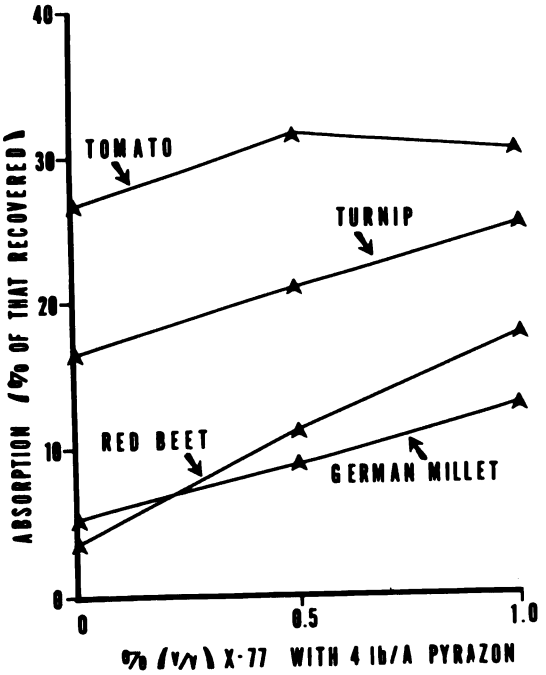
Treatment		Species		
Chemical (3 lb/A)	Surfactant (1% v/v)	red beet	German millet	tomato
None	X-77	1.0 a	1.3 a	1.3 a
pyrazon	none	1.6 a	1.3 a	6.0 b
pyrazon	X-77	2.0 a	5.3 b	9.0 c

1/ Ratings followed by unlike letters are significantly different at the .01 level.

Figure 2. Herbicidal injury ratings for red beet and German millet treated with pyrazon (80% wp) and several concn of surfactant X-77. The F value for the interaction, red beet vs millet x linear increase in X-77 concn was significant at the .01 level.

Figure 3. Absorption of ^{3}H-pyrazon by red beet, German millet, turnip, and tomato with several concn of surfactant X-77. The F values for differences between species and between rates of X-77 were significant at the .05 level.


9
6
3
INJURY RATING
---GERMAN MILLET
—RED BEET
0
0.33
0.67
1.0
1.33
% [v/v] X-77 WITH 3 lb/A PYRAZON


40
30
20
10
0
ABSORPTION (% OF THAT RECOVERED)
TOMATO
TURNIP
RED BEET
GERMAN MILLET
0
0.5
1.0
% (v/v) X-77 WITH 4 lb/A PYRAZON

Influence of surfactant X-77 on the foliar absorption of pyrazon: The foliar absorption of ^{3}H-pyrazon increased linearly with increasing concentrations (% v/v) of surfactant X-77 for all species except tomato (Figure 3). When pyrazon was applied in a 1.0% (v/v) X-77 solution, the percent absorption was 30% for the susceptible tomato, 25% for the susceptible turnip, 18% for the tolerant beet and 13% for the moderately tolerant millet. Again, there was no movement of the pyrazon out of the treated leaf in any of these species.

From the results of the root uptake and foliar absorption studies, it was apparent that species which absorb pyrazon most readily via the leaves or species which readily translocate pyrazon from the root to shoot are most susceptible. If foliar absorption is increased with a surfactant, injury increases to all species. However, red beet was tolerant to greater quantities of pyrazon absorbed by the leaves than millet.

Metabolism of Pyrazon in Tolerant and Susceptible Species

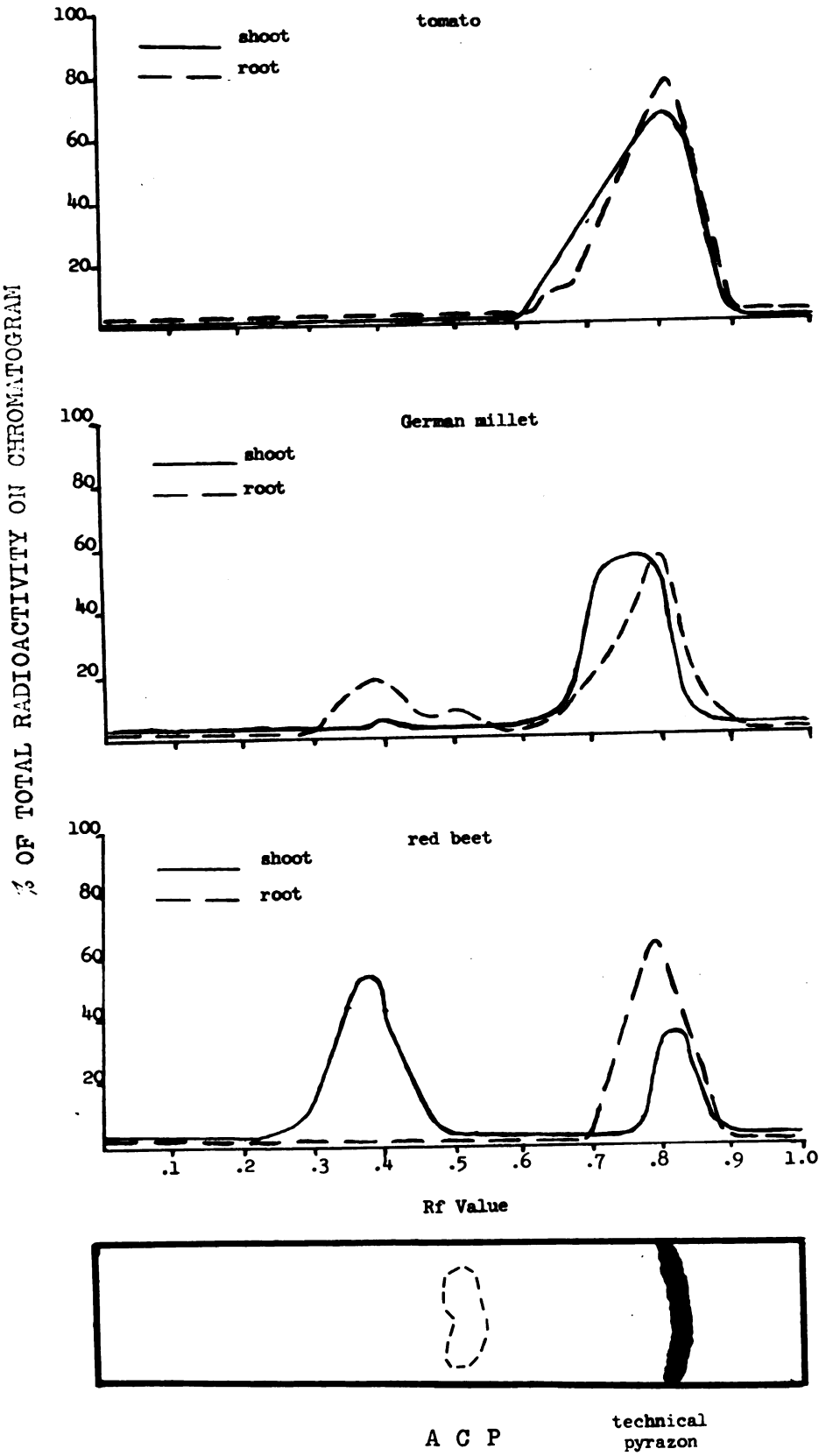
Metabolite detection: The Rf values for both ^{3}H-pyrazon and pyrazon were 0.8 and 0.5 in solvent system I (benzene-ethanol 75:25 v/v) and solvent system II (chloroform-ethanol 95:5 v/v), respectively. The Rf values for ACP in the same two systems, were 0.53 and 0.30, respectively. The Rf value of ^{3}H-pyrazon was determined by measuring the radioactivity in each of the chromatogram sections and those for technical pyrazon and ACP were determined by the diazotiazation color reaction.

Metabolism of pyrazon applied to the roots: After treatment for 72 hr with ^{3}H-pyrazon in the root solutions, essentially all of the radioactivity in the roots and shoots of tomato seedlings, shoots of millet and roots of red beet was chromatographically identifiable as pyrazon. The Rf values were 0.8 in solvent system I (Figure 4) and 0.5 in solvent system II. Obvious differences were apparent in the shoot of the tolerant red beet. In this species only 40% of the radioactivity present had the same Rf value as authentic pyrazon, the remainder had an Rf value of 0.37 in solvent system I and 0.15 in solvent system II. This ^{3}H-labeled metabolite has been identified (44) as N-(2-chloro-4-phenyl-3(2H)-pyridazinone)-glucosamine (N-glucosyl pyrazon).

Metabolism of pyrazon applied to the foliage: When the extracts of the leaves treated with ^{3}H-pyrazon and X-77 were examined for metabolites essentially the same results were obtained. In leaves of the tolerant red beet, 55% of the absorbed radioactivity was in the form of N-glucosyl pyrazon (Rf 0.37) while the remaining radioactivity in the beet leaf and all of that absorbed by the leaves of the susceptible millet, turnip, and tomato had the same Rf (0.8) as authentic pyrazon (Table 6).

Metabolism study with species susceptible to pyrazon: This study was conducted to test the hypothesis: The conversion of pyrazon to N-glucosyl pyrazon in the leaves of red beet is a detoxication process. Since all of these susceptible species, except corn, were known to be capable of converting

Figure 4. Rf values of the radioactive substances in the root (---) and shoot (——) extracts of (a) tomato (b) millet and (c) red beet plants treated with ^{3}H-pyrazon. Each graph represents the mean of 3 chromatograms, developed with solvent system I.


% OF TOTAL RADIOACTIVITY ON CHROMATOGRAM
tomato
shoot
root
100
80
60
40
20
German millet
shoot
root
red beet
shoot
root
.1
.2
.3
.4
.5
.6
.7
.8
.9
1.0
Rf Value
A C P
technical pyrazon

amiben to N-glucosyl amiben (8), there seemed to be a strong possibility that some may also make N-glucosyl pyrazon. If this had been the result, the above hypothesis would have been rejected. However, all of the pyrazon in the red beet seedlings had been metabolized to N-glucosyl pyrazon after 20 hr but not one of the other species was capable of making this conversion (Table 7).

Table 6. Relative amounts of [1]/pyrazon and N-glucosyl pyrazon in leaves of red beet, millet, turnip and tomato treated with ^{3}H-pyrazon.

Radioactive substance	Rf [2]/ value	% of total cpm on the chromatogram Species			
		red beet	millet	turnip	tomato
Unknown	origin	12	13	6	12
N-glucosyl pyrazon	0.37	55	0	0	0
Pyrazon	0.80	33	87	94	88

[1]/Data represent the mean of 3 replicates.

[2]/Rf values are for solvent system I.

Metabolism of pyrazon in inbred lines of red beet: This study was initiated in an attempt to find inbred lines of red beet which differed in the capacity to metabolize pyrazon. Such a system could provide evidence pertinent to the hypothesis: Tolerance to pyrazon is directly related to the rate of conversion of pyrazon to N-glucosyl pyrazon. After incubation of the infiltrated leaf discs of 24 inbred lines for 10 hr, considerable variation was observed in the

conversion of pyrazon to N-glucosyl pyrazon. From these 24 lines, two lines, W223 and W285 were selected for a replicated time course study. In this study, approximately 20% more of the pyrazon had been converted to N-glucosyl pyrazon in leaf discs from line W223 compared to line W285, after 4 and 8 hr (Figure 5).

Influence of pyrazon on the growth of two inbred beet lines: The tolerance of the above inbred lines of red beet to pyrazon dissolved in the nutrient solution was determined. The concentrations of pyrazon in the nutrient solution were 0, 11 and 22 μM.

