# MOVEMENT AND PERSISTENCE OF A HERBICIDE, 5-AMINO-4-CHLORO-2-PHENYL-3(2H)-PYRIDAZINONE (PYRAZON), IN SOIL

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This is to certify that the

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William Meggitt
Major professor

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ABSTRACT

MOVEMENT AND PERSISTENCE OF A HERBICIDE, 5-AMINO-4-CHLORO-2-PHENYL-3(2H)-PYRIDAZINONE (PYRAZON), IN SOIL

by Dudley T. Smith

Pyrazon (5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone) was applied alone and in combination with TCA (trichloroacetic acid) in commercial sugar beet fields to determine pyrazon movement and persistence in soil under natural environmental conditions. Soils were sampled through the growing season and bioassayed with mustard (Brassica juncea L). In laboratory studies, pyrazon treated soils were incubated at 4.5, 12.5, 21.0, and 29.5 C for 20 weeks to determine bacteria and actinomycete populations in response to pyrazon. Radioactive labelled pyrazon was incubated in warm, moist soil to determine herbicide disappearance and possible breakdown products. Pyrazon adsorption was determined in nine soils ranging from 2.5 to 11.4% organic matter and 11 to 37% clay.

Pyrazon adsorption was correlated with soil organic matter ($r=0.92$) but not with clay content ($r=0.09$) or soil pH ($r=-0.08$) between 6.3 and 7.9. In the field 70 to 90% of the pyrazon residue was retained in the upper 2 inches of soil. Movement in the profile was retarded by a high

organic matter content and was not related to rainfall intensity in these soils. Pyrazon did not accumulate in field soils at lower depths as a result of movement. Residues and phytotoxicity were low in soils containing 20 to 30% clay and 2.5 to 4.3% organic matter where rainfall was well distributed after herbicide application. Pyrazon was more persistent in soils containing organic matter in excess of 10% and where rainfall was low or poorly distributed. The persistence of phytotoxicity was related to soil organic matter; however, the level or intensity of phytotoxicity appeared to be associated with the clay content. Following a 6 lb/acre application, pyrazon decreased from 2.5 to 0.25 µg/g of soil between 2 and 4 months after applications on a sandy loam containing 10% clay and 2.9% organic matter. Under the same environmental conditions pyrazon decreased from 2.75 to 2.25 µg/g in a sandy clay loam containing 26% clay and 12.9% organic matter.

In three soils bacterial response to pyrazon was maximal after 5 weeks of incubation at 21.0 C. With lower incubation temperatures more time was required for bacteria to respond to pyrazon. Bacteria increased gradually with time in amended soil incubated at 4.5 C.

In a laboratory incubation study pyrazon decreased from 11.6 to 9.6 mµ moles/g between 3 and 74 days of incubation. Part of the decrease in pyrazon was accounted for by the formation of a breakdown product that increased

from 0.04 to 0.94 mμ moles/g over the same period. The breakdown product did not contain the phenyl ring of pyrazon but retained the primary amino group. The degradation product was identified as 5-amino-4-chloro-3-pyridazinone and was subject to additional degradation in the soil.

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By

Dudley T. Smith

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# INTRODUCTION

The accumulation and persistence of organic residues is a common feature of soils (17), particularly since soils are generally regarded as a dumping ground for waste materials (2). Usually residues arise from plant and animal sources but in recent years man has added increasing amounts of organic residues to soils, primarily in the form of pesticides.

Chemical week control is an essential agricultural practice today, extending into all areas of crop and plant production. Efficient growth of desirable plants is still a simple basic requirement for man's existence. Man must be intelligent enough to observe his natural environment and manipulate it properly in order to survive. There has been an increasing concern regarding the use of biologically active materials in our environment.

Herbicide behavior in soils has been investigated in numerous laboratory, greenhouse, and field studies; however, there are few studies where laboratory and greenhouse findings have been directly correlated with field observations. This study was conducted to investigate the movement, persistence, and possible accumulation of pyrazon in sugar beet soils under field conditions. Adsorption, degradation,

and microbial response to pyrazon in soil were investigated in a laboratory to investigate and explain phenomena observed in the field.

# REVIEW OF LITERATURE

## Introduction

In 1907 Schreiner and Reed (54) found that phytotoxic materials such as terpenes, courmarin, and vanillin, resulting from microbial degradation of plant material, contributed to low productivity in soil. These investigators showed that toxic substances could be removed by carbon black and recognized soil organic matter as a complex adsorbing material. In 1946 (19), during the initial development of chemical weed control, the leaching, disappearance, and phytotoxicity of herbicides in soil was investigated at Camp Detrick. After observing the disappearance of two phenoxyacetic acids and a phenylcarbamate in soil, De Rose (19) stated, "At the present time [1946] no information is available to explain this difference in persistence." Now, less than 25 years later, there are extensive reviews concerning the behavior and disappearance of herbicides in soil.

Aldrich (1), in an early review, discussed herbicide persistence in relationship to microorganisms, retention by soil colloids, leaching, and decomposition in soil. More recent reviews concern the nature of the herbicide adsorption phenomena in soil (6, 17), relationships with soil microorganisms (2, 8, 40), effects of

climatic and environmental factors and agricultural practices on herbicides in soil (55, 58, 60) and the practical significance of pesticides in the soil environment (22, 43, 48).

## Factors Affecting Soil Microorganism Degradation of Herbicides

A portion of all herbicide applications eventually reach the soil, either directly or indirectly from crop residues (57). Alexander (2) emphasized that despite man's concept of microbial infallibility, soil organisms do have limitations in the materials degraded. Furthermore, microbes may generate metabolic products that are more toxic than the original substance. As explained by Kearney (40), soil microbes are in reality complex enzyme systems capable of degrading a large number of organic pesticides. Frequently a lag period occurs between the time of pesticide application and the first observed loss while there is a buildup of adaptive organisms. Schlegel et al. (53) regard the mixed microbial populations of soil and water as a community of competing species and metabolic types. Growth of one or a few species will be favored at the expense of others as external conditions change.

Aldrich (1) and Bollen (8) pointed out that herbicide persistance is mainly determined by conditions affecting microbial activity in soil. Techniques in isolating soil organisms were reviewed by Schegel et al. (53). The

importance of bioassays in herbicide persistance studies involving soil microorganisms were pointed out by Alexander (2).

Structure

Molecular structure is of major importance in determining the susceptibility of herbicides to microbial attack. In chlorinated phenoxy compounds, resistance to degradation is governed more by the position of the halogen on the ring rather than the number (3, 42), whereas the number of halogens is more important in the case of benzoic acids (42). Kearney et al. (41) indicated that chlorosubstitution, the addition of large electronegative atoms, influenced the disappearance of aliphatic acids as well as aromatic compounds. In a classical experiment Jenson (36) observed an 80% loss of mono- and dichloroacetic acids in 2 to 4 and 8 to 14 days, respectively, in soil, whereas 14 to 28 days were required for a comparable loss of trichloroacetic acid (TCA). It was emphasized (36) that only a limited number of soil bacteria develop dechlorinating enzymes.

Kaufman (39) showed that the presence of one molecular structure influenced the degradation of another. Amitrole apparently interfered with the proliferation of organisms capable of degrading dalapon, a chlorinated aliphatic acid, however, the converse was not observed with this combination.

## Temperature and Moisture

Effects of temperature on microorganisms were reviewed by Farrell et al. (24). Burnside (10) and Ercegovich et al. (23) showed relationships between soil temperature and herbicide disappearance. The breakdown of an s-triazine was clearly temperature dependent. Atrazine was not observed after 1, 2, and 9 months in soils incubated at 35, 25, and 15 C, respectively. Amitrole disappeared more rapidly as soil temperature increased from 8 to 100 C and also disappeared more rapidly in moist soil than in dry soil (23).

Persistance of amiben (10) and atrazine (10, 42) in laboratory incubation studies was contrary to that usually observed under field conditions. Bollen (8) discussed herbicide losses in incubation studies in relation to losses observed under field conditions and indicated that some variables, such as unequal herbicide distribution in the field micro-environment, soil drainage, and rhizosphere conditions could not be duplicated in laboratory incubation studies.

## Soil pH and Fertility

Persistance may be affected by soil pH and fertility. Corbin and Upchurch (16) determined the optimum soil pH for detoxification of several herbicides. Marked differences were observed. Cochrane (13) pointed out that

actinomycetes tend to be sensitive to phosphates in the soil; hence, soil pH is a factor in regulating the growth and activity of those organisms. Bacteria generally respond best in soil near a neutral pH while fungi are more predominant in acid soils.

## Retention of Herbicides in Soil

Bailey and White (6) discussed the nature of soil colloids, pH, moisture, temperature, and herbicide formulation with regard to herbicide sorption phenomena. Dean (17) indicated that hydrogen bonding, polar molecules, and basic amino groups are all probably involved in retaining and stabilizing organic pesticides in soil. Although not all compounds are particularly resistant to decomposition, many exhibit some degree of stability in the soil colloid system.

## Herbicide Structure

In a study with five s-triazines, Harris (31) indicated that there was little relationship between herbicide structure and adsorption in soil. However, it was suggested that electron distribution in the s-triazine molecule, as affected by substitution on the ring, may account for some differences observed in the adsorption of those compounds. Ward and Upchurch (62) studied adsorption of 52 related N-phenylcarbamates, acetanilides, and anilines on nylon, cellulose, and cellulose acetate.

Adsorption occurred by hydrogen bonding between the amido hydrogen of the adsorbate and the carbonyl oxygen of the adsorbent.

Soil Colloids

There has been some controversy as to the relative importance of clay in comparison to organic matter in herbicide adsorption. In an objective study, Harris and Sheets (33) stated that there was no single factor from which herbicide adsorption could be predicted. Montmorillonite clay may be important in Midwestern areas while organic matter may dictate the extent of adsorption in Southern soils (34). Doherty (20) showed that adsorption may vary with different types of organic matter, depending on the degree or stage of decomposition of the adsorbent. The importance of the organic matter in herbicide adsorption is evident in many instances (20, 34, 35, 56) but the importance of clays should not be underestimated (61) since there is ample evidence of the role of clay in herbicide adsorption (9, 30, 34, 45, 46). Harris et al. (34) point out that herbicide research is at a stage where broad generalizations regarding adsorption are not very useful.

Soil pH

The influence of pH on adsorption was shown by Harris et al. (34) where DNBP and an s-triazine were readily

adsorbed by a bentonite clay at pH 2.3 but not at pH 8.4. Adsorption of the two herbicides by muck and a cation exchange resin was intermediate between the two extreme values, depending on the pH of the adsorbent. Adsorption of three unrelated herbicides was not related to soil pH in 32 soils, ranging from pH 4.3 to 7.7 (33). Nearpass (46) observed that adsorption of an s-triazine was correlated with titratable acidity, which was a better indicator of adsorption than soil pH. In a later study (47), adsorption was related to the degree of base saturation rather than to a predominance of any specific cation on clay. With increased base saturation apparently more sites were occupied by cations and less herbicide adsorption occurred. Competition for adsorption sites has been observed in other instances (18, 30) in which less adsorption occurred when water was present and occupied the sites.

Leaching

Movement of herbicides in soil has been related to the adsorption and water solubility of herbicides. In a study of s-triazines (31), mobility in soil was more related to adsorption than to water solubility. The mobility of s-triazines has also been related to pH dependent adsorption (46). Hilton et al. (35) reported that although a substituted urea was adsorbed by soil, the herbicide was readily leached by water. Bayer (7) in studying the

role of surfactants in leaching of herbicides concluded that differences in herbicide movement were due to the wetting ability of surfactants and not their ionic character.

### Photodecomposition and Volatility

Jordan et al. (37) showed that after exposure to ultra violet light the spectrophotometric adsorption of herbicides was altered, indicating that photodecomposition occurred. Photodecomposition may be more of a problem in warm sunny areas than in those of frequent rainfall and low sunlight. The phytotoxic properties of a specific herbicide may be modified after exposure to sunlight or specific artificial light but the relative importance of photodecomposition under field conditions has not been demonstrated (60).