Line W223, which most effectively metabolized pyrazon, was also the most tolerant to high concentrations of this chemical (Figure 6). These results supported the hypothesis: Tolerance to pyrazon is directly related to the rate of conversion to N-glucosyl pyrazon.

Table 7. Relative amounts [1/] of pyrazon and N-glucosyl pyrazon in red beet and 5 susceptible species 20 hr after infiltration with ^{3}H-pyrazon.

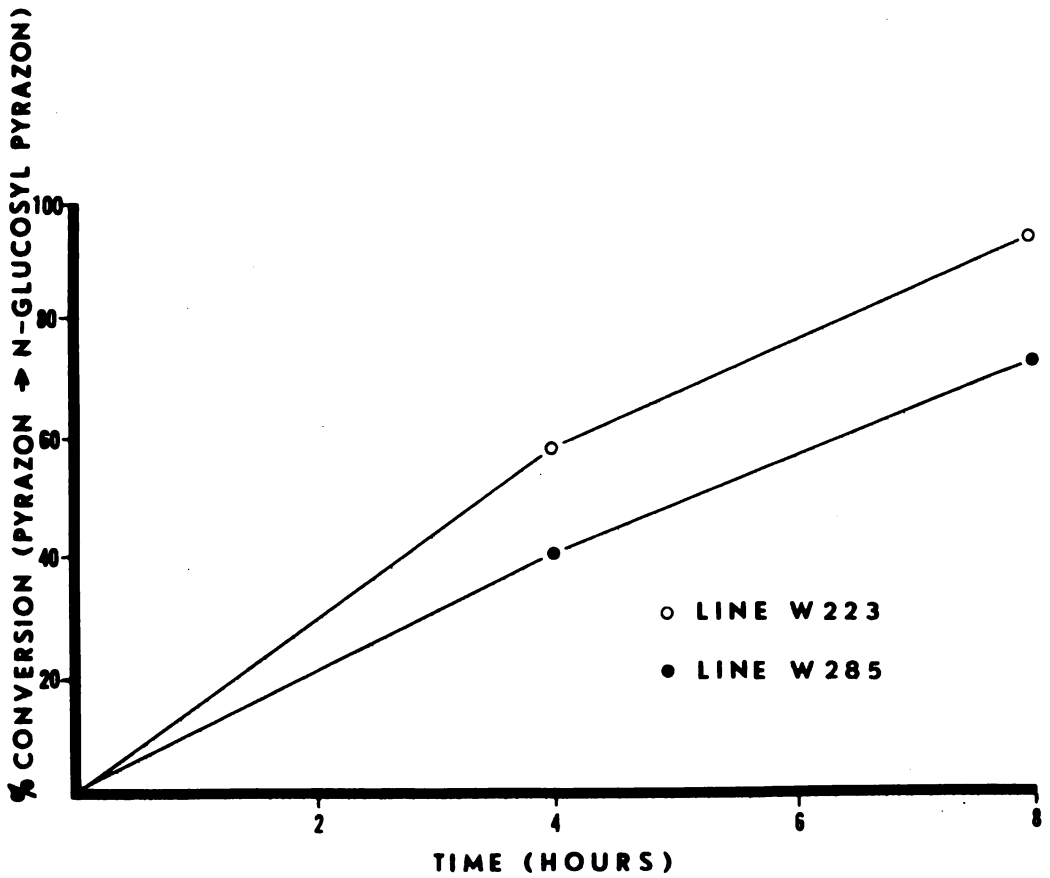
Radioactive substances	Rf [2/] value	% of total cpm on the chromatogram					
		Species					
		red beet	squash	soybean	cucumber	pea	corn
Unknown	origin	21	18	7	5	10	6
N-glucosyl pyrazon	0.37	79	0	0	0	0	0
Pyrazon	0.80	0	82	93	95	90	94

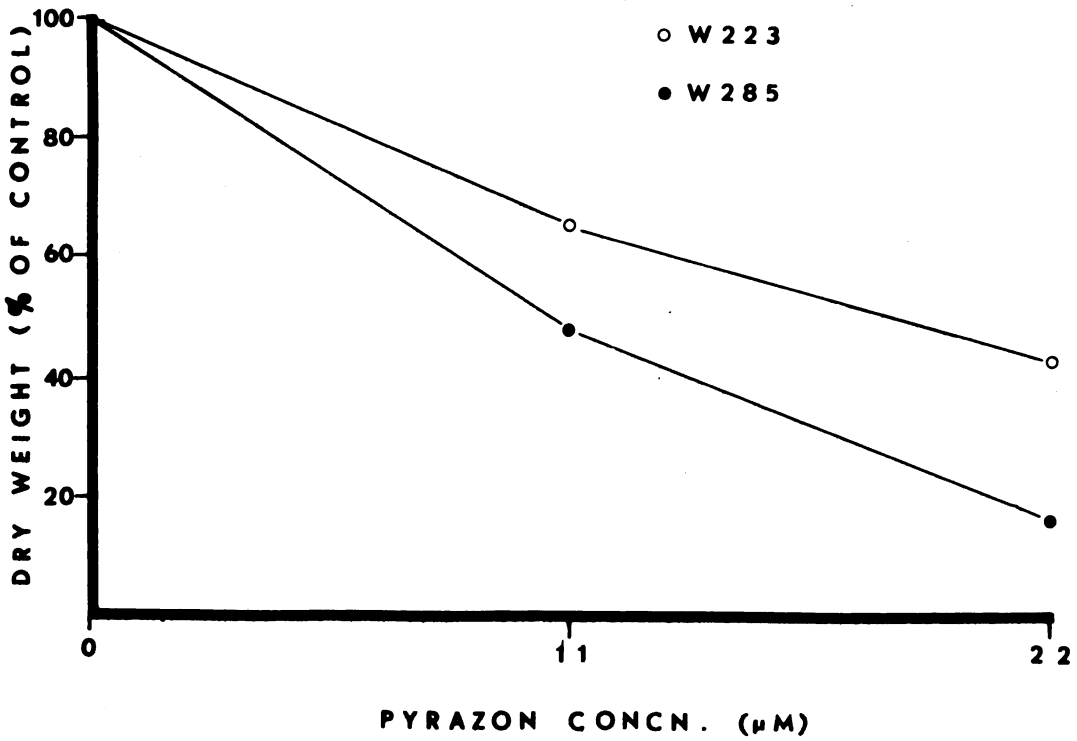
[1/] Percent of total radioactivity on chromatograms of 3 replicate treatments.

[2/] Rf values in solvent system I.

Figure 5. Percent conversion, pyrazon to N-glucosyl pyrazon in leaf discs from two inbred lines of red beet. Graph represents the percent of the total cpm on the chromatogram at Rf 0.4 in solvent system I. F value for the interaction, W223 vs W285 x linear time, was significant at the .01 level.

Figure 6. Dry wt (% of control) of two inbred lines of red beet treated for 10 days with several concn of pyrazon in the root nutrient solution. The F value for the interaction, W223 vs W285 x linear increase in pyrazon concn, was significant at the .05 level.


% CONVERSION (PYRAZON → N-GLUCOSYL PYRAZON)
100
80
60
40
20
2
4
6
8
TIME (HOURS)
○ LINE W223
● LINE W285


DRY WEIGHT (% OF CONTROL)
100
80
60
40
20
0
11
22
PYRAZON CONCN. (μM)
○ W223
● W285

Influence of Light on the Metabolism of Pyrazon in Red Beet

Study with intact plants: Even though there was greater uptake of pyrazon in light compared to dark by red beet seedlings, there was greater percent conversion of pyrazon to N-glucosyl pyrazon in the light after all harvest times (Table 8). One possible explanation for this enhancement of N-glucosyl pyrazon formation in the light was that the formation of this metabolite was dependent on the concentration of photosynthate in the leaf tissue. This would have been higher in the light.

Table 8. Uptake and conversion of pyrazon to N-glucosyl pyrazon in leaves of beet plants in the light or dark. [1/]

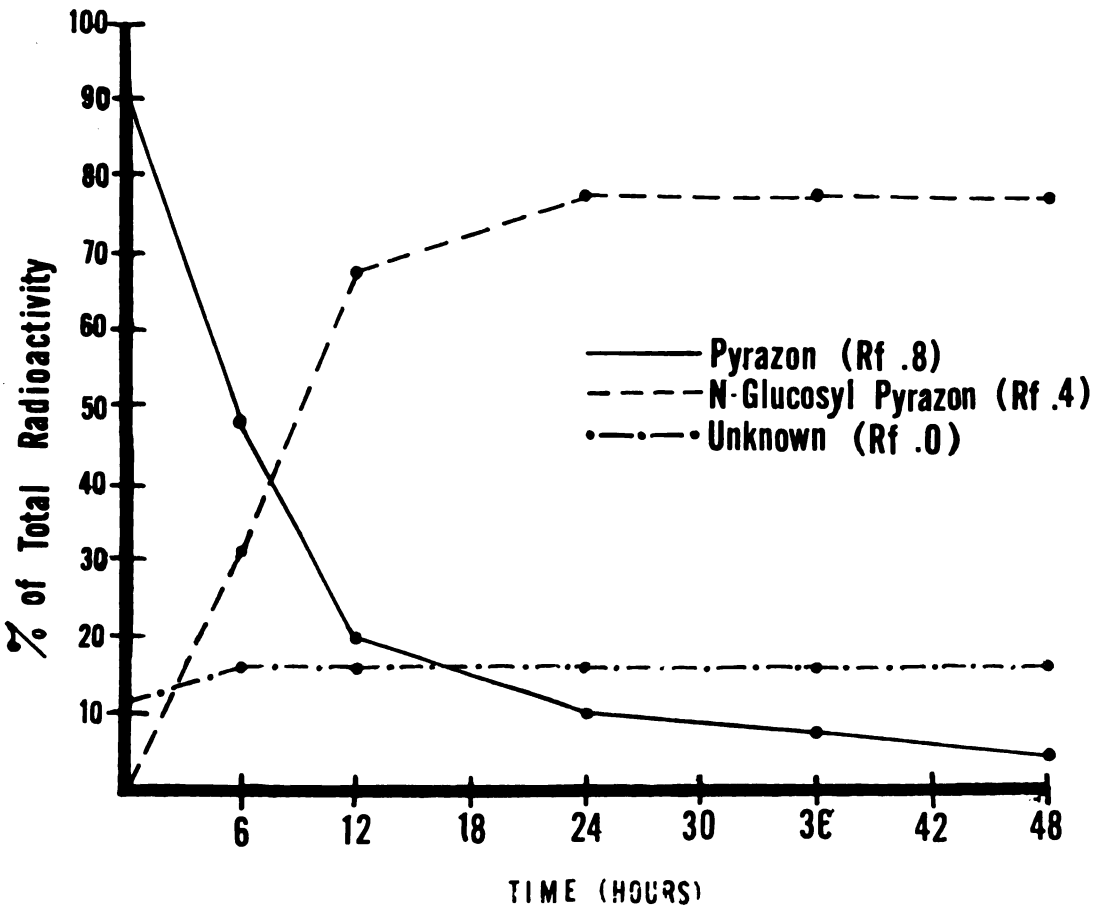
	Dark		Light	
Time	dpm/mg dry wt	% conversion [2/]	dpm/mg dry wt	% conversion
24	2124	0	3630	12.5
48	3402	1.6	4330	16.7
72	7802	22.4	8364	30.1

[1/] Data represent the mean of 5 replications. The F values for differences between light and dark were significant at the .01 level for percent conversion and at the .05 level for dpm/mg dry wt.

[2/] Obtained by thin-layer chromatography (cpm at Rf 0.4 ÷ total cpm on chromatogram) with solvent system I.

Studies with infiltrated leaf discs: The metabolism of pyrazon in vacuum infiltrated beet leaf discs was first examined under conditions of a normal photoperiod (Figure 7). It was apparent that with time there was a rapid increase in N-glucosyl pyrazon. This was coincident with an equally rapid

Figure 7. Conversion of pyrazon to N-glucosyl pyrazon in beet leaf discs infiltrated with ^{3}H-pyrazon. The graph represents the mean of 3 chromatograms developed in solvent system I.


100
90
80
70
60
50
40
30
20
10
% of Total Radioactivity
6
12
18
24
30
36
42
48
TIME (HOURS)
Pyrazon (Rf .8)
N-Glucosyl Pyrazon (Rf .4)
Unknown (Rf .0)

decrease in the radioactivity identifiable as pyrazon. After infiltration of the leaf discs with 9.0 μM ^{3}H-pyrazon, only 12 hr was required to convert 70% of the pyrazon to N-glucosyl pyrazon.

The percent conversion of pyrazon to N-glucosyl pyrazon in leaf discs obtained from plants which received 12 hr light was essentially the same in the light compared to the dark for 6 hr after infiltration (Figure 8). However, after 6 hr, the percent conversion was significantly higher in the light. It is conceivable that the concentration of photosynthate, specifically glucose, may have become limiting after 6 hr in the dark.

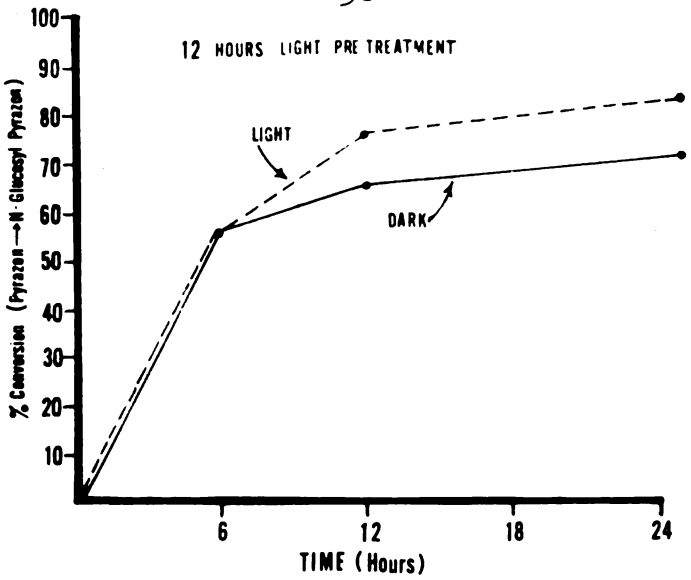
When leaf discs were obtained from plants which had received 24 hr dark and then infiltrated with ^{3}H-pyrazon, the percent conversion to N-glucosyl pyrazon in light was essentially the same as with light pretreatment (Figure 9). However, the metabolism in the dark was even more limited. After 22 hr only 50% of the pyrazon had been converted to N-glucosyl pyrazon in the dark compared to 80% conversion in the light.

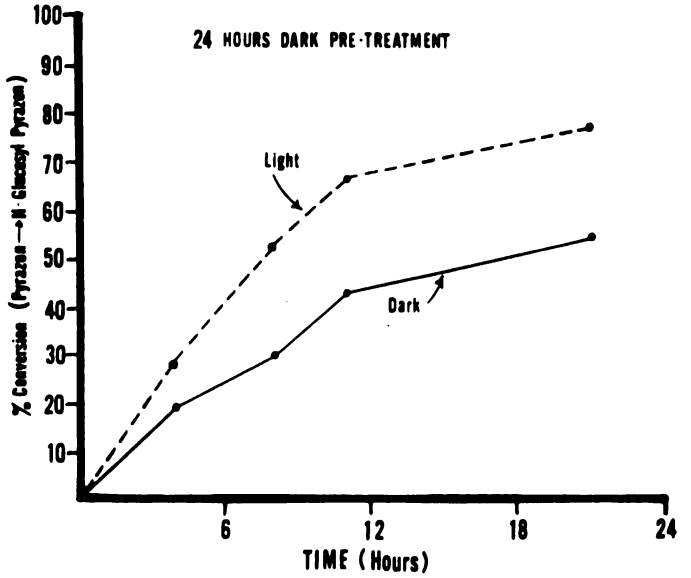
Influence of supplemental sucrose on pyrazon metabolism: When leaf discs from plants pretreated with 48 hr dark were infiltrated with pyrazon and 0.05 or 0.10 M sucrose, the percent conversion in the dark after 4 hr was the same as that in the light without supplemental sucrose (Figure 10). This series of experiments indicated that the metabolism of pyrazon in leaves of red beet was enhanced in the light compared to the dark. Furthermore, it appeared that this enhancement was due to a higher concentration of carbohydrate in the light, since inhibition in the dark was overcome by the addition of

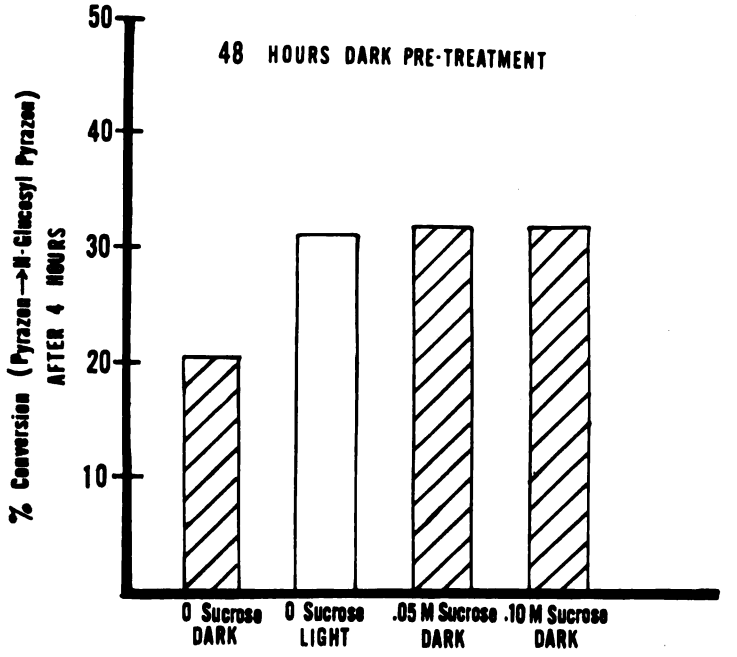
Figure 8. Conversion of pyrazon to N-glucosyl pyrazon in the light and dark in leaf discs from beet plants pretreated with 12 hr light. The graph represents cpm at Rf 0.4 ÷ total cpm on chromatogram (solvent system I). The F value for the interaction, light vs dark x linear time, was significant at the .05 level.

Figure 9. Conversion of pyrazon to N-glucosyl pyrazon in the light and dark in leaf discs from beet plants pretreated with 24 hr dark. The graph represents cpm at Rf 0.4 ÷ total cpm on chromatogram (solvent system I). The F value for the interaction, light vs dark x linear time, was significant at the .01 level.

Figure 10. Conversion of pyrazon to N-glucosyl pyrazon in leaf discs from beet plants pretreated with 48 hr dark, in the light and in the dark with 0, 0.05, and 0.10 M supplemental sucrose. The graph represents cpm at Rf 0.4÷ total cpm on the chromatogram (solvent system I). The F value for the comparison, 0 sucrose, dark vs others, was significant at the .01 level.


12 HOURS LIGHT PRE TREATMENT
% Conversion (Pyrazon→N-Glucosyl Pyrazon)
LIGHT
DARK
TIME (Hours)


24 HOURS DARK PRE-TREATMENT
% Conversion (Pyrazon→N-Glucosyl Pyrazon)
Light
Dark
TIME (Hours)


48 HOURS DARK PRE-TREATMENT
% Conversion (Pyrazon→N-Glucosyl Pyrazon)
AFTER 4 HOURS
0 Sucrose DARK
0 Sucrose LIGHT
.05 M Sucrose DARK
.10 M Sucrose DARK

supplemental sucrose to the leaf tissue.

Enzymatic Studies

The dry wt of the acetone powder obtained from 32.0g fresh beet leaves was 1.25g. Kjeldahl protein content was 24.91 mg/100 mg powder while alcohol insoluble and TCA precipitable protein contents were 22.80 and 20.52 mg/100 mg powder, respectively. The alcohol soluble N per 100 mg powder was 10.40 mg.

The water extractable protein content of the acetone powder enzyme extract was 2.74 mg and 0.88 mg/ml. before and after elution through Sephadex G-25, respectively. The protein content of the crude extract of fresh tissue was 2.37 mg/ml before and 0.58 mg/ml after elution through Sephadex G-25.

Conversion of pyrazon to N-glucosyl pyrazon was examined in vitro, using the four types of enzyme preparations discussed above with each of the three glucosyl donors, UDPG, ADPG, and TDPG. Portions of each of the reaction mixtures (approx. 4,000 cpm) were applied to thin-layer chromatograms after 30 min and 1 hr of incubation at 25 C. However, after developing and counting each of the chromatogram sections, all of the radioactivity had the same Rf as as pyrazon (solvent system I).

From these results, it was apparent that the mechanism for the conversion of pyrazon to N-glucosyl pyrazon may be different than the mechanism for the conversion of amiben to N-glucosyl amiben. However, before UDPG, ADPG, and TDPG can be excluded as donors for N-glucosyl pyrazon, other

buffers, different pH's, and different enzyme preparations should be examined. The activity of these systems should also be tested by examining the conversion of amiben to N-glucosyl amiben.