In regard to herbicide losses by volatility, Upchurch (60) pointed out that volatile herbicides are generally lost more readily in moist soil, due to competitive adsorption with water, than in dry soil. Effects of competitive adsorption of water on the loss of a volatile herbicide have been demonstrated (18).

## Climatic Factors and Field Studies on Herbicide Persistence

The research previously described in this review was performed primarily under laboratory and greenhouse

conditions. Upchurch (60) indicated that although individual "component reactions" should be understood, weed researchers have been slow to conduct herbicide experiments considering all the diverse conditions encountered in the field. Bailey and White (6) indicated that soil structure, moisture, and temperature affected the persistence of herbicides indirectly but were of major importance, nevertheless.

Sheets and Harris (58) indicated that favorable weather both before and after application is important in the degradation of herbicides. The significance of herbicide residues was also discussed in relationship to soil properties and subsequent crop production. Rainfall, soil properties, and the total environment must be considered when evaluating herbicide disappearance in soil (55). In one investigation (61) phytotoxicity of five herbicides was studied over a three year period at 17 field sites. Approximately 60% of the variability in plant response was accounted for on the basis of soil and climatic factors. Disappearance of a substituted benzoic acid herbicide was accounted for by geographical differences in soil type and rainfall (11). Wide variation in the loss of pichloram, a highly persistent herbicide, was observed (29) but rates of disappearance were correlated with soil and environmental factors.

The persistence and mobility of endothall, a herbicide used in sugar beets, was investigated in an arid region (14). Movement depended on irrigation methods and soil clay content and was not affected by the amount of soil moisture at application. Phytotoxic residues disappeared rapidly in soils with low retention capacity and were not found at the end of the growing season.

## Pyrazon in Plants and Soil

### Development and Use

In 1962 Fischer (25) reported that a group of phenylpyridazones were effective herbicides in sugar beets. Pyrazon (1-phenyl-4-amino-5-chloro-6-pyridazone) exhibited the best herbicidal properties of 500 derivatives investigated. Accepted nomenclature for pyrazon in the United States is 5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone or PCA. In Michigan (44) in 1963 pyrazon applied preemergence in sugar beet fields was most effective at 3 and 4 lbs/acre in combination with TCA, indicating that low rates of this herbicide were effective in humid regions in the United States. Comparable performance was observed in Ontario, Canada (27). Use, application methods, and performance of pyrazon and other herbicides used in sugar beets were reviewed by Alley (4).

Soil Microorganisms

Jung (38) reported that $CO_2$ evolution from soils amended with glucose, starch, or cellulose was not adversely affected by 60 and 660 ppm of pyrazon in the soil. In soils amended with glucose or wheat straw, $CO_2$ evolution was stimulated slightly by the addition of pyrazon, indicating that microbial activity was enhanced by the presence of the herbicide. Nitrification in soil was not affected by pyrazon at rates up to 100 kg/ha, however, respiration and nitrification were reduced by rates equivalent to 1/2 and 1 ton/acre.

Wolcott (63) observed that 250 ppm of pyrazon had no effect on nitrification in combined pure cultures of Nitrosomonas europaea and Nitrobacter agilis. In fungal cultures Pommer (50) observed that the growth and reproduction of Aspergillus, Penicillium, Trichoderma, and Rhizopus were not affected by 10 ppm of pyrazon. Development and reproduction of naturally occurring fungi in soils at pH 5.3 and 7.5 were not affected by 100 ppm of pyrazon.

Fate in Plants

Stephenson et al. (59) showed that roots of susceptible species absorbed and accumulated pyrazon more readily than roots of a tolerant species, red beet. Translocation to the shoot and foliar absorption of pyrazon was also greater in two susceptible species

than in red beet. A metabolite was extracted from red beet tissue but was not found in susceptible species. This compound was later conclusively identified as N-glucosyl pyrazon (52). It appears that in addition to differential uptake of the herbicide there is an additional mode of selectivity, in which the herbicide may be detoxified in tolerant plants. The importance of the presence and the position of the amino group on the pyridazine ring for herbicidal activity has been shown (26).

In contrast to work in the United States (52, 59), other researchers (21, 26) have observed a different metabolite in sugar beets. Dephenylated pyrazon or 5-amino-4-chloro-3-pyridazinone (metabolite A), was found in beet extracts five days after the herbicide was applied (26). Apparently the metabolite was not phytotoxic although no evidence was offered.

Fate in Soils

Drescher (21) stated that the breakdown of pyrazon was slower in soils than in plants. In laboratory trials 40 weeks were required for the dissipation of 12 ppm of pyrazon in incubated loamy sand. No residues from a 20 ppm application were detected in potted soil after 12 weeks of exposure outdoors. In field experiments pyrazon was applied at 3.2 kg/ha and immediately after application a residue of 4 ppm was observed in the upper 4 inches

of soil. This residue decreased to 0.2 ppm within 11 weeks. Fischer (26) reported that the residual phytotoxicity of pyrazon in loamy sand was shorter than that of monuron or simazine and that corn or small grain was not injured when planted 8 to 10 weeks after pyrazon was applied. Metabolite A has been observed in soil shortly after pyrazon application (26).

Cooke (15) found that pyrazon adsorption in five pure clays was highest with hectorite, intermediate with illite and attapulite, and lowest with halloysite and montmorillonite. When pyrazon was added at 500 µg/g in 0.01 M $CaCl_2$ hectorite adsorbed 100 µg/g while montmorillonite adsorbed 4 µg/g; however, both of these are a type of montmorillonite clay. Doherty (20) studied pyrazon adsorption by organic matter and bentonite by mixing 1% of the adsorbent with quartz sand. Adsorption was determined by a bioassay and was in the order of fibrous peat > muck soil >> sphagnum moss > bentonite clay. The fibrous peat was highest in organic matter while the sphagnum was least decomposed. Indications were that adsorption was related to the stage of decomposition as well as the percentage of organic matter. Adsorption of pyrazon was not directly related to surface area or cation exchange capacity of the adsorbents. In Frank's work (28) soil type accounted for differences in phytotoxicity from pyrazon.

Phytoxicity in bioassays was greater in a sandy loam than in two clay soils, indicating that less adsorption occurred in the lighter soil.

Movement

Although pyrazon was adsorbed by soil colloids (15, 20) extensive leaching has been observed. In one study (15) pyrazon was applied at 6 lbs/acre to a moist sandy loam soil in a column. After 4 inches of water were applied over a 4 hour period the column was split longitudinally and bioassayed. Pyrazon trailed from the surface to 5 1/2 inches below the surface.

TCA, which is frequently applied with pyrazon, exhibits an ionic character in water, is highly soluble, and adsorption is extremely limited (41). The mobility of this herbicide was demonstrated by Ogle et al. (49), in which TCA moved 4 to 6 inches in fine sand, silt loam, and a muck soil after an application of 2 inches of water. This high degree of mobility has been confirmed by others (41), but there was no report of the effect of this herbicide on the movement of another less mobile compound. The rapid loss of TCA in field soils is associated with its mobility.

Photodecomposition and Volatility

Zabik (64) subjected a pyrazon solution to irradiation from a mercury arc lamp. After exposure,

approximately six different fragments or breakdown products were observed with about 80% existing as one product. Although fragments were not positively identified, pyrazon was not recovered. This work indicated that photodecomposition of pyrazon may occur if exposed to sunlight under field conditions. Fischer (26) negated the possibility of any significant loss of pyrazon by volatilization since the vapor pressure of the compound was low at temperatures usually encountered. At 40 C the vapor pressure was 0.074 mm of Hg.

## MATERIALS AND METHODS

### Field Experiments

Pyrazon residues were evaluated in several soils during 1965 and 1966 under natural environmental conditions. In 1965 pyrazon was applied at three field locations at 0, 2, 3, 4, and 6 lbs/acre and, except for the 2- and 6-pound rates, at the same rates in combination with TCA at 8 lbs/acre. Pyrazon was also applied at 10 lbs/acre at location 2. In 1966 treatments were applied at five locations at 2, 3, 4, and 6 lbs/acre of pyrazon in combination with TCA at 8 lbs/acre and at 0, 4, and 6 lbs/acre of pyrazone alone. Herbicide treatments were applied between May 1 and 13 in 1965 and April 15 and May 10 in 1966, in three replications in commercial sugar beet fields in Michigan. All herbicides were applied in 23 gal/acre with a tractor mounted plot sprayer. Plots were 4 rows wide (approximately 10 ft.) by 50 to 100 ft. long. Soil composition was determined from a composite sample at the respective locations (Table 1).

Soil samples were obtained three times during 1965 in a two to five month period after application to determine residual concentrations of pyrazon at three soil

depths. Samples were obtained from 0 to 2, 2 to 4, and 4 to 8 inches below the surface by dividing 15 to 20 soil core probes from each plot into three segments. During 1966 soil samples were obtained two to three times between 2 and 6 months following application from the upper 2 inches of soil. One pound samples were sealed in wax cups and stored at 4.5 C or lower for 3 to 7 months until bioassayed. Daily distribution and intensity of rainfall

TABLE 1.--Characteristics of soils in field studies.

| Soil No. | Soil Texture | Soil Characteristics | | | | |
|---|---|---|---|---|---|---|
| | | Sand | Silt | Clay | Organic Matter | pH |
| | | % | % | % | % | |
| 1 | Clay loam | 44.0 | 24.0 | 32.0 | 3.8 | 6.8 |
| 2 | Clay loam | 32.0 | 29.0 | 39.0 | 6.8 | 6.7 |
| 3 | Sandy loam | 67.0 | 24.0 | 9.0 | 13.7 | 7.0 |
| 4 | Sandy clay loam | 54.0 | 21.6 | 24.4 | 4.2 | 7.3 |
| 5 | Sandy clay loam | 48.2 | 23.8 | 28.0 | 4.3 | 7.5 |
| 6 | Sandy clay loam | 52.8 | 19.0 | 28.2 | 4.2 | 6.2 |
| 7 | Sandy clay loam | 46.6 | 26.8 | 26.6 | 12.9 | 7.6 |
| 8 | Sandy loam | 79.1 | 10.9 | 10.0 | 2.9 | 7.3 |

during the season was determined from data compiled by regional weather stations (5).

## Soil Analysis

All soils were crushed and sieved through a 10 mesh screen prior to laboratory analyses. Soil mechanical analyses were determined using the Bouyoucos hydrometer method from which soil texture was determined. Organic matter was determined from 15 to 20 g of oven dried soil (105 C) by weight difference after combusion at 400 C. Soil pH was determined from a soil-water suspension (1:1) with a glass-calomel electrode system, one hour after initial mixing. Water holding capacity was determined by the water retained by 100 g of dry soil after six hours of free drainage. All analyses and determinations were made in duplicate. Cation exchange capacity was determined from the amount of cations extracted from soil with 1.0 N ammonium acetate, pH 7.0 and from buffer suppression by hydrogen ions, in nine soils in an adsorption study.

## Biological Assay for Pyrazon

Since many field samples may have contained TCA as well as pyrazon, a preliminary experiment was conducted to determine the selectivity and sensitivity of brown mustard (*Brassica juncea* (L) Cosson, var. Florida Broadleaf) for pyrazon. Pyrazon and TCA were added alone and in combination up to 5 µg/g of soil in four replications. Plant

growth was observed and fresh weight of mustard plants was determined 3 weeks after planting.

Pyrazon was determined in soil samples with a mustard bioassay. Samples from field plots were crushed and sieved and 250 g of air-dried soil were placed in a wax cup with holes punched to allow drainage. Mustard seed was covered with coarse washed sand and seedlings were thinned to ten plants per cup at an early cotyledon stage. Fresh weight and plant injury were determined 30 to 40 days after planting. Visual injury from pyrazon was evaluated on a scale of 0 to 10 where 0 indicated no apparent injury while 10 indicated that all plants were killed. Injury ratings between 0 and 10 were based on the intensity of damage and area of chlorosis on the foliage.