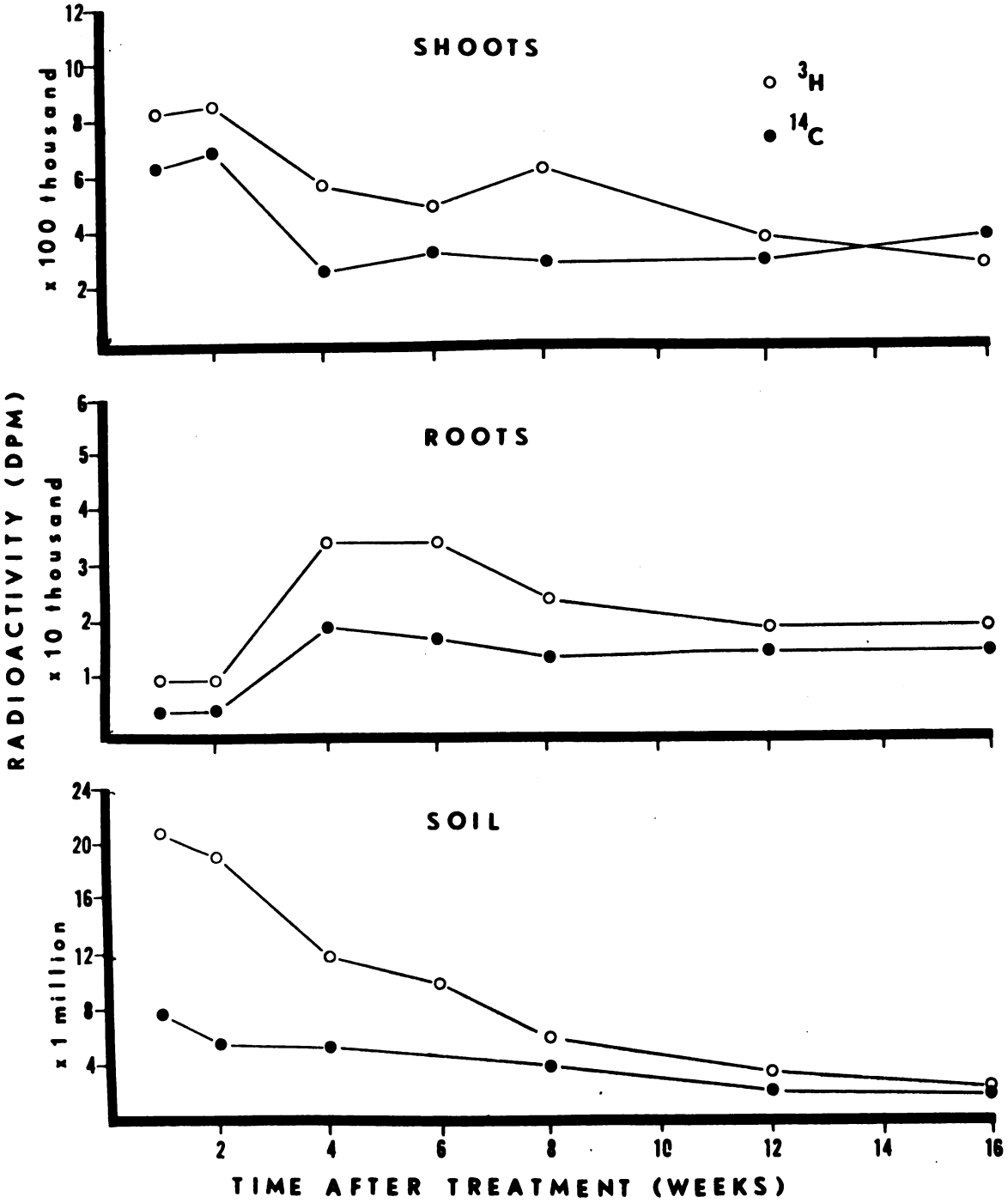
Long Term Metabolism Study with ^{14}C and ^{3}H-Labeled Pyrazon in Sugar Beets and Soil

Recovery of 50% ethanol extractable radioactivity: Pyrazon, both ^{3}H and ^{14}C-labeled, was taken up from the soil and accumulated in the shoots of sugar beet. At all harvest times, there was approximately a 100:10:1 relationship between the 50% ethanol extractable radioactivity in the soil, shoots and roots, respectively (Figure 11). The extractable radioactivity increased sharply and reached a peak in the shoots at 2 weeks and in the roots at 4 weeks after treatment. From this point on, there was actually a decrease in the extractable ^{3}H and ^{14}C-labeled compounds in the plant.

In the soil, there was a gradual decline in the 50% ethanol extractable radioactivity throughout the experiment in both ^{3}H-pyrazon and ^{14}C-pyrazon treated soils (Figure 11). However, this decrease in recoverable radioactivity from the soil was greater for ^{3}H-labeled compounds than for ^{14}C-labeled compounds.

Of the approximately 24,528,000 dpm ^{3}H-pyrazon applied per pot, 89% and 10% was recoverable by extraction after 1 week and 16 weeks, respectively. One week and 16 weeks after the application of 11,610,000 dpm ^{14}C-pyrazon per pot, 72% and 23%, respectively, were recovered by extraction.

Figure 11. 50% ethanol extractable radioactivity in the soil and in roots and shoots of sugar beet, 1 to 16 weeks after the application of pyrazon-(phenyl-^{3}H) and pyrazon-(4, 5-^{14}C to the soil. The decrease in extractable radioactivity in the soil with time was greater for ^{3}H-labeled compounds than for ^{14}C-labeled compounds (.01 level). However, this comparison was not significant for either the roots or shoots.


SHOOTS
^{3}H
^{14}C
x 100 thousand
ROOTS
x 10 thousand
SOIL
x 1 million
RADIOACTIVITY (DPM)
TIME AFTER TREATMENT (WEEKS)

The schöniger combustion procedure was used to estimate the non-extractable radioactivity in the insoluble residues of the ^{14}C-pyrazon treated soil, root and shoot samples from the 16 week treatment. The efficiency of the combustion process was found to be 65%. A total of 6.49% of the applied radioactivity was recovered by combustion of the extracted samples. Most of this non-extractable radioactivity was recovered from the soil (Table 9). It was apparent from these data that after 16 weeks there was a net loss of at least 70% of the radioactivity applied as ^{14}C-pyrazon.

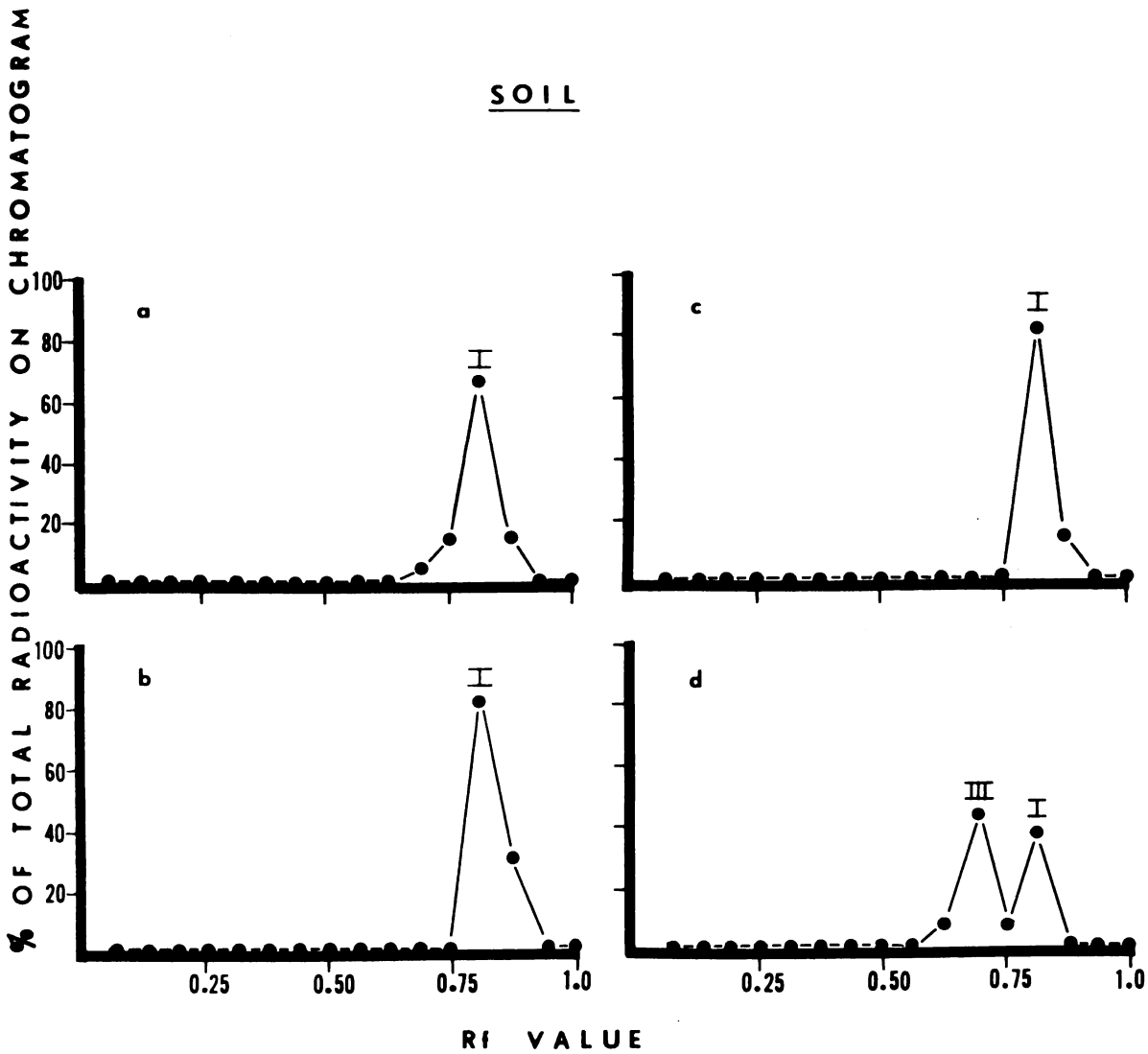
Table 9. Percent recovery of applied radioactivity [1/] 16 weeks after the application of ^{14}C-pyrazon.

	Percent recovery of applied radioactivity	
	50% ethanol [1/] extraction	Schöniger combustion [2/] of insoluble residues
Soil	18.96	5.95
Shoot	3.78	0.47
Root	0.15	0.07
Total	22.89	6.49

[1/] Data for extraction represents the mean of 3 replications, that for combustion represents the mean of 6 observations, 2 per replicate.

Detection and identification of ^{3}H-labeled and ^{14}C-labeled pyrazon derivatives: After one week, only one radioactive compound (Compound I) was detectable in the soil. It had an Rf value of 0.81 in solvent system III and it was labeled with both ^{3}H (Figure 12a) and ^{14}C (Figure 12c). At

Figure 12. Rf values of labeled compounds in the extracts of soil, 1 and 16 weeks after the application of ^{14}C-pyrazon. The graphs are the mean of 3 chromatograms (solvent system III). Points on the graph represent the percent of the total cpm on the chromatogram at each of the 16 Rf values: (a) ^{3}H-pyrazon, 1 week; (b) ^{3}H-pyrazon, 16 weeks; (c) ^{14}C-pyrazon, 1 week and (d) ^{14}C-pyrazon, 16 weeks.


SOIL
% OF TOTAL RADIOACTIVITY ON CHROMATOGRAM
a
b
c
d
100
80
60
40
20
0.25
0.50
0.75
1.0
I
III
Rf VALUE

16 weeks there were two ^{14}C labeled metabolites, Compound I, again at Rf value 0.81, and Compound III with an Rf value of 0.69. Compound III was labeled with ^{14}C (Figure 12d) but not with ^{3}H (Figure 12b).

In the shoot of sugar beet, two radioactive compounds were detectable one week after treatment. A small amount of Compound I was present and in addition to this, Compound II, with an Rf of 0.44, was detected in high concentrations. Compound II was labeled with both ^{3}H (Figure 13a) and ^{14}C (Figure 13c). At 16 weeks, three ^{14}C-labeled substances were detectable in the shoot; Compounds II, III, and IV at Rf values 0.44, 0.69 and 0.25, respectively (Figure 13d). Of these three ^{14}C labeled substances, only Compound II was also labeled with ^{3}H (Figure 13b and Figure 13d).

The Rf values of Compounds I, II, III, and IV were determined in solvent system III [benzene-ethanol 60:40 (v/v)] and solvent system IV [acetone-dioxane-NH_4OH (25%) (v/v/v)] (Table 10). The Rf values obtained were compared to the Rf values for known pyrazon derivatives in the same two solvent systems. These controls included technical pyrazon, technical ACP, and ^{3}H-N-glucosyl pyrazon (Table 11). From these results, it was established that Compounds I, II, and III were chromatographically identical to pyrazon, N-glucosyl pyrazon, and ACP, respectively. The fact that Compounds I and II were labeled with both ^{3}H and ^{14}C, indicating the presence of both phenyl and pyridazinone rings, strongly supported this conclusion. Likewise, Compound III was labeled only with ^{14}C. This indicated the absence of the ^{3}H-labeled phenyl ring and confirmed its identity as ACP. Compound IV, the last metabolite to appear in the beet shoot,

Figure 13. Rf values of radioactive substances in shoot extracts of sugar beets grown in soil treated for 1 and 16 weeks with ^{3}H-pyrazon and ^{14}C-pyrazon (solvent system III). The points on the graphs represent the percent of the total cpm on the chromatogram at each of the 16 Rf values: (a) ^{3}H-pyrazon, 1 week: (b) ^{3}H-pyrazon, 16 weeks; (c) ^{14}C-pyrazon, 1 week; and (d) ^{14}C-pyrazon, 16 weeks.

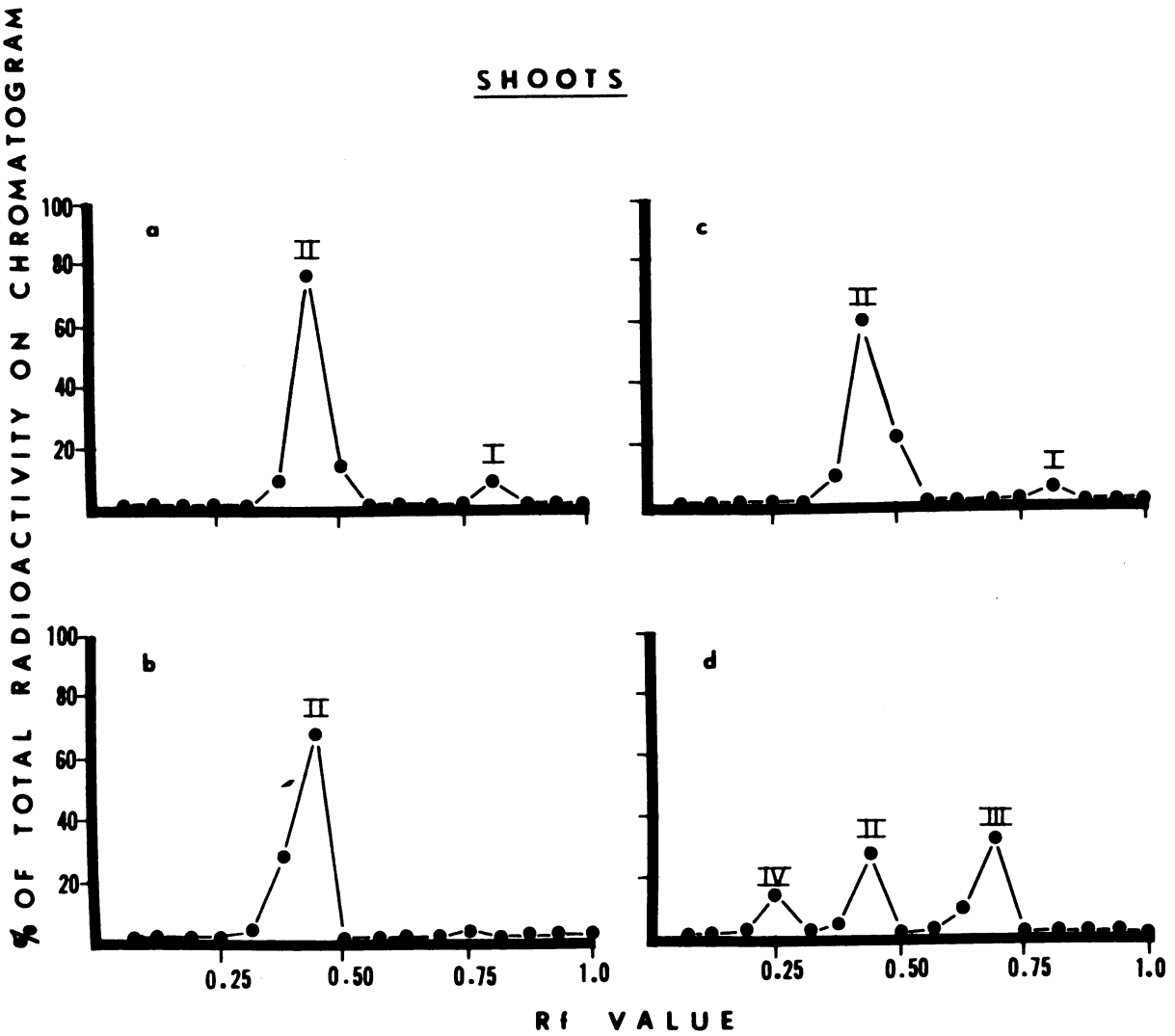

SHOOTS
% OF TOTAL RADIOACTIVITY ON CHROMATOGRAM
a
b
c
d
I
II
III
IV
100
80
60
40
20
0.25
0.50
0.75
1.0
Rf VALUE

Table 10. Thin-layer chromatographic Rf values of the ^{14}C-labeled pyrazon derivatives.

Number of ^{14}C-labeled compound	Rf values in solvent system III	IV
I	0.81	0.75
II	0.44	0.38
III	0.69	0.63
IV	0.25	0.20

Table 11. Thin-layer chromatographic Rf values of known pyrazon derivatives.

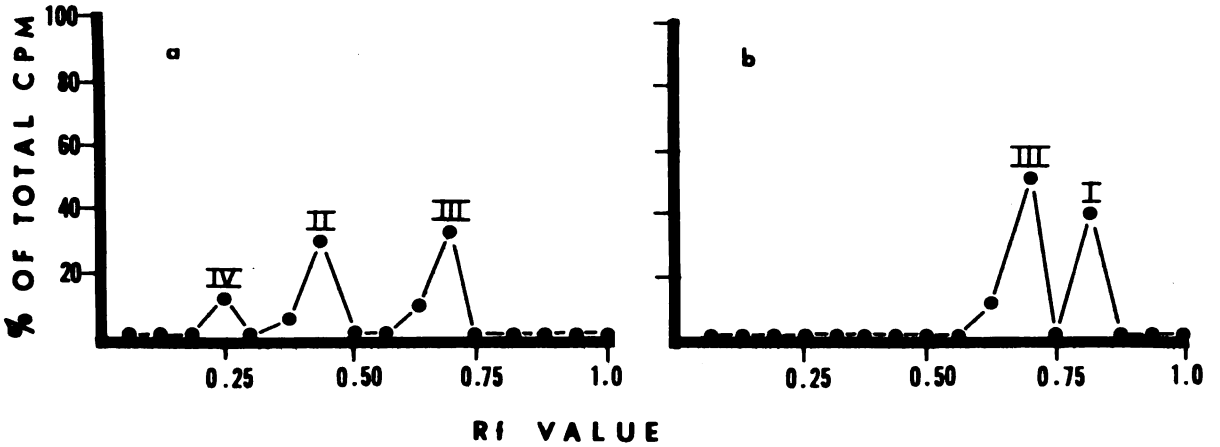
Pyrazon derivative	Rf values in solvent system III	IV
Pyrazon	0.84	0.74
N-glucosyl pyrazon	0.44	0.38
5-amino-4-chloro-3(2H) pyridazinone (ACP)	0.71	0.63

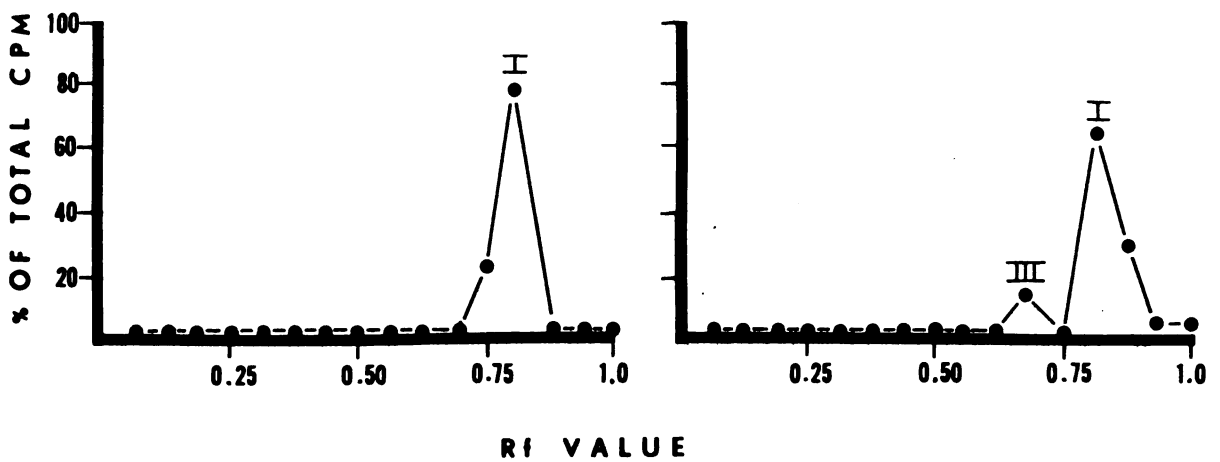
appeared to be a derivative of ACP since it was labeled with only ^{14}C and thus lacked the phenyl ring (Figure 13b and Figure 13d).

When portions of the beet shoot extracts containing N-glucosyl pyrazon, ACP, and Compound IV were treated with 0.2N HCl and heat (100 C) for 30 min and then chromatogrammed in system III, only pyrazon and ACP were detected (Figure 14a and Figure 14b). N-glucosyl pyrazon and compound IV were apparently converted by hydrolysis to pyrazon and ACP, respectively.

Figure 14. Rf values of the radioactive substances in extracts of sugar beet shoots grown for 16 weeks in soil treated with ^{14}C-pyrazon (solvent system III). The points on the graphs represent the percent of the total cpm on the chromatogram at each of the 16 Rf values (a) before exposure of the extracts to acid and heat and (b) after exposure of the extracts to acid and heat.

Figure 15. (a) Rf value of ACP (Compound III) isolated from a 16 week soil extract by preparative thin-layer chromatography. (b) Rf values of radioactive substances in extracts of beet leaf discs 4 hr after infiltration with ACP-^{14}C + 0.10 M β-D(+) glucose and 0.25 M mannitol. Graphs are the mean of 3 chromatograms. Points on the graphs represent the percent of the total cpm on the chromatogram at each of the 16 Rf values. Solvent system III was used. (See Fig. 17, page 78)