Soil containing known amounts of pyrazon was included in the bioassay of all samples, except those from location 1 in 1965, to access residual concentration of pyrazon in field samples. Pyrazon was added to untreated soil from each location at rates ranging from 0.0 to 5.0 µg/g. Twenty-five ml of prepared pyrazon standards were pipetted on 250 g of soil spread out on paper. Mixing was accomplished by lifting alternate corners and sides of the paper, tumbling the soil back and forth across the surface. Clumps of moist soil were broken and redistributed during mixing. Excessive soil wetting, loss of herbicide

and contamination were reduced or avoided by this method while soil structure was maintained. Standards were replicated three times and were randomized with samples from the same soil during the bioassay. Pyrazon concentrations in field samples were determined by comparison of bioassay injury ratings to a standard curve from the same soil.

## Response of Soil Microorganisms to Pyrazon

The influence of temperature and time of incubation on the response of microorganisms to pyrazon was investigated in three soils. A sandy clay loam (Table 2) was obtained from a sugar beet field after recent tillage and was crushed, sieved, and thoroughly mixed prior to treatment. Treatments consisted of two soil treatments; an untreated control, and soil amended with pyrazon, four incubation temperatures; 4.5, 12.5, 21.0, and 29.5 C, and four times of incubation; 5, 10, 15, and 20 weeks. In addition, soil at 21.0 C was removed after 2 weeks of incubation. Samples incubated at 4.5 C were not obtained at the 15 week interval. Soil was amended with pyrazon at the rate of 4 µg/g. The amount of herbicide necessary for soil treatment was first mixed with washed quartz sand and then sprinkled over the soil with intermittent soil mixing. Mixing was accomplished as previously described in connection with the bioassay standards. Two

hundred g of soil were weighed into incubation containers. Containers were prepared by punching holes in the side of low-form wax cups near the lip and plugging holes with sterile cotton. Sterilized water was added to soil, equivalent to 60% of the water-holding capacity, and containers were covered with tight fitting lids during incubation.

TABLE 2.--Characteristics of soils in the pyrazon incubation study.

| Soil Characteristics | | | |
|---|---|---|---|
| Texture | Clay | Organic Matter | WHC[1] |
| | % | % | g/100g |
| Sandy clay loam | 23 | 4.8 | 35 |
| Sandy loam (soil 3) | 25 | 13.7 | 41 |
| Clay loam (soil 1) | 32 | 3.8 | 30 |

[1]Soil water-holding capacity.

Two additional soils, a sandy loam and a clay loam, were prepared and treated in the same manner described above, with some exceptions. These soils had been in a dry, dormant condition in a greenhouse for 7 months prior to treatment. In this study these soils were amended with pyrazon at 3 µg/g, incubated at 21 C, and samples were removed after 2, 5, 10, and 15 weeks of incubation.

Time and soil treatments were randomized within each temperature treatment. All cups were weighed

periodically and soil moisture was maintained at 40 to 60% of water-holding capacity by the addition of sterilized water. Only soils incubated at the highest temperature longer than 10 weeks required appreciable amounts of water. Bacteria and actinomycete populations were determined from soil as samples were removed from incubation.

Microbe populations were determined by plating 2 ml of $10^{-4}$, $10^{-5}$, and $10^{-6}$ dilutions of moist soil in triplicate in a soil extract agar (52). Plates were incubated in the dark at room temperature for 7 to 10 days before colonies were counted. Colony characteristics were recorded. Bacteria colonies were counted from a $10^{-6}$ dilution while actinomycetes were determined from a $10^{-5}$ dilution. Counts were corrected to a dry soil basis. Data were statistically analyzed on the basis of counts per g of soil but bacteria counts were expressed graphically as a percentage of the untreated control from the same incubation time and temperature treatment to evaluate response to pyrazon. This percentage expression removed population fluctuations in amended soils occurring as a result of natural microbial responses to incubation environments. Soil pH was determined in untreated soils for each time and temperature treatment.

## Degradation of Pyrazon in Soil

Radioactive labelled pyrazon was incorporated in a sandy loam and incubated in a warm moist environment to

observe herbicide degradation in the soil. This soil contained 18.4% clay, 3.5% organic matter, and had a holding capacity of 29 g/100g of soil. One mg of pyrazon, uniformly tritiated in the phenyl ring, was applied in 20 ml of water to 200 g of soil by the mixing process previously described. In preliminary isotope work, differences between pyrazon concentrations in soil were reduced from 41 to 4% after 10 and 20 minutes of mixing by the soil rolling process. In this study soil was mixed for 20 minutes and uniform herbicide distribution was confirmed. Pyrazon, labelled with $^{14}C$ in positions 4 and 5 of the pyridazine ring, was added to a second batch of soil in a similar manner. Hence, two soils were amended with 5 µg of pyrazon/g (22.6 mµ moles/g) with a radioisotope label in each ring of the compound. Specific activity after dilution was 14.6 µc/mg of $^{3}H$-pyrazon and 19.6 µc/mg of $^{14}C$-pyrazon. Both compounds were synthesized by BASF Corporation.

Amended soils and an untreated control were incubated in closed glass containers at approximately 20 C at 60% of the water-holding capacity. Ten g samples of soil were removed after 3, 10, 15, 30, and 74 days of incubation. Duplicate soil samples containing $^{3}H$-pyrazon were obtained after 30 days of incubation while duplicates of soil amended with $^{14}C$-pyrazon were obtained after 30 and 74 days. Sample moisture was calculated from changes in

soil weight during the experiment. Soils were frozen until all samples were obtained.

Samples were extracted with 10 ml of absolute ethanol for 25 minutes on a horizonal shaker and centrifuged at 2000 x g for 10 minutes. Radioactivity in the supernatant was determined by counting 100 µl dissolved in 2 ml of absolute ethanol in 15 ml of a BBOT (2,5,bis (2-tert-buytlbenzoxazolyl) thiophene)-toluene) counting system (4 gm/L). All counting was done in a Packard 3003 Liquid Scintillation Spectrometer. There was no evidence of quenching by materials extracted from soil. One hundred µl of each supernatant solution were also chromatogrammed to determine the percentage of the source(s) of radioactivity in the soil extracts. Chromatographic techniques described by Stephenson et al. (60) were employed, utilizing Eastman prepared silica gel Chromagrams for all chromatograms. Both $^{3}$H-pyrazon and $^{14}$C-pyrazon were added to aliquots of supernatant solutions from untreated controls and were co-chromatogrammed as standards. Chromatograms were developed in a benzene-ethanol (3:1, v/v) solvent system (solvent system I).

Extracts from soils incubated for 74 days with $^{3}$H-pyrazon and $^{14}$C-pyrazon (250 and 350 µl, respectively) were co-chromatogrammed with a standard of $^{14}$C-pyrazon and non-labelled 5-amino-4-chloro-3-pyridazinone; (metabolite A). After development in a benzene-ethanol (85:15, v/v) solvent (solvent system II) chromatograms

were visualized by diazotizing and coupling the primary amine on the pyridazine ring to produce an azo compound. Developed chromatograms were exposed to nitrous acid fumes for 5 minutes by adding $NaNO_2$ to concentrated HCl. Chromatograms were sprayed with a beta-napthol solution (1 g napthol, 10 g urea, and 50 ml each of water and ethanol) and exposed to $NH_4OH$ fumes to observe color. Colored bands on chromatograms were outlined, and chromatograms were segmented and counted to establish the position(s) of radioactivity in relation to the bands observed.

Supernatants from soils incubated for 74 days with $^{14}C$-pyrazon were combined and concentrated approximately ten-fold, at 35 C, to confirm the presence of a $^{14}C$ labelled compound occurring in that extract. Five hundred µl of the concentrated greenish liquid were chromatogrammed in solvent system I and visualized by the color method procedure previously described. Colored bands and intermediate areas were scraped off and counted for radioactivity. The concentrated extract and a standard containing pyrazon and metabolite A were also co-chromatographed in benzine-chloroform-methanol (3:1:1, v/v/v) (solvent system III).

Pyrazon Adsorption in Soils

The relationship between soil composition and pyrazon adsorption was determined in nine soils with different

properties (Table 3). Clay content ranged from 11 to 36% while organic matter ranged from 2.5 to 11.3%. Soils were obtained from untreated areas in sugar beet fields and were air-dried, crushed, and sieved through a 40 mesh screen. One g amounts were weighed into 15 ml polycarbonate centrifuge tubes. Tritium labelled pyrazon (specific activity after dilution, 1.91 µc/mg) was added at rates of 50 and 500 µg/g of soil in 10 ml of 0.01 M $CaCl_2$. In a preliminary experiment there was no difference in pyrazon adsorption after 5 and 15 hours of equilibration time. In this experiment soils were placed on a horizonal shaker at a high speed for 6 hours of equilibration at 21 $\pm$ 1 C and then centrifuged at 2000 x g. Radioactivity was determined by counting 50 µl of the supernatant solution, dissolved in 1 ml of absolute ethanol. Herbicide adsorption in the soil was assumed to be the difference between the initial concentration added and that found in the supernatant after equilibration. Pyrazon was not absorbed by polycarbonate tubes or caps. Adsorption was expressed as µg of pyrazon/g of dry soil.

The supernatant solution from the soil with the highest organic matter content was chromatogrammed on silica gel in solvent systems I and II to confirm pyrazon as the source of radioactivity in the solution. Labelled pyrazon, in 0.01 M $CaCl_2$, was co-chromatographed as a standard. A multiple regression analysis was employed

to evaluate the relationship between various soil properties and adsorption from two concentrations of pyrazon.

TABLE 3.--Characteristics of soils in the pyrazon adsorption study.

| Texture | Clay | Organic Matter | pH | C.E.C. |
|---|---|---|---|---|
| | % | % | | me/100g |
| Clay loam | 32.0 | 3.21 | 7.34 | 13.2 |
| Sandy clay loam | 24.4 | 2.54 | 7.84 | 11.3 |
| Sandy clay loam | 28.4 | 3.91 | 7.95 | 15.0 |
| Sandy loam | 18.4 | 3.50 | 7.81 | 13.5 |
| Clay loam | 36.8 | 5.08 | 6.35 | 13.6 |
| Sandy loam | 11.2 | 3.06 | 6.57 | 6.6 |
| Sandy loam | 16.2 | 4.94 | 6.73 | 11.6 |
| Sandy clay loam | 26.6 | 11.38 | 7.92 | 23.7 |
| Sandy loam | 16.7 | 8.44 | 6.76 | 24.5 |

## Statistical Analyses

Computer programs written by the Department of Agricultural Economics, Michigan State University, were utilized for analyses of variance and regression analysis of experimental data. Significant differences in analyses of variance were determined at the 5% level while significance in correlation coefficients was determined

at the 1% level. Treatment means were separated using the Duncan multiple range test.

## RESULTS AND DISCUSSION

### Mustard as a Bioassay for Pyrazon

Brown mustard was an excellent bioassay species for pyrazon and exhibited many desirable characteristics. Plants were easy to establish, uniform in growth, and specific and sensitive to pyrazon.

Pyrazon added at 0.5 and 1.5 µg/g reduced mustard growth significantly whereas TCA showed little or no effect on plant growth (Table 4). When pyrazon and TCA were applied in combination, the resultant fresh weight in each treatment was comparable to that observed from the same rate of pyrazon applied alone.

Plant injury was not observed from TCA treatments; however, injury from pyrazon was evident and could be evaluated visually. The size of chlorotic areas on leaf tips and the peripheral distance of chlorosis around the leaf margin increased proportionally with higher pyrazon rates. Consequently the intensity of plant injury could be evaluated on an empirical scale of 0 to 10. In standards from soil 2 there was a significant correlation between herbicide concentrations and injury ratings ($r = 0.85$) and fresh weight ($r = -0.93$). Approximately

TABLE 4.--Fresh weight of mustard from soil treated with pyrazon, TCA, and combinations of both herbicides.[1]

| Control | | Pyrazon | | TCA | | Pyrazon + TCA | |
|---|---|---|---|---|---|---|---|
| Rate | Weight | Rate | Weight | Rate | Weight | Rate | Weight |
| µg/g | g/cup | µg/g | g/cup | µg/g | g/cup | µg/g | g/cup |
| 0 | 2.3a | 0.25 | 2.1a | 1.5 | 2.3a | | |
| | | 0.5 | 1.2b | 3.0 | 2.5a | 0.5 + 1.5 | 1.1b |
| | | 1.5 | 0.1c | 5.0 | 1.9a | 1.5 + 3.0 | 0.1c |

[1]Means with the same letter are not significantly different ($P < 0.05$).