% OF TOTAL CPM
100
80
60
40
20
a
b
IV
II
III
III
I
0.25
0.50
0.75
1.0
Rf VALUE

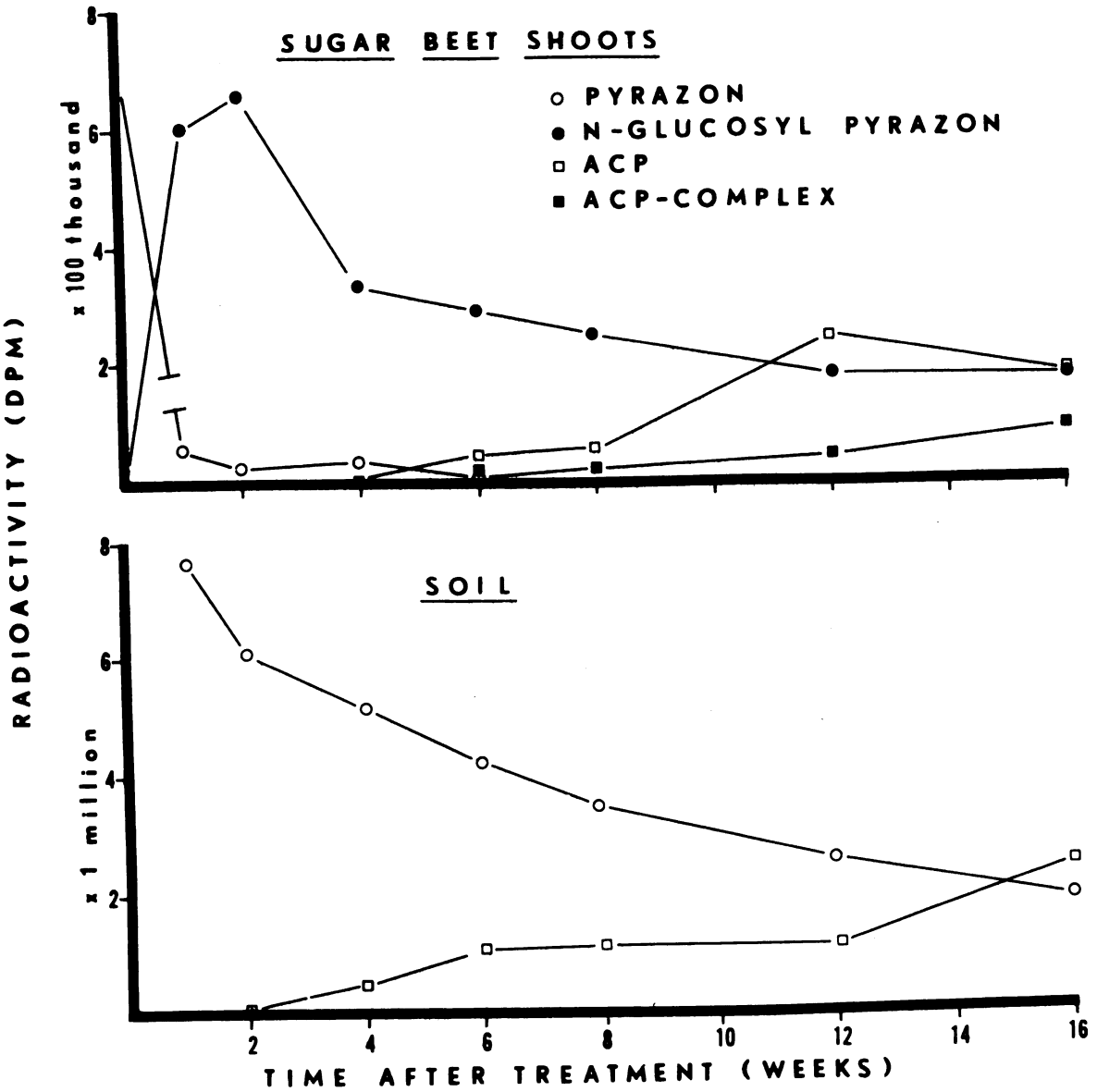

% OF TOTAL CPM
100
80
60
40
20
I
III
I
0.25
0.50
0.75
1.0
Rf VALUE

Compound IV was separated from Compounds II and III by thin-layer chromatography (solvent system III). The Compound IV peak was located (Rf 0.20-0.35), scraped from the plate, and eluted with ethanol. When this ethanol extract was rechromatogrammed after acid hydrolysis, all of the radioactivity (400 cpm) peaked at an Rf value of 0.67, comparable to that for ACP. When leaf discs of sugar beet were infiltrated with ^{14}C-labeled ACP (obtained from a 16 week soil extract) and β-D(+)-glucose (0.10M) in mannitol (0.25M), Compound IV was detectable after 4 hr incubation (Figure 15a and Figure 15b).

These studies supported the hypothesis that Compound IV was a conjugate of ACP. The natural moiety in this complex was not identified, however glucose, as in N-glucosyl pyrazon is a logical possibility.

Time course for the metabolism of pyrazon in the soil and in the plant: The soil and shoot samples were examined for the presence of pyrazon, N-glucosyl pyrazon, ACP and ACP-complex at all harvest times from 1 to 16 weeks after treatment. The percent of the radioactivity in the form of each of these compounds was determined by thin-layer chromatography of the extracts from ^{14}C-pyrazon treatments (solvent system III). The dpm for each compound was determined by multiplying these percentages times the total dpm in each extract (Figure 16).

Figure 16. Time course for the appearance of ^{14}C-pyrazon in the soil and in sugar beet plants from 1 to 16 weeks after the application of ^{14}C-pyrazon to the soil. The total dpm in the form of each of the pyrazon derivatives was determined by multiplying the % of the radioactivity in the form of each (obtained by thin-layer chromatography) times the total dpm in three replicates.


SUGAR BEET SHOOTS
PYRAZON
N-GLUCOSYL PYRAZON
ACP
ACP-COMPLEX
x 100 thousand
8
6
4
2
SOIL
x 1 million
8
6
4
2
2
4
6
8
10
12
14
16
TIME AFTER TREATMENT (WEEKS)
RADIOACTIVITY (DPM)

The concentration of pyrazon in the soil decreased steadily from 1 to 16 weeks after treatment. At two weeks, ACP was detectable in all three replicates of the soil extracts. Approximately 4% of the radioactivity in these extracts formed a peak, at an Rf of 0.69, which was clearly separate from the major pyrazon peak at 0.80. At 4 weeks, this compound accounted for 8.7% of the radioactivity in the soil. The concentration of ACP in the soil increased slowly, but this increase was not equivalent to the decrease in pyrazon concentration.

Pyrazon was taken up by the beet plant and quickly converted to N-glucosyl pyrazon. Pyrazon represented only a small portion of the radioactivity in the shoot at one week and from 6 weeks on, it was not detectable. At 2 weeks after treatment, ACP was not detectable in the shoots of sugar beet. However, at 4 weeks, 2 weeks after the appearance of ACP in the soil, 2.9% of the radioactivity in the shoot was in the form of ACP. On a quantitative basis, 186,300 and 364,800 dpm of the radioactivity in the soil were in the form of ACP at 2 and 4 weeks, respectively. In the shoot 0 and 6,699 dpm were in the form of ACP at these same harvest times. These data strongly support the conclusion that ACP appeared in the plant by root uptake rather than by degradation of pyrazon or N-glucosyl pyrazon in the plant.

The concentration of ACP in the shoot increased with time and reached a maximum at 12 weeks. Compound IV (ACP-complex) was first detected in the plant at 6 weeks, 2 weeks after the appearance of ACP. The ACP-complex

increased in concentration from 6 weeks on. This increase coincided with a decrease in ACP from 12 to 16 weeks.

Because of a low concentration of radioactivity and a high concentration of sugar in the root extracts, the radioactive substances could not be separated by direct thin-layer chromatography. However, using a benzene-50% ethanol partition technique, an estimate of the relative amounts of water soluble and insoluble derivatives in the root extracts was obtained for the 16 week harvest. This technique was based on the high solubility of pyrazon in benzene compared to the water soluble N-glucosyl pyrazon (44). To obtain this estimate, the total benzene extractable radioactivity was determined before and after exposure of the 50% ethanol root extracts to 0.2N HCl and 100 C for 30 min (Table 12). These conditions should have resulted in hydrolysis of any water soluble complexes present, e. g., N-glucosyl pyrazon or ACP-complex. Using these procedures, it was established that 80% of the radioactivity in the roots was in the form of water soluble compounds which could be extracted with benzene after acidifying the aqueous extracts (Table 12).

After concentration of the benzene extracts, an extract free of sugar was obtained. Thin-layer chromatographic analysis of this extract revealed that 91% of the radioactivity was in the form of pyrazon and only 7% in the form of ACP (Figure 17). Based on the solubility of the radioactivity in benzene before and after hydrolysis, it was apparent that pyrazon and ACP may appear in the root as water soluble complexes.

Figure 17. Rf values of the radioactive substances in benzene extracts of sugar beet roots from plants grown in soil treated for 16 weeks with (a) ^{3}H-pyrazon and (b) ^{14}C-pyrazon. The graphs are the mean of 3 chromatograms developed in solvent system III. The points on the graph represent the percent of the total cpm on the chromatogram at each of the 16 Rf values.

(See Fig. 15, page 71)

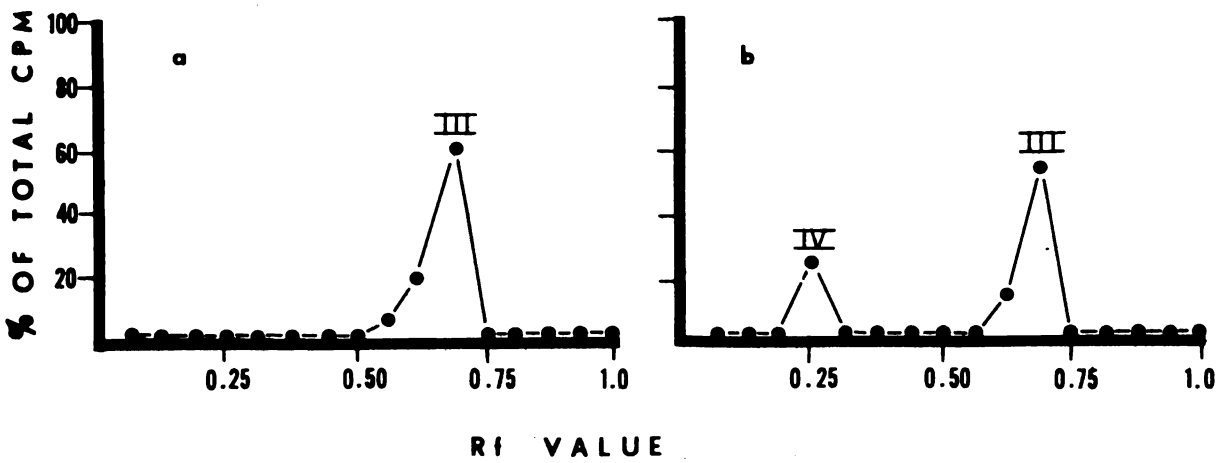

% OF TOTAL CPM
100
80
60
40
20
a
III
0.25
0.50
0.75
1.0
b
IV
III
0.25
0.50
0.75
1.0
Rf VALUE

Table 12. Solubility of radioactive compounds in the roots after 16 weeks, before and after acid hydrolysis. [1/]