70 to 85% of the differences in injury ratings and fresh weights could be attributed to differences in pyrazon in the soil.

In some instances mustard vigor and fresh weight production in bioassay standards increased when low rates of pyrazon were added to soil. This enhancement was most apparent with applications of 0.2 to 0.5 µg/g in four soils (Table 5), but was evident at higher concentrations in some instances. Fresh weight of mustard increased 10 to

TABLE 5.--Enhancement of mustard growth by pyrazon added to four soils.[1]

| Pyrazon Added | Soil Number | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2 | | 3 | | 6 | | 7 | |
| µg/g soil | g/cup | %[2] | g/cup | % | g/cup | % | g/cup | % |
| 0.0 | 1.0b | | 1.4ab | | 1.6ab | | 1.0ab | |
| 0.25[3] | 1.3a | 30 | | | 1.7ab | 6 | 1.7a | 70 |
| 0.5[3] | 1.2ab | 20 | 1.5ab | 7 | 2.4a | 50 | 1.7a | 70 |
| 1.0[3] | 1.0b | | 2.2a | 57 | 1.5b | | 1.3ab | 30 |
| 1.5[3] | 0.7c | | 1.7ab | 21 | 1.4b | | 0.9b | |
| 2.0 | 0.4d | | 1.8a | 28 | 1.1b | | 0.7b | |
| 2.5 | 0.3d | | 0.9b | | 0.8b | | 0.7b | |

[1]Means with the same letter in the same column are not significantly different ($P < 0.05$).

[2]Percentage increase in fresh weight compared to untreated control.

[3]In soils 2 and 3 pyrazon added at 0.2, 0.4, 0.8 and 1.2 µg/g, respectively.

70% as a result of some pyrazon treatments; however, the presence of small amounts of pyrazon could be detected by observing foliar injury.

## Pyrazon Distribution and Movement

Distinct patterns in pyrazon distribution and movement were evident in two of the three soils during 1965. In two clay loam soils, pyrazon residues were significantly different due to rate of application, depth, and time of sampling (Tables IV and VII), as well as soil composition. Interactions between application rates and concentrations at various depths, and between time of sampling and soil depth were also meaningful. These trends were more discernible when pyrazon was applied at 4 and 6 lbs/acre since bioassay responses were greater, particularly in samples below the surface layer of soil. Since rainfall intensity and distribution were comparable at field locations 1 and 2, it was assumed that differences in herbicide residues were due primarily to treatments and soil properties and were not due to local environmental conditions. In a sandy loam (soil 3) pyrazon residues were not affected by herbicide treatments (Table I) and herbicide distribution and movement patterns were not observed in this soil (Table 7).

Distribution

Approximately 70 to 90% of the residual pyrazon observed in the clay loam soils remained in the upper 2 inches of soil (Table 6). The amount of pyrazon in both the surface layer and soil 2 to 4 inches below the surface tended to increase with higher rates of application. Consequently the percentage of total residue at each soil level remained constant for a given soil and was not affected by the rate of application. While 70% of the total residue in soil 1 resided in the upper 2 inches of soil, approximately 30% was located in the adjacent 2- to 4-inch zone. In soil 2, with a higher clay and organic matter content, nearly all of the residue was located in the top layer of soil and only 10% or less occurred below 2 inches. Pyrazon was not found in either soil between 4 and 8 inches below the surface, even where the herbicide was applied at 6 or 10 lbs/acre. There was no indication that TCA applied in combination with pyrazon affected the distribution of the latter herbicide in the soil profile.

Movement

Herbicide concentrations decreased with increasing soil depth and in soil 1 were different in each layer from those in adjacent zones (Table 7). Between the first and second sampling dates in soil 1, pyrazon concentrations approximately doubled 2 to 4 inches below the surface. No

TABLE 6.--Distribution of pyrazon in two clay loam soils in 1965 as accessed by bioassay injury ratings.[1]

| Soil Depth | Rate of Pyrazon Applied (lbs/acre) | | | | | Distribution (Range) | Mean[2] |
|---|---|---|---|---|---|---|---|
| | 0 | 4 | 4 + TCA | 6 + TCA | 10 | | |
| in | IR | IR | IR | IR | | % | % |
| | | | Clay Loam (Soil 1) | | | | |
| 0-2 | 0.3 | 3.5 | 3.2 | 5.3 | | 70-71 | 73A |
| 2-4 | 0.0 | 1.4 | 1.3 | 2.2 | | 29-30 | 25B |
| 4-8 | 0.0 | 0.0 | 0.1 | 0.1 | | 0- 1 | 2C |
| | | | Clay Loam (Soil 2) | | | | |
| 0-2 | 0.8 | 3.8 | 4.1 | 9.0 | 9.8 | 90-97 | 90A |
| 2-4 | 0.2 | 0.2 | 0.1 | 0.8 | 0.9 | 3- 8 | 8B |
| 4-8 | 0.1 | 0.0 | 0.0 | 0.2 | 0.0 | 0- 2 | 2B |

[1]Bioassay injury ratings (IR) shown, where 0 indicated no injury and 10 indicated that all plants were killed. Means within a soil with the same letter of the same case are not significantly different ($P < 0.05$).

[2]Includes pyrazon applied at 0, 2, and 3 pounds per acre with and without TCA, in addition to treatments shown.

additional movement was observed after the second sampling date and pyrazon residues at all depths decreased after that time. In contrast to soil 1, herbicide movement was not detected in soil 2 although more rainfall occurred after the initial sampling. There was essentially no residue in soil below 2 inches and residues below the surface layer did not differ during the growing season.

TABLE 7.--Pyrazon residues at three depths in three soils as related to time of sampling as accessed by bioassay injury ratings.[1]

| Time After Application | Soil Depth, inches | | | Mean | Rainfall Prior To Sampling |
|---|---|---|---|---|---|
| | 0-2 | 2-4 | 4-8 | | |
| months | IR | IR | IR | | in |
| | | Clay Loam (Soil 1) | | | |
| 2.4 | 3.0 | 0.6 | 0.0 | 1.2b | 5.5 |
| 3.4 | 2.8 | 1.2 | 0.1 | 1.4a | 1.2 |
| 4.6 | 1.9 | 0.7 | 0.0 | 0.9c | 7.2 |
| | | Clay Loam (Soil 2) | | | |
| 2.0 | 4.3 | 0.1 | 0.0 | 1.5c | 3.5 |
| 3.0 | 4.7 | 0.6 | 0.1 | 1.8a | 2.9 |
| 4.2 | 3.0 | 0.2 | 0.1 | 1.1c | 7.5 |
| | | Sandy Loam (Soil 3) | | | |
| 2.2 | 0.6 | 0.2 | 0.1 | 0.3a | 3.1 |
| 3.3 | 0.2 | 0.3 | 0.3 | 0.3a | 2.6 |
| 4.4 | 0.4 | 0.0 | 0.1 | 0.2a | 5.5 |

[1]Bioassay injury ratings (IR) shown, where 0 indicated no injury and 10 indicated that all plants were killed, based on the mean of all rates. Means (of all treatments), within a soil with the same letter are not significantly different ($P < 0.05$).

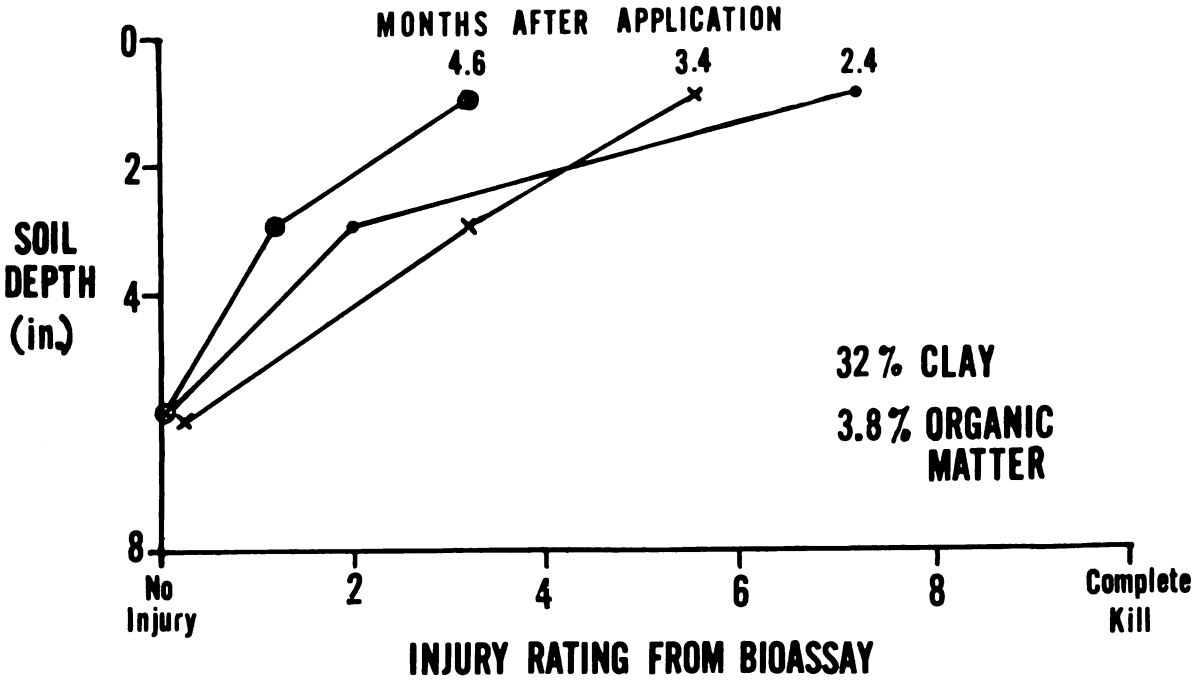
[2]Rainfall between time of application and first sampling date and between sampling dates.

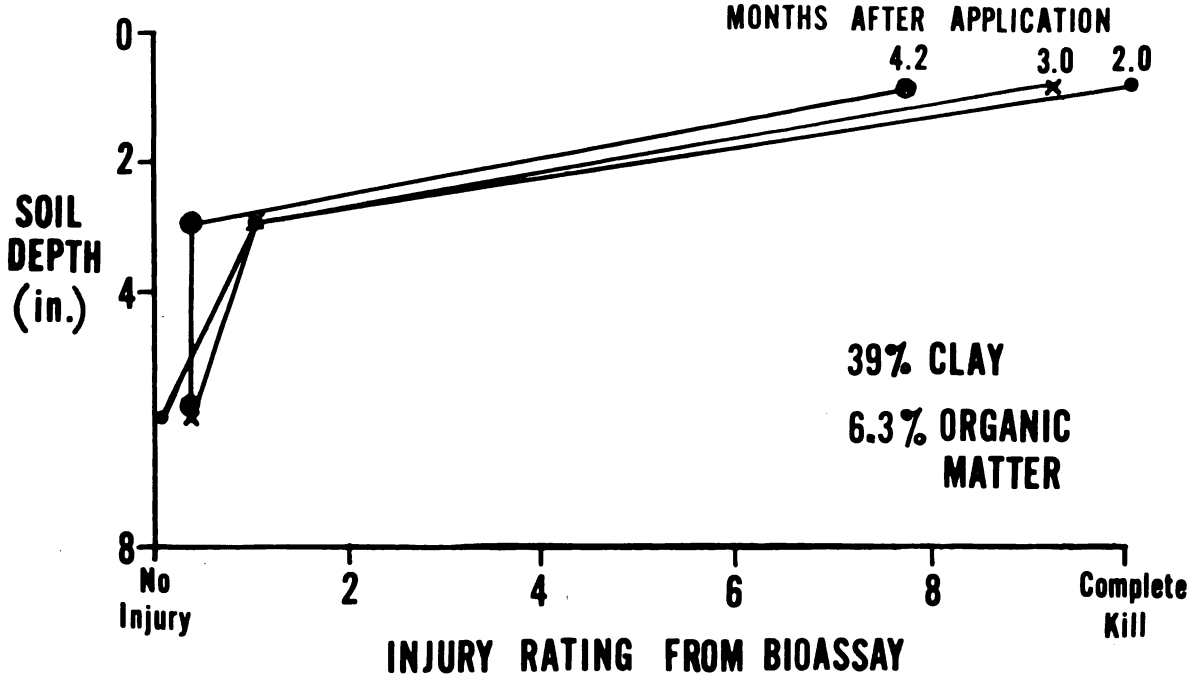
In the sandy loam extremely low amounts of pyrazon were observed at all depths. Although a time-depth interaction was statistically significant for this soil, these differences were not considered to be meaningful since there was no difference in bioassay responses between application rates. The range of residues observed was generally below the level of bioassay sensitivity. Less rainfall occurred at this location than at the two previous field sites.

The movement of pyrazon in the two clay loam soils during the sampling period was most dramatic when pyrazon was applied at 6 lbs/acre (Figure 1). As pyrazon decreased in the surface layer of soil 1 (3.8% organic matter) between 2.4 and 3.4 months after application, a comparable increase in residue was observed in the 2- to 4-inch layer. During the second sampling period disappearance of pyrazon in the lower layer of soil was comparable to that in the surface layer and additional net movement was not observed. After 4.6 months pyrazon residues at all depths were less than one-half of the maximum residue observed previously at that depth. There was no indication of herbicide accumulation at lower depths late in the season.

Pyrazon concentrations in the top 2 inches of soil 2 (6.8% organic) were higher than those observed in the previous soil and little herbicide was present in the adjacent layer of soil. Although more herbicide was

**FIGURE 1. Pyrazon movement in two clay loam soils with varying clay and organic matter contents, 6 pounds per acre applied.**



present in the surface layer initially than in soil 1, pyrazon was retained in the upper 2 inches. Although some movement may have occurred in this soil, no net movement occurred since there was no increase in residues below the surface during the sampling period.

## The Persistence and Disappearance of Pyrazon From the Upper 2 Inches of Soil

There were wide variations between soils in the initial pyrazon residues observed (2 to 2.5 months after application) and in the rate of herbicide disappearance. Differences in pyrazon concentrations were related to soil properties and rainfall intensity and distribution (Figure 2). Soils were classified into four groups on the basis of pyrazon residues and disappearance patterns observed after herbicide applications of 4 and 6 lbs/acre.

### 1. Little or No Pyrazon

Soil 3.--Measureable herbicide residues were low but persistent throughout the period of observation. Pyrazon residues did not approach 0 at 4 months following application but remained at the same level observed initially. This soil was characterized by a low clay content (less than 10%), with organic matter in excess of 10%. Rainfall between the time of application and the last sampling date totaled 7.8 inches.

FIGURE 2. Pyrazon persistence in the upper two inches of seven soils with varying clay and organic matter contents, 0, 4, and 6 pounds per acre applied.

FIG. 2A- 39% CLAY; 6.8% ORGANIC MATTER

SOIL 2

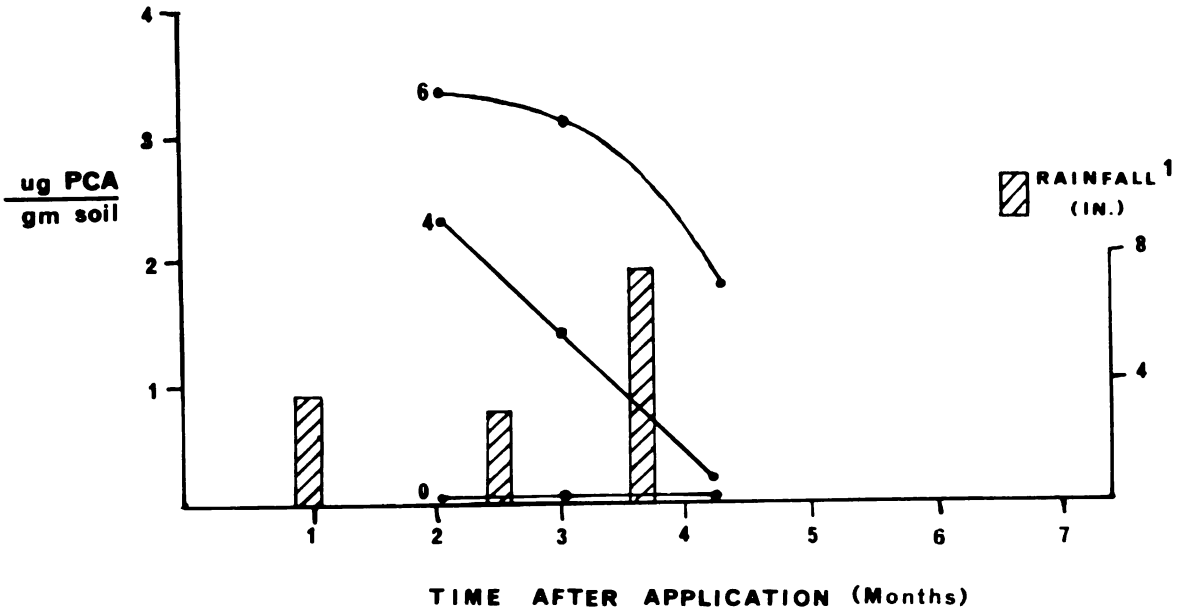
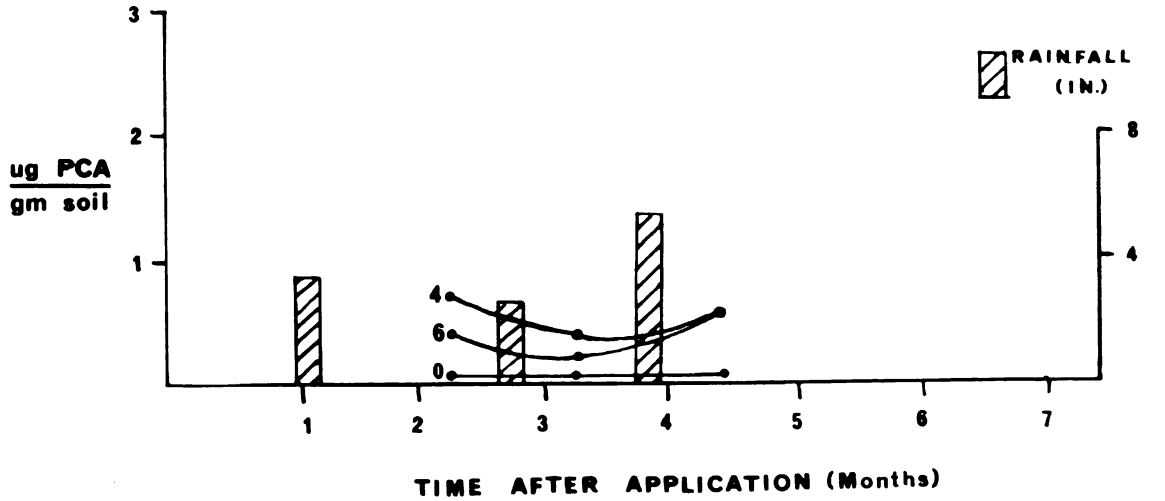