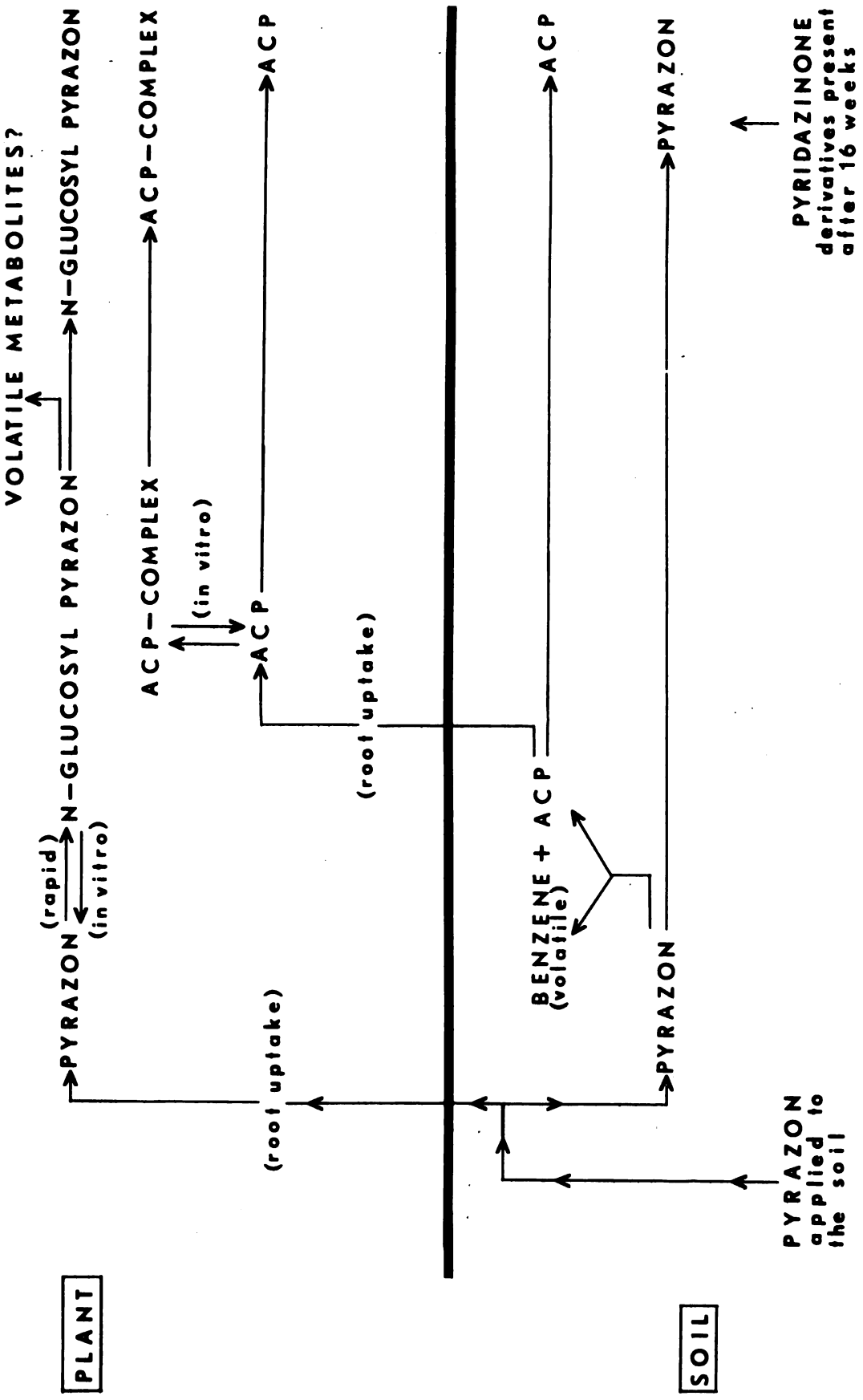
Treatment	Total dpm in extract	% Extractable		
		Before hydrolysis		After hydrolysis
		50% ethanol	benzene	benzene
^{3}H-pyrazon	20,330	82	15	88
^{14}C-pyrazon	18,500	84	12	90

[1/]Data represent the mean of 3 replications. The F value for the comparison, ^{3}H and ^{14}C, was not significant.

Summary: The net loss of over half of the radioactivity applied as either ^{3}H-pyrazon or ^{14}C-pyrazon cannot be totally explained by results obtained in this study. The efficiency of the extraction procedure was acceptable (72% for ^{14}C and 89% for ^{3}H) at the one week harvest and did not decrease during the experiment, since the non-extractable radioactivity at 16 weeks was only 6.49%. The only way radioactive compounds could have left the system was by volatilization since a closed system in other respects was maintained throughout the experiment. The loss of pyrazon by volatilization was not likely because of its low vapor pressure (0.074 mm Hg at 0 C). However, the loss of volatile metabolites of pyrazon from either the soil or the plant remained a possibility. The greater loss of ^{3}H compared to ^{14}C, from the soil could be explained by volatilization of ^{3}H-benzene after the dephenylation of pyrazon.

Based on the results of this study, the metabolic fate of pyrazon in soil and in plants is best described by the following diagram (Figure 18).

Figure 18. Diagramatic scheme, summarizing the metabolic fate of soil applied pyrazon in soil and in tolerant sugar beets.


VOLATILE METABOLITES?
N-GLUCOSYL PYRAZON
ACP-COMPLEX
ACP
PYRAZON
(rapid)
(in vitro)
(root uptake)
BENZENE + ACP
(volatile)
PYRIDAZINONE
derivatives present
after 16 weeks
PYRAZON
applied to
the soil
PLANT
SOIL

SUMMARY AND CONCLUSIONS

Red beet, German millet, and tomato seedlings were tolerant, intermediate, and susceptible, respectively to pyrazon applied to the roots. The concentrations of pyrazon causing this selective toxicity were 0.45 and 4.5 μM. The uptake of pyrazon via the roots and accumulation in the shoots was most rapid in the susceptible tomato. The tolerant red beet had the lowest rate of uptake and accumulation in the shoots and the moderately susceptible German millet was intermediate. Thus, susceptibility to pyrazon was directly related to the rate of root uptake and accumulation in the shoot.

In the foliar absorption studies, tomato again absorbed pyrazon at the greatest rate and was the most susceptible species to foliar applications. The addition of the surfactant X-77 to pyrazon solutions increased the rate at which pyrazon was absorbed by the leaves of all species tested. Increased foliar absorption was correlated with increased injury to tomato and German millet. However, red beet was more tolerant than German millet to pyrazon applied to the foliage, even though the rate of foliar absorption of pyrazon was similar, with and without a surfactant. There was no basipetal movement of pyrazon applied to the foliage in any species tested. This was evidence that pyrazon was not mobile in the phloem. These results indicated that in all species except red beet, pyrazon toxicity was directly related to the amount of pyrazon absorbed by the leaves.

When the extracts of tolerant and susceptible plants treated with ^{3}H-pyrazon were examined, the most important factor responsible for the selective action of pyrazon became evident; i.e., the ability of the tolerant beet plant to convert pyrazon to some other compound. This compound has been identified as N-(2-chloro-4-phenyl-3(2H)-pyridazinone)-glucosamine or N-glucosyl pyrazon (44). After absorption of pyrazon, the rate of synthesis of N-glucosyl pyrazon in leaves of red beet was very rapid and was dependent on the concentration of carbohydrate in the leaf tissue. The contention that the conversion of pyrazon to N-glucosyl pyrazon was a detoxication mechanism and responsible for the tolerance of beets to pyrazon was supported by the following: Unlike other cases in which herbicides have been converted to glucosides, most plants susceptible to pyrazon were not capable of converting it to N-glucosyl pyrazon; the susceptible species examined were tomato, German millet, turnip, squash, soybean, cucumber, pea and corn. Four of these species; tomato, squash, soybean and cucumber convert amiben to N-glucosyl amiben (4, 10, 11). However, except for some evidence of N-glucosyl pyrazon formation in roots of millet, none of these susceptible species converted pyrazon to N-glucosyl pyrazon. Furthermore, in a study involving two inbred lines of red beet, that line which most readily converted pyrazon to N-glucosyl pyrazon was also the most tolerant to this herbicide.

The research of others also supports the conclusion that the conversion of pyrazon to N-glucosyl pyrazon in plants is a detoxication mechanism. First of all, in a study of the structure-activity relationships of chloropyridazinone

compounds, Fischer (20) found that the free amino group was essential for both phytotoxicity and beet tolerance. The attachment of glucose to this amino group in red beet leaves could either directly destroy the toxic structure or permit removal of this molecule from the site of toxic action. Secondly, the conversion of pyrazon to N-glucosyl pyrazon was rapid; 12 hr after the infiltration of beet leaf discs with 9.0 μM ^{3}H-pyrazon, over 70% had been converted to N-glucosyl pyrazon. This correlated perfectly with the work of Van Oorschot (59). He treated beets with pyrazon until the rate of CO_2 fixation was inhibited by 50% and found that only 12 hr was required for complete recovery after the pyrazon treatment was terminated. The rate of CO_2 fixation by susceptible oat was also inhibited but did not recover after termination of the pyrazon treatment. Frank (22) showed that pyrazon inhibited the Hill reaction equally in chloroplasts isolated from tolerant beets or susceptible Chenopodium album L. However, pyrazon inhibited photosynthesis (O_2 evolution) to a much greater extent in Chenopodium sp. compared to beet, when leaf discs rather than isolated chloroplasts were used. All of this research can best be related by the following interpretation: The site of action is equally sensitive to pyrazon in tolerant as well as susceptible plants. However, in beet leaves, the conversion of pyrazon to N-glucosyl pyrazon prevents the accumulation of toxic concentrations of pyrazon at the site of action.

The in vitro conversion of pyrazon to N-glucosyl pyrazon was attempted but was not successful. In this study, UDPG, ADPG, and TDPG were examined

as possible glucosyl donors for the metabolite.

Other pyrazon metabolites have also been detected in the soil and in plants (7, 19, 48). In fact, the conversion of pyrazon to ACP in plants has been interpreted as the detoxication mechanism responsible for the selective action of pyrazon (7, 19, 20, 58). A better understanding of the relationship between pyrazon, N-glucosyl pyrazon, and other metabolites in the soil and in the beet plant was provided by the long term metabolism study with radioactively labeled pyrazon (phenyl-^{3}H and 4, 5-^{14}C). This study clearly indicated that N-glucosyl pyrazon was the first metabolite of pyrazon formed and that this conversion prevented the accumulation of high concentrations of pyrazon in the plant. ACP was first detected in the soil 2 weeks after treatment and was not detectable in the plant until 2 weeks after it appeared in the soil. After its appearance in the plant, ACP was in turn complexed with some natural plant metabolite. The natural moiety in the ACP-complex was not identified, but glucose, as in N-glucosyl pyrazon, is a possiblility. These latter metabolites of pyrazon may not be phytotoxic, but any contention that they were related to the selective action of pyrazon would be unfounded. This conclusion was obvious since ACP was formed in the soil, 2 to 4 weeks after treatment, and not in the plant. Thus, it would have been equally available to all plants which survived the 2 to 4 weeks treatment with pyrazon.

LIST OF REFERENCES

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1. Anon. 1965. Amchen products technical service data sheet H-95.

2. Ashton, F. M. 1964. Fate of amiben-^{14}C in carrots. Weeds, 14: 55-57.

3. Aya, F. O. 1967. The influence of oils on the toxicity of 3-amino-1, 2, 4-triazole on quackgrass (Agropyron repens (L.) Beauv.). M.S. Thesis, Michigan State University, East Lansing, Michigan.

4. Baker, R. S., and G. F. Warren. 1962. Selective herbicidal action of amiben on cucumber and squash. Weeds, 10: 219-224.

5. Beinhauer, H. 1964. Wirkung von pyramin auf unkräuter. (The effect of pyrazon on weeds.) pp. 25-31. In Vortrage anlässlich der wissenschaftlichen unkrautbekämpfung in zuckerrüben mit pyramin. Badische Anilin- and Soda-Fabrik AG. Ludwigshafen am Rhein, Germany.

6. Brian, R. C. 1964. The metabolism of herbicides. Weed Res. 4, 105-117.

7. Burger, T. F. 1967. Untersuchungen über den abbau von ^{14}C-markiertem pyrazon in zuckerrüben. (Investigation of the degradation of ^{14}C-labeled pyrazon in sugar beets.) Fourth International Weed Control Congress. 30:8.

8. Colby, S. R. 1966. The mechanism of selectivity of amiben. Weeds, 14: 197-201.

9. _____. 1966. The metabolism of amiben derivatives in plants and soils. Abstracts, 152nd Meeting of the ACS, Div. of Agr. and Fd. Chem., New York.

10. _____. 1965. N-glycoside of amiben isolated from soybean plants. Science, 150:619-620.

11. _____., G. F. Warren and R. S. Baker. 1964. Fate of amiben in tomato plants. Jour. of Agr. and Fd. Chem., 12:320-321.