FIG. 2B - 9% CLAY; 13.7% ORGANIC MATTER

SOIL 3




1. AMOUNT of rainfall between application date and first sampling and between samplings shown as: ▨

FIG. 2c - 24% CLAY; 4.2% ORGANIC MATTER
SOIL 4

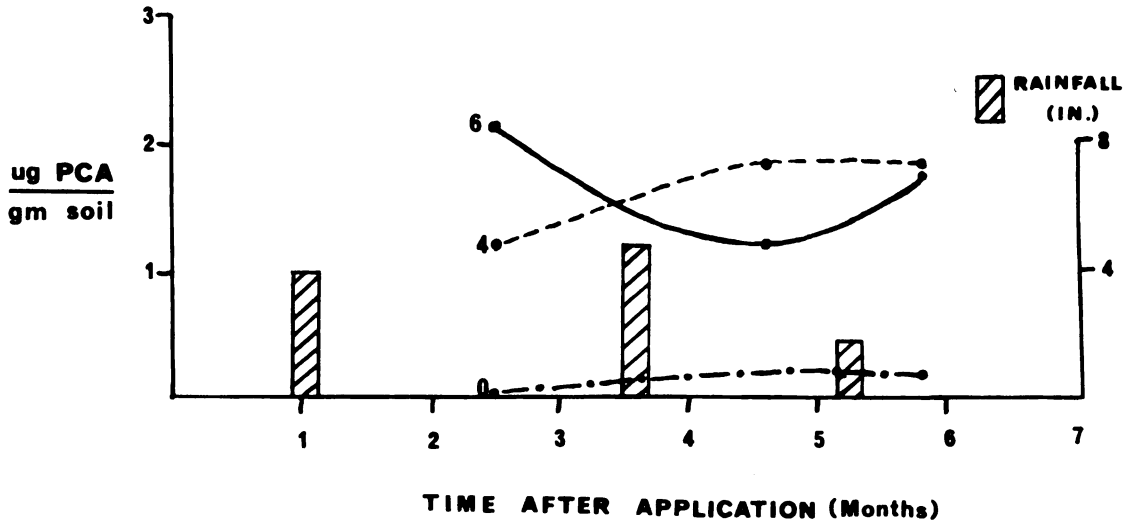



FIG. 2D - 28% CLAY; 4.3% ORGANIC MATTER
SOIL 5

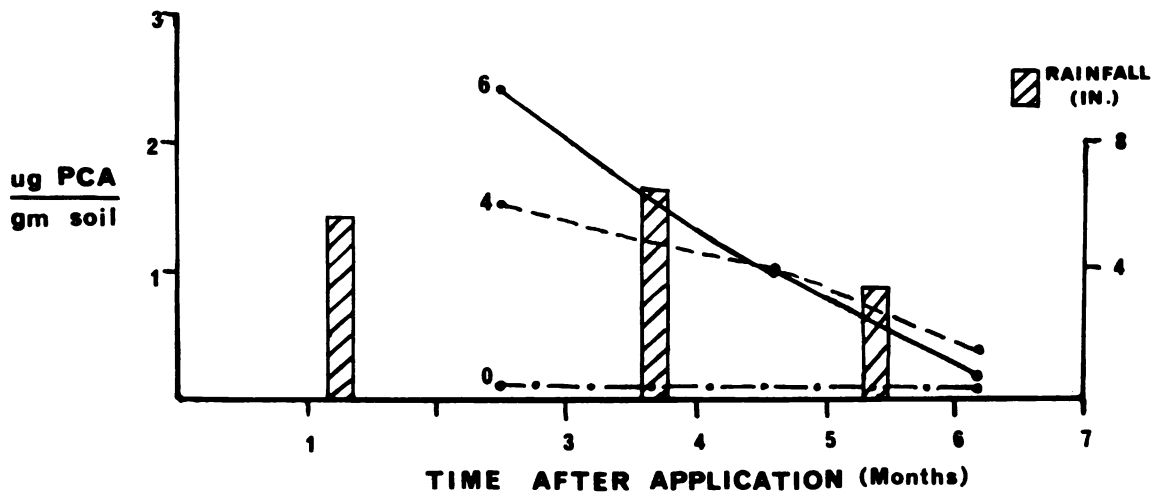


FIG. 2E - 28% CLAY; 4.2% ORGANIC MATTER

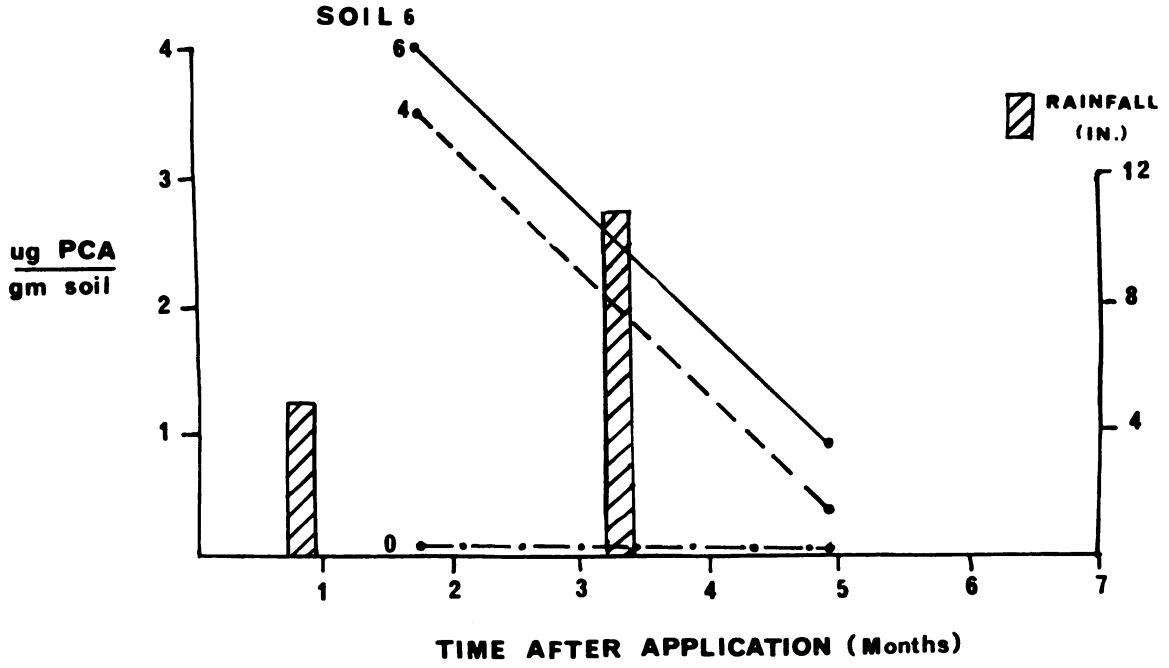



FIG. 2F - 26% CLAY; 12.9% ORGANIC MATTER

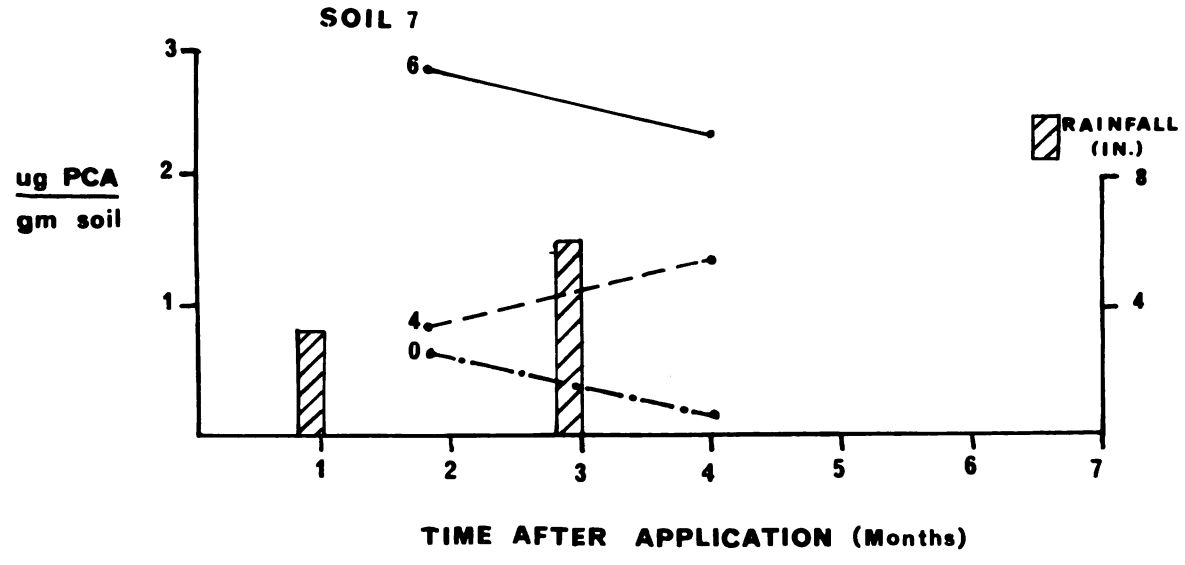
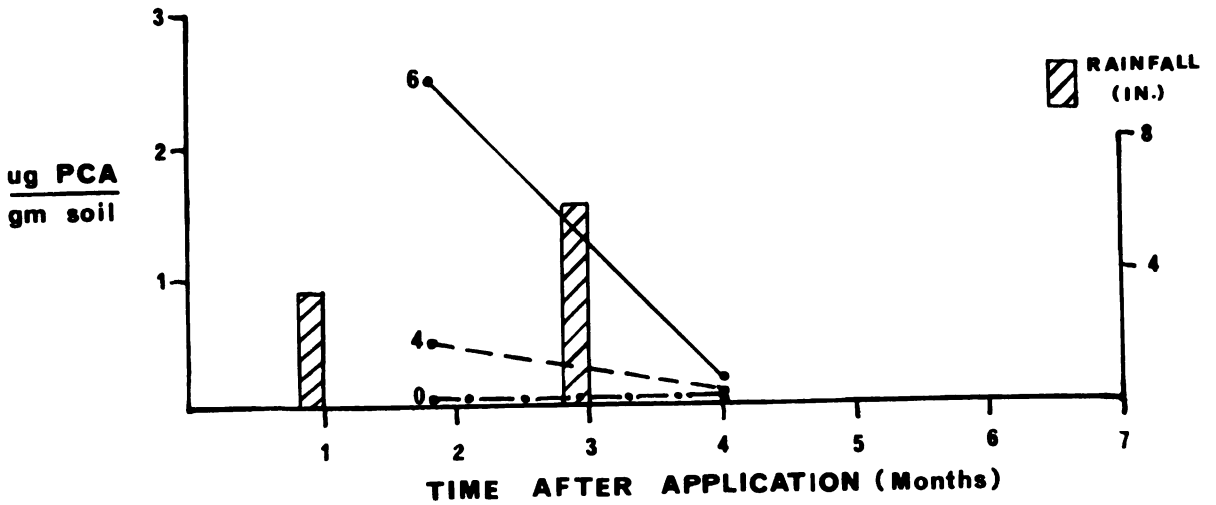


FIG. 2G - 10% CLAY; 2.9% ORGANIC MATTER
SOIL 8



2. Little or No Loss of Pyrazon

Soil 4.--Pyrazon concentrations remained between 1.2 and 2.2 µg/g of soil throughout the sampling period and did not decrease. Rainfall prior to sampling was comparable to that at other locations but was lower during the rest of the season. This soil contained intermediate amounts of clay and organic matter compared to other soils investigated.

Soil 7.--There was no change in residues in this soil between 1.8 and 4 months after application. Pyrazon decreased slightly following an application of 6 lbs/acre but residues increased by a comparable amount where pyrazon was applied at a 4-pound rate. Soil organic matter was in excess of 10% and rainfall was well distributed throughout the season.

3. Intermediate Loss of Pyrazon

Soil 1.--When applied at 6 lbs/acre, pyrazon disappeared linearly with time but at 4.5 months after application the residue was approximately one-half of that observed initially. Pyrazon did not decrease during the period of observation when applied at 4 lbs/acre (Table VII). Clay and organic matter contents were 24 and 3.8%, respectively. Rainfall was low (1.2 inches) between 2.4 and 3.4 months after application.

Soil 2.--Pyrazon disappeared rapidly when applied at 4 lbs/acre, however, disappearance was slow following a 6-pound application during the first sampling period compared to that observed during the last period or at other locations. Disappearance was more rapid during the last sampling period with increased rainfall. In this soil herbicide disappearance appeared to be associated with rainfall intensity and distribution. This soil contained 6.8% organic matter and the highest amount of clay of any soil in the study.

4. Consistent Loss of Pyrazon

Soil 5.--Pyrazon disappeared linearly with time and after 6 months following application residues were negligible. This soil contained intermediate amounts of clay and organic matter and rainfall was well distributed throughout the growing season.

Soil 6.--Two months after application pyrazon exceeded 3 µg/g of soil but disappeared rapidly during the next 3 months. High rainfall was well distributed throughout the season. Clay and organic matter contents were intermediate compared to other soils.

Soil 8.--Initial pyrazon concentrations were 0.5 and 2.5 µg/g following applications of 4 and 6 lbs/acre, respectively. Disappearance was rapid following the 6-pound application and residues from both application

rates were negligible 4 months after application. Rainfall was well distributed throughout the season. Soil was exceedingly low in clay (10%) and contained less than 3% organic matter.

## Adsorption of Pyrazon in Soil

Adsorption ranged from 1.7 to 103.2 µg/g of soil and was related to soil properties. Pyrazon adsorption was correlated with organic matter and cation exchange capacity to the same extent; however, cation exchange capacity appeared to be dependent on soil organic matter (Table 8). Adsorption was not related to clay content

TABLE 8.--Simple correlation coefficients (r) between pyrazon adsorption and soil properties.

| | Soil Properties | | | |
|---|---|---|---|---|
| | pH | Clay Content | C.E.C. | Organic Matter |
| Adsorption from | | | | |
| 50 µg/g soil | -0.08 | 0.09 | 0.87* | 0.88* |
| 500 µg/g soil | -0.01 | 0.02 | 0.92* | 0.97* |
| Exchange Capacity (C.E.C.) | 0.22 | 0.15 | 1.00 | 0.87* |

*Indicates significant correlation coefficients ($P < 0.01$).

between 11 and 37%. Organic matter content was the only significant independent variable in a multiple regression analysis.

There was close agreement between the relative increase in soil organic matter and pyrazon adsorption when organic matter was 3.2% or higher (Table 9). Adsorption was greater when more pyrazon was added in the equilibrating solution in soils that contained 3.5% or more organic matter. The interaction for adsorption, between organic matter and the concentration of pyrazon added to soil was significant since proportionally more adsorption occurred from the higher concentration as organic matter increased.

Since pyrazon was equilibrated with soil in a solution containing $Ca^{++}$, differences in pH between soils may have been negated if soils were saturated with calcium. However, in two soils with the same organic matter content (4.94 and 5.08%), adsorption was lower in the soil with the lower pH and higher clay content (pH 6.3 and 37% clay).

Radioactivity in an equilibrated water extract cochromatogrammed with a pyrazon standard which indicated that activity in the equilibrated solutions was from $^{3}$H-pyrazon.

TABLE 9.--Relationship between soil organic matter and herbicide adsorption from two concentrations of pyrazon.[1]

| Organic Matter % | Pyrazon Adsorbed (µg/g) | |
|---|---|---|
| | 50 µg/g Added | 500 µg/g Added |
| 2.54G | 3.94 | 4.17 |
| 3.06F | 3.85 | 1.74 |
| 3.21EF | 7.84 | 11.37 |
| 3.50E | 8.50 | 13.72 |
| 3.91D | 9.66 | 27.67 |
| 4.94C | 14.08 | 40.34 |
| 5.08C | 11.44 | 37.72 |
| 8.44B | 19.20 | 90.90 |
| 11.38A | 18.98 | 103.21 |
| Mean | 10.80a | 36.80b |

[1]Means followed by the same letter of the same case are not significantly different ($P < 0.05$).