12. Cooke, A. R. 1966. Unpublished data. Amchem Products, Inc., Ambler, Penn.

13. Doherty, P. J. 1967. The adsorption of four herbicides by different types of organic matter and a bentonite clay. M. S. Thesis, Purdue Univ., Lafayette, Ind.

14. Drescher, N. 1964. Bestimmung der rückstände von pyramin in pflanze und boden. (The determination of pyrazon residues in plants and soil.) pp. 78-84. In Vorträge anlässlich der wissenschaftlichen aussprache über chemische unkrautbekämpfung in zuckerrüben mit pyramin. Badische Anilin-and Soda-Fabrik AG. Ludwigshafen am Rhein, Germany.

15. Edgerton, L. J. 1961. Inactivation of 2,4-dichlorophenoxy acetic acid by apple. Science, 134:341-342.

16. Ennis, W. B. Jr. 1964. Selective toxicity in herbicides. Weed Res., 4:93-104.

17. Eshel, Y. 1968. Unpublished data. The Hebrew Univ. of Jerusalem. Rehovot, Israel.

18. Fang, S. C. and J. S. Butts. 1954. Studies in plant metabolism. III. Absorption, translocation, and metabolism of radioactive 2,4-D in corn and wheat plants. Plant Physiol., 29:56.

19. Fischer, A. 1967. Untersuchungen über den abbau von 1-phenyl-4-amino-5-chlor-pyridazon-6 (PCA) im boden und pflanze. Journees Int. d'etude desherb. selectif cult. better., 9-10 3. Marly-Le-Roi, 213-219.

20. _____. 1964. Die wirkungsweise von pyramin. (The mode of action of pyrazon.) pp. 19-24. In Vorträge anlässlich der wissenschaftlichen aussprache über chemische unkrautbekämpfung in zuckerrüben mit pyramin. Badische Anilin-and Soda-Fabrik AG. Ludwigshafen am Rhein, Germany.

21. _____. 1962. 1-phenyl-4-amino-5-chloro-pyridazinone-6 (PCA) als ein neues rubenherbizid. (Pyrazon, a new beet herbicide.) Weed Res., 2:177-184.

22. Frank, Richard. 1968. The mode of action of pyrazon. Ph.D. Thesis, Univ. of Guelph, Guelph, Ont., Canada

23. _____. 1967. Pyrazon, a selective herbicide for sugar beets. Weeds, 15:197-201.

24. Frear, D. S. 1968. Herbicide metabolism in plants I. Purification and properties of UDP-glucose: aryl amine N-glucosyl transferase from soybean. Phytochem., 7:381-390.

25. Freed, V. H., and M. L. Montgomery. 1963. The metabolism of herbicides in plants and soils. Residue Reviews, 3:1-18.

26. Gallup, H. A., and F. G. Gustafson. 1952. Absorption and translocation of radioactive 2,4-dichloro-5-IODO^{131}-phenoxy acetic acid by green plants. Plant Physiol., 27:603-612.

27. Geissbühler, H., C. Haselbach, H. Aebi, and L. Ebner. 1963. The fate of N-(4-chloro phenoxy)-phenyl-N,N-dimethylurea (C-1983) in soils and plants. III. Breakdown in soils and plants. Weed Res. 3:277-297.

28. Hanf, M. 1964. Die chemische unkrautbekampfung in zuckerrüben in westeuropa. (Chemical weed control in sugar beets in West Europe.) pp. 14-18. In Vorträge anlasslich der wissenschaftlichen unkrautbekampfung in zuckerrüben mit pyramin. Badische Anilin-and Soda-Fabrik AG. Ludwigshafen am Rhein, Germany.

29. Hogue, E. J., and G. F. Warren. 1968. Selectivity of linuron on tomato and parsnip. Weed Sci. 16:51.

30. Holly, K. 1964. Herbicide selectivity in relation to formulation and application methods. pp. 423-263. In The physiology and biochemistry of herbicides. Academic Press, London and New York.

31. Kaslander, J., A. Kaars Sijpesteijn and G. J. M. Van Der Kerk. 1961. Transformation of dimethyldithiocarbamate into β-glucoside by plant tissues. Biochem. Biophys. Acta. 52:396-397.

32. Kearney, P. C. 1965. Purification and properties of an enzyme responsible for hydrolyzing phenylcarbamates. Jour. of Agr. and Fd. Chem. 13:561-563.

33. Kirkwood, R. C., M. M. Robertson, and J. E. Smith. 1966. Differential absorption as a factor influencing the selective toxicity of MCPA and MCPB. pp. 47-57. In Isotopes in weed research. IAEA, Vienna.

34. Klingman, G. C. 1961. Weed control as a science. John Wiley and Sons, Inc., New York.

35. Kuhr, R. J. and J. E. Casida. 1967. Persistent glycosides of metabolites of methyl carbamate insecticide chemicals formed by hydroxylation in bean plants. Jour. Agr. and Fd. Chem. 15:814-824.

36. Langbein, H. 1964. Die wirkung von pyramin auf rüben bei verschiedenen anwendungsterminen. (The effect of pyrazon on beets at different stages of growth.) pp. 32-36. In Vorträge anlasslich der wissenschaftlichen aussprache über chemische unkrautbekämpfung in zuckerrüben mit pyramin. Badische Anilin-and Soda-Fabrik AG. Ludwigshafen am Rhein, Germany.

37. Lowry, O. H., N. J. Roseborough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin-phenol reagent. Jour. of Biol. Chem. 193:265-275.

38. Luckwill, L. C., and C. P. Lloyd-Jones. 1960. Metabolism of plant growth regulators. I. 2,4-dichloro phenoxy acetic acid in leaves of red and of black current. Ann. of Appl. Biol. 48:613-625.

39. Makovcova, O. and L. Sindelar. 1966. A contribution to knowledge of the mechanism of selectivity of pyrazon. Biologia Pl., 8:260-262.

40. Meggitt, W. F. 1966. Environmental variables and weed control with pyrazon in sugar beets. Abstracts, Weed Soc. of Amer., p. 11.

41. Mitchell, J. W. and P. J. Linder. 1963. Absorption, translocation, exudation, and metabolism of plant growth-regulating substances in relation to residues. Residue Reviews, 2:51-56.

42. Moreland, D. E. 1967. Mechanism of action of herbicides. Ann. Rev. of Plant Physiol. 18:365-386.

43. Riden, J. R. and T. R. Hopkins. 1962. Formation of a water soluble 3-chloroaniline-containing substance in Barban-treated plants. Jour. of Agr. and Fd. Chem. 10:455-458.

44. Ries, S. K., M. J. Zabik, G. R. Stephenson and T. M. Chen. 1968. N-glucosyl metabolite of pyrazon in red beets. Weed Sci. 16:40-41.

45. Sargent, J. A. 1966. The physiology of entry of herbicides into plants in relation to formulation. Proc. 8th Br. Weed Control Conf. 3:804-811.

46. Schrodt, A. D., J. A. Gibbs and R. E. Cavanaugh. 1965. Quench correction by automatic external standardization. Packard Instrument Co. Bull.

47. Shimabukuro, R. H. 1967. Atrazine metabolism and herbicidal selectivity. Plant Physiol. 42:1269-1276.

48. Smith, D. T. 1968. Movement and persistence of a herbicide, 5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone (Pyrazon), in soil. Ph.D. Thesis, Michigan State Univ., East Lansing, Mich.

49. Smith, J. W., and T. J. Sheets. 1967. Uptake, distribution, and metabolism of monuron and diuron by several plants. Jour. of Agr. and Fd. Chem. 15:577-581.

50. Stephenson, G. R. and S. K. Ries. 1967. The movement and metabolism of pyrazon in tolerant and susceptible species. Weed Res. 7: 51-60.

51. Still, Gerald G. 1968. Metabolism of 3,4-dichloro-propionanilide in plants: The metabolic fate of the 3,4-dichloro aniline moiety. Sci. 159:992-993.

52. Stoller, E. W. 1968. Differential phytotoxicity of an amiben metabolite. Weed Sci. 16:384-386.

53. _____., and L. M. Wax. 1968. Amiben metabolism and selectivity. Weed Sci. 16:283-288.

54. Swanson, C. R. and H. R. Swanson. 1968. Metabolic fate of monuron and diuron in isolated leaf discs. Weed Sci. 16:137-143.

55. _____. 1966. Recent research on the fate of herbicides in crop plants. pp. 135-146. In Isotopes in weed research. IAEA, Vienna.

56. _____., R. H. Hodgson, R. E. Kadunce and H. R. Swanson. 1966. Amiben metabolism in plants. Weeds, 14:323-327.

57. _____. 1965. Metabolic fate of herbicides in plants. U.S.D.A., ARS., 34-66.

58. Van Der Zweep, W. 1964. The reaction of sugar beet and other test plants upon herbicides. pp. 70-78. In Vorträge anlassliche der wissenschaftlichen aussprache über chemische unkrautbekämpfung in zuckerrüben mit pyramin. Badische Anilin-and Soda-Fabrik AG. Ludwigshafen am Rhein, Germany.

59. Van Oorschot, J. L. P. 1965. Selectivity and physiological inactivation of some herbicides inhibiting photosynthesis. Weed Res. 5:84-97.

60. Wain, R. L. 1964. The behavior of herbicides in the plant in relation to selectivity. In The physiology and biochemistry of herbicides. Academic Press, London and New York.

61. Wang, C. H. and D. L. Willis. 1965. Radiotracer methodology in biological science. Prentice Hall. New Jersey

62. Williams, R. T. 1959. In Detoxication mechanisms, Chapman and Hall, Ltd. London, 796 p.

63. Wood, J. W., J. W. Mitchell, and G. W. J. Irving. 1947. Translocation of a radioactive plant growth regulator in bean and barley plants. Sci. 105:337.

64. Zabik, M. J. 1968. Unpublished data. Michigan State Univ., East Lansing, Mich.

65. Zemanek, J., E. Mydlilova, and M. Bylinska. 1966. Influence of pyrazon on catalase activity in the leaves of mustard (Sinapis alba L.) and sugar beet. Biologia Pl., 8:122-126.

APPENDIX

Common and Chemical Names of Herbicides

ACP *	5-amino-4-chloro-3(2H)-pyridazinone
amiben	3-amino-2, 5-dichlorobenzoic acid
amitrole	3-amino-1, 2, 4-triazole
atrazine	2-chloro-4-ethylamino-6-isopropylamino-s-triazine
barban	4-chloro-2-butynyl m-chlorocarbanilate
chloroxuron	N'-4-(4-chlorophenoxy)phenyl-N, N-dimethyl urea
CIPC	Isopropyl N-(3-chlorophenyl) carbamate
dalapon	2, 2-dichloropropionic acid
dinoben	3-nitro-2, 5-dichlorobenzoic acid
linuron	3-(3, 4-dichlorophenyl)-1-methoxy-methyl urea
MH	1, 2-dihydropyridazine-3, 6-dione (maleic hydrazide)
MCPA	2-methyl-4-chlorophenoxyacetic acid
MCPB	4-(2-methyl-4-chlorophenoxy)butyric acid
monuron	3-(p-chlorophenyl)-1, 1-dimethylurea
propanil	3', 4'-dichloropropionanilide
pyrazon	5-amino-4-chloro-2phenyl-3(2H)-pyridazinone
simazine	2-chloro-4, 6-bis(ethylamino)-s-triazine
TCA	trichloroacetic acid
2, 4-D	2, 4-dichlorophenoxyacetic acid

*Author's abbreviation for a pyrazon derivative.


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