## Response of Soil Microorganisms to Pyrazon

In three soils the response of bacteria to pyrazon was maximal after approximately 5 weeks of incubation at 21 C, when compared to untreated controls (Figure 3). The highest plate counts in treated soils also occurred at that time except in the sandy clay loam. In this soil bacteria counts in treated soil were highest after 10 weeks of incubation but when compared to the population

**FIGURE 3.** Changes in bacteria populations in three soils amended with pyrazon and incubated at 21 C.

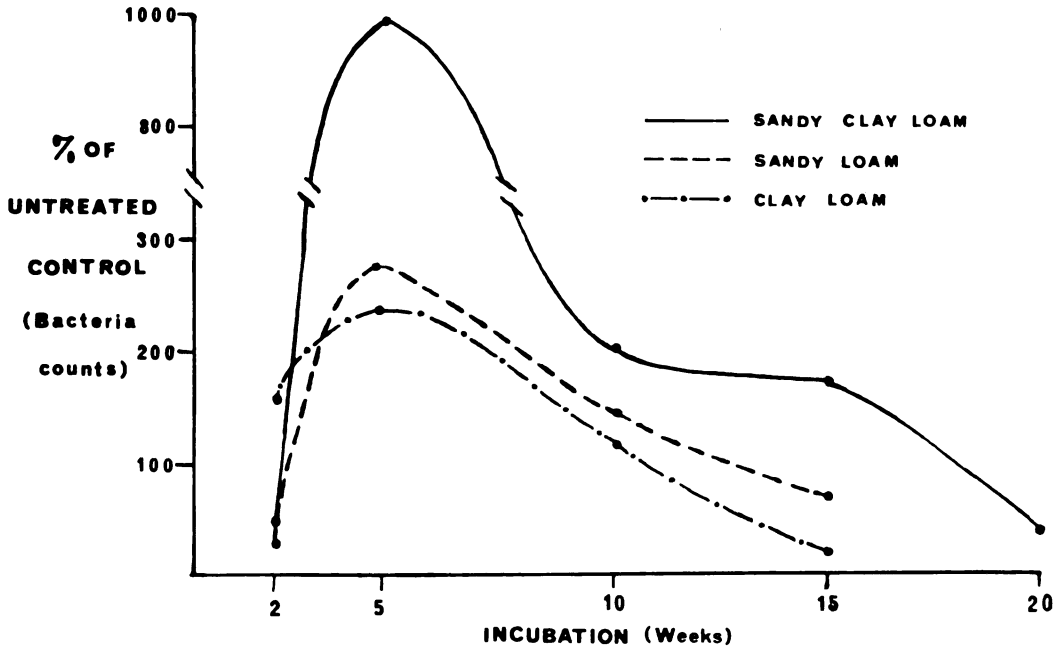
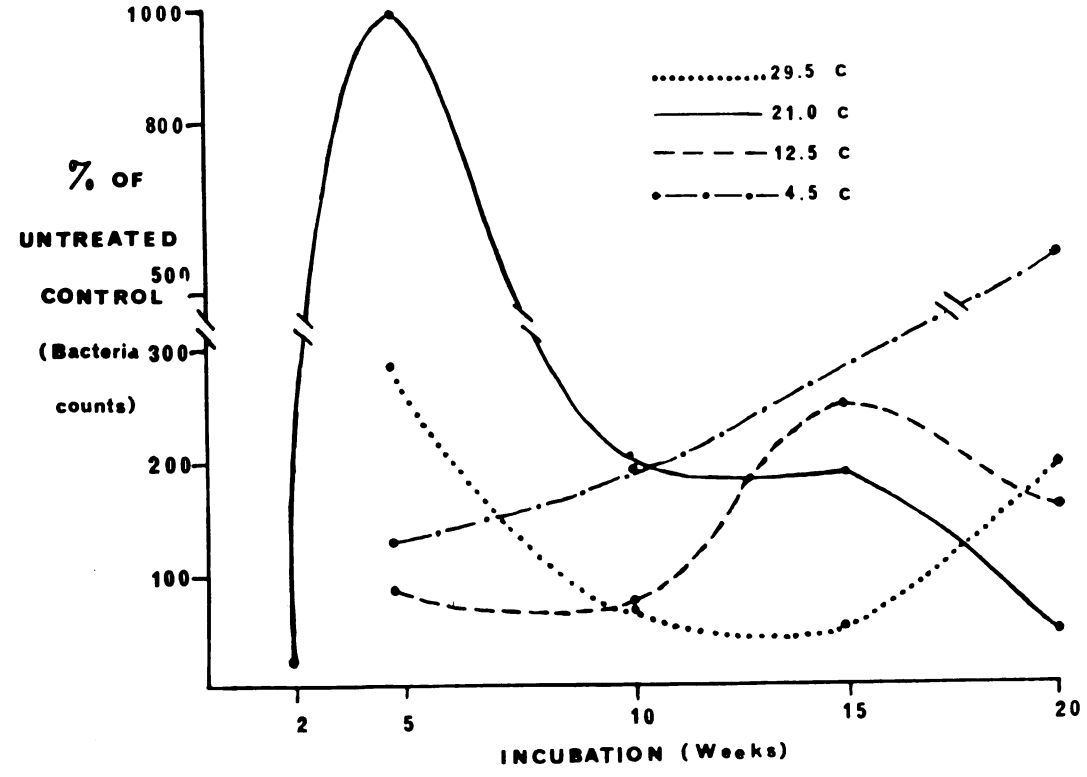



**FIGURE 4.** Changes in bacteria populations in a sandy clay loam amended with pyrazon and incubated at four temperatures.



in the control the greatest enhancement from pyrazon occurred prior to that time. In the amended clay loam soil bacterial counts were significantly higher in treated soil after 5 weeks of incubation than at other dates or in untreated soil. In the sandy loam the response to pyrazon after 5 weeks of incubation was considered as being meaningful although plate counts were not statistically different. Bacteria populations in amended soils ranged from 10 to 384 X $10^6$/g while counts in untreated controls ranged from 10 to 163 x $10^6$/g. In both soil treatments bacteria developed as convex circular colonies predominantly on the surface of the agar. Colonies were generally 2 to 6 mm in diameter and were whitish, cream, or light yellow. These bacteria were considered to be aerobic, based on colony growth characteristics and location. Only a limited number of colonies were embedded in the agar below the surface. Bacteria populations in each soil were not considered to be a result of minor pH fluctuations.

Bacteria in amended sandy loam also responded to different incubation temperatures. With higher incubation temperatures less time was required for the organisms to respond to pyrazon (Figure 4). Bacteria in amended soil at 29.5 C decreased from 280 to 65% of the untreated control between 5 and 10 weeks of incubation and continued to decrease after that. This trend was assumed to be a

decline of a maximum population achieved sometime prior to the first sampling at 5 weeks. Soil incubated at this temperature dried out after 15 weeks and the increased population observed at 20 weeks was believed to be a cyclic response from an addition of water at week 18. When amended soil was incubated at 21 C a maximum response occurred at 5 weeks, as previously explained, and occurred after the peak that probably occurred in soil incubated at 29.5 C. In amended soil incubated at 12.5 C 15 weeks were required for organisms to show a response to pyrazon in the soil media.

There was a general decline in the number of bacteria between 5 and 10 weeks of incubation at 4.5 C. In the following 10 week period at this temperature the population in the untreated control remained the same; however, there was a two and one-half fold increase in bacteria in the treated soil. This response in the amended soil indicated that the species surviving and functioning at the lowest incubation temperature eventually responded to pyrazon in the soil media.

In soils incubated at 29.5 and 12.5 C bacteria colonies were similar to those described previously in soils incubated at 21 C and were considered to be aerobic. Colonies from soil incubated at 4.5 C were distinctly different from those in soil at higher temperatures. Colonies developed both in and on the agar and were

minute, usually one mm in diameter, white or yellow in color, with occasional colonies displaying a pink pigmentation. The difference in the actual number, response, and type of colonies observed at this temperature compared to those at higher temperatures was undoubtly due to the difference in species that survived and responded in each environment.

Actinomycete populations ranged from 0 to 70 X $10^5$/g; however, most counts were between 3 and 15/g at a $10^{-5}$ dilution. Colonies were crusty, formed only on the surface and usually developed a tan or brownish pigment with limited mycelium. Actinomycete development and populations were not related to any particular soil treatment but populations were higher in soil incubated at 29.5 C than at other temperatures. There was no evidence that actinomycetes responded to or were inhibited by the addition of pyrazon to soils.

## Disappearance of Pyrazon in Soil

Total radioactivity recovered from soils decreased between 3 and 74 days of soil incubation. Total activity decreased more consistently in soils containing $^{14}$C-pyrazon than in soils amended with $^{3}$H-pyrazon. Approximately 50% of the pyrazon added initially was recovered. On thin layer chromatograms, 89 and 97% of the activity from $^{3}$H-pyrazon and $^{14}$C-pyrazon standards, respectively, was located at $R_f$ 0.73 to 0.78 in solvent system I

(Table 10). Activity from soil extracts at this location was assumed to be pyrazon on the basis of these standard controls. Differences in recovered identifiable pyrazon ranged from 5.7 to 11.0% between duplicate soil extracts.

TABLE 10.--Recovery of radioactivity from soils amended with $^{3}H$ and $^{14}C$ labelled pyrazon.[1]

| Time of Incubation | $^{3}H$-Phenyl Labelled Pyrazon | | $^{14}C$-Pyridazine Labelled Compounds | | | |
|---|---|---|---|---|---|---|
| | | | $R_f$ 0.47 | | Pyrazon | |
| days | % | mμ moles/g | % | mμ moles/g | % | mμ moles/g |
| 3 | 88.5 | 10.45 | 0.3 | 0.04 | 95.6 | 11.65 |
| 10 | 76.9 | 8.28 | 0.9 | 0.11 | 96.4 | 11.65 |
| 15 | 91.2 | 10.25 | 1.7 | 0.19 | 95.9 | 10.90 |
| 30 | 96.1 | 10.85 | 2.9 | 0.32 | 95.1 | 10.45 |
| 30 | 91.4 | 10.25 | 2.9 | 0.30 | 94.0 | 9.85 |
| 74 | 92.8 | 9.48 | 8.7 | 0.99 | 88.6 | 10.15 |
| 74 | | | 8.2 | 0.84 | 89.0 | 9.10 |
| Standard | 89.6 | | 0 | | 97.2 | |

[1]Based on the distribution of radioactivity on thin layer chromatograms.

The percentage of activity as $^{3}H$-pyrazon in soil extracts varied irregularly and was particularly low after 10 days of incubation. Other than pyrazon, there was no indication that any other tritium labelled compound was present in the soil. Radioactivity as $^{14}C$-pyrazon decreased consistently with longer periods of soil

incubation and was accompanied by an increase in radioactivity in a compound at $R_f$ 0.47.

During the 71 day incubation period $^{3}H$-pyrazon decreased from 10.45 to 9.48 mμ moles/g, a difference of 0.97. During the same period $^{14}C$-pyrazon decreased from 11.6 to 9.6 mμ moles/g, a difference of 2.08. Part of this decrease was accounted for by the formation of a $^{14}C$ labelled compound which increased from 0.04 to 0.94 mμ moles/g. The concentration of this new compound after 74 days of incubation amounted to less than 10% of the pyrazon extracted while pyrazon decreased 18% during the same period.

Initial evidence for the formation and presence of a new compound from $^{14}C$-pyrazon was obtained from the distribution of radioactivity on chromatograms. In solvent system I this compound chromatogrammed at $R_f$'s of 0.4 and 0.5, with a distinct decrease in activity at $R_f$ 0.6 prior to the pyrazon peak at $R_f$ 0.7 and 0.8. The distribution of radioactivity on chromatograms was distinct in extracts after 30 and 74 days of soil incubation (Table 11). The low activity between two peaks indicated that the unidentified compound was, in fact, different from pyrazon and was not the result of trailing activity from pyrazon.

TABLE 11.--Distribution of radioactivity on chromatograms of extracts from soil incubated for 30 to 74 days with $^{14}$C-pyrazon.[1]

| Position on Chromatogram | Days of Incubation | |
|---|---|---|
| | 30 | 74 |
| $R_f$ | % | % |
| 0.1 | 1.5 | 1.4 |
| 0.2 | 0.0 | 0.1 |
| 0.3 | 0.1 | 0.2 |
| 0.4 | 0.8 | 2.0 |
| 0.5 | 2.0 | 6.4 |
| 0.6 | 0.7 | 0.8 |
| 0.7 | 64.9 | 74.1 |
| 0.8 | 29.6 | 14.7 |
| 0.9 | 0.0 | 0.0 |
| 1.0 | 0.0 | 0.0 |

[1]Percentage of total activity on chromatograms from duplicate samples.

Two distinct colored bands were observed when a standard containing $^{14}$C-pyrazon and non-labelled 5-amino-4-chloro-3-pyridazinone was co-chromatogrammed in solvent system II. Nearly 90% of the total activity corresponded to an intense dark maroon band occurring at $R_f$ 0.65. A less intensely colored region was observed at $R_f$ 0.43 but contained no activity and was considered to be the dephenylated pyrazon (metabolite A). Both bands tended

to trail due to the high concentration applied. On the chromatogram of $^{14}$C-pyrazon (incubated in soil 74 days), 81% of the total activity occurred in an extremely faint pink band at $R_f$ 0.62 while 7.3% of the activity occurred at $R_f$ 0.42. Although no color was observed in the second band the positions of activity on this chromatogram corresponded to the activity and the color observed in the standard. Pyrazon was the only source of radioactivity on the chromatogram of the $^{3}$H-pyrazon extract but was not colored since less material was spotted initially.

On the chromatogram containing 500 µl of the concentrated $^{14}$C-pyrazon extract three irregularly shaped bands were observed (Figure 5). Band 1 appeared as the same yellow-greenish color observed in the concentrated extract at $R_f$ 0.78 but contained no radioactivity. Band 2 was immediately behind and along the sides of band 1 and appeared as an intense dark maroon color at approximately $R_f$ 0.73. Some trailing of the maroon color was observed. This maroon band contained 84% of the total activity and was considered to be pyrazon based on the $R_f$ and color observed. Between bands 2 and 3 there was a distinct break and change of color. Band 3 was light pink, well defined, and occurred at $R_f$ 0.45, the same position where activity had been observed on other chromatograms of $^{14}$C-pyrazon extracts. Chromatogram sections 4 and 5, corresponding

**FIGURE 5.** Correspondence of colored bands and $^{14}C$ radioactivity on a thin layer chromatogram after incubation of $^{14}C$-pyrazon in a sandy loam soil for 74 days.

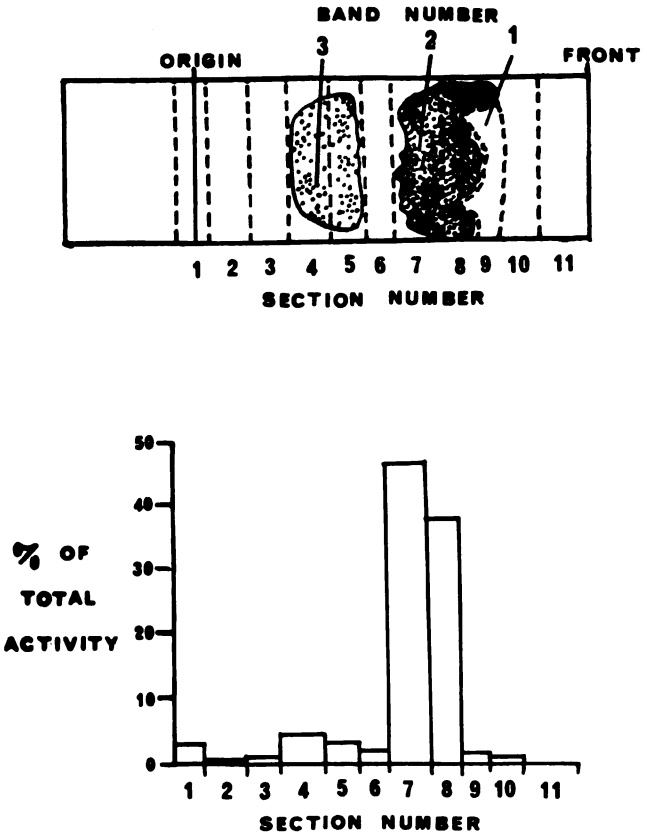
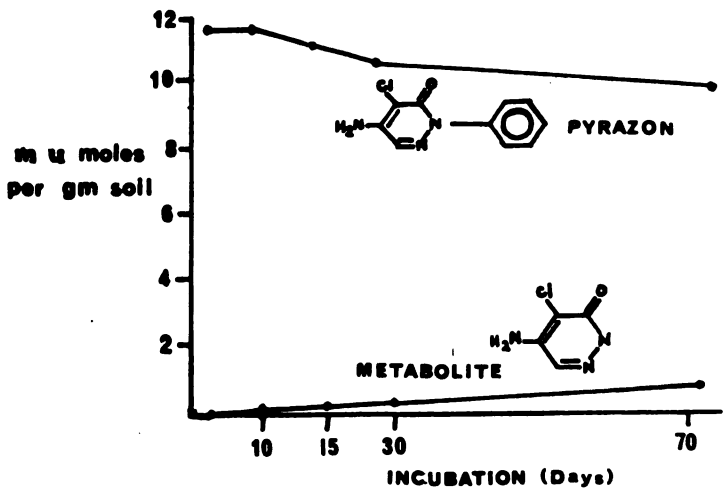



**FIGURE 6.** Recovery of pyrazon and a metabolite from a sandy loam soil amended with $^{14}C$ labelled pyrazon.



to band 3, contained 3.7 and 3.8% of the total activity, respectively, or a total of 7.5% of the activity; the same percentage of activity observed in this region on previous chromatograms. Radioactivity between bands 2 and 3 amounted to 1.98% of the total. Total activity on the chromatogram exceeded $6.5 \times 10^4$ dpm, which may have resulted in the slight activity observed between the two peaks on the chromatogram.

When the concentrated $^{14}$C-pyrazon extract and a standard were co-chromatographed in solvent system III, pyrazon in the standard migrated in a sharp band to $R_f$ 0.63. Ninety per cent of the activity from the extract occurred in the same position; however, the remaining activity, observed previously as a separate peak in other solvent systems, tended to trail from the pyrazon peak and separation was not achieved.

Although the presence of the primary amine on the pyridazine ring was confirmed in the product formed from pyrazon and the product corresponded to 5-amino-4-chloro-3-pyridazinone on chromatograms; the other substituents were not positively identified (Figure 6). However, breakdown product probably contained the chloro and carbonyl groups since the product co-chromatogrammed identically with a standard that contained these substituents, in two solvent systems.

# SUMMARY AND CONCLUSIONS

Pyrazon residues were evaluated by both fresh weight and plant injury; however, greater reliance was placed on injury ratings. Low concentrations of the herbicide were evaluated more accurately in all soils by the injury rating system since fresh weight of the bioassay was enhanced 10 to 70% in some soils by small amounts of pyrazon.

There was little or no TCA present in soil at the time samples were obtained. TCA is not persistent in soil at the rates applied in this study and leaches readily (41). However, if any TCA was present in samples the bioassay species would have responded only to pyrazon. Differences in the movement or persistence of pyrazone due to TCA could have been detected by the method of evaluation. There was no evidence that pyrazon movement or residues were affected by TCA.

In the pyrazon adsorption study herbicide retention was associated with the organic matter content of soil and was not related to clay content. The influence of organic matter in pyrazon movement and persistence was evident in the field studies. The importance of organic matter in the adsorption of pyrazon has been noted previously (20).

In observations on the distribution and movement of pyrazon in two clay loam soils, the herbicide was retained in the upper 2 inches of the soil with highest organic matter and clay content. Less pyrazon was retained in the upper 2 inches of the soil with less organic matter and clay, and significant movement occurred during the season. It was concluded that the difference in pyrazon movement between soils was due to differences in soil organic matter. The small percentage change in clay content between the two soils would not account for the vast differences in the movement observed.

Since the herbicide was retained near the soil surface, primarily in the upper 2 inches of the profile, degradation of pyrazon would occur in the plow-depth layer of soil. Under most field conditions pyrazon is available for biological use and degradation in the soil and is not lost as a result of leaching or movement in soil. However, only a portion of the total amount of pyrazon applied is available for biological systems, due to adsorption by organic matter.

Although there was no direct evidence that bacteria were involved in the degradation of pyrazon, it is highly probable that soil bacteria are responsible for the degradation of pyrazon. In three soils these organisms responded to pyrazon, indicating that bacteria were capable of utilizing the herbicide. Significant losses

of labelled pyrazon did not occur until after 10 days of incubation but, corresponded to the increased proliferation of bacteria observed previously.

Different bacteria species at four soil temperatures also responded to the herbicide in the soil media. No doubt pyrazon is exposed to a similar variety of organisms in the natural environment as a result of increasing soil temperature after early spring applications of the herbicide. There was no indication that actinomycetes responded to or utilized pyrazon. Fungi would not be highly functional at the pH levels usually encountered in sugar beet soils due to competition from soil bacteria.

It was concluded that the high initial loss of labelled pyrazon in incubated soils, between the time of application and the first sampling, was due to adsorption. After the initial loss of pyrazon due to adsorption the herbicide disappeared slowly in incubated soil. One would expect to observe a slow rate of disappearance considering the length of persistence and weed control observed in the field and the general reluctance of soil organisms to utilize chlorinated compounds.

Persistence and disappearance of pyrazon in field soils may be attributed to factors that enhance microbial activity and contribute to herbicide adsorption. In all instances, the rate of disappearance was highest in soils that contained 2.9 to 4.3% organic matter where

rainfall was well distributed throughout the season. Pyrazon concentrations remained high or did not disappear as rapidly in soils that received low rainfall or contained high amounts of organic matter.

Uniform distribution of rainfall is essential in maintaining favorable soil moisture for microbial activity while water movement in the soil would tend to remove soluble toxic by-products. The complete loss of phytotoxicity would be prolonged in soils with high organic matter contents since the herbicide would be replenished in the soil constantly by desorption. Microbial degradation of organic matter may also release the herbicide.

Increased clay content may affect herbicide persistence by maintaining adequate soil moisture for microbial development; however, in this study it appeared that clay may have aided in maintaining phytotoxicity. In comparing residues in two soils that contained organic matter in excess of 10%, phytotoxicity did not change during the growing season; however, there was a marked difference in the level of phytotoxicity. In the soil with a low but persistent level of phytotoxicity the clay content was less than 10%. In contrast, the second soil contained two and one-half times more clay, and phytotoxicity was higher. The persistence of phytotoxicity was related to soil organic matter content. However, the level or intensity

of phytotoxicity in the soil appeared to be associated with the clay content. Although there is no clear explanation for this phenomena, clay may tend to stabilize the herbicide in the soil after desorption from organic matter.

A breakdown product of pyrazon was observed in incubated soil. The product was first observed after 10 days of incubation and continued to increase. Loss of a radioactive labelled phenyl ring and retention of the labelled pyridazine ring indicated that the compound lacked the phenyl substituent of pyrazon. The primary amine on the pyridazine ring was confirmed and since the breakdown products co-chromatogrammed with a known metabolite of pyrazon, it was concluded that the product was 5-amino-4-chloro-3-pyridazinone. Since the single breakdown product did not completely account for the total loss of radioactivity from pyrazon, it was assumed that the identified product undergoes additional degradation in the soil.

The slow loss of pyrazon after 30 days of incubation may be due to an accumulation of the breakdown product. The breakdown product(s) may be toxic to some soil microorganisms but may be removed from the biological environment in the field by rainfall.

In summary, the movement and persistence of pyrazon, herbicide was investigated in field environments. In

laboratory studies adsorption of pyrazon was correlated with soil properties and the degradation of pyrazon in soil and response of soil microorganisms to pyrazon was determined in long-term incubation studies. Many of the changes in pyrazon concentrations in field soils could be explained after consideration of soil properties and rainfall in relation to laboratory findings. Significant findings of this study were:

1. Adsorption of pyrazon is correlated with soil organic matter ($r = 0.9$) and is not dependent on clay content.
2. Pyrazon is immobile in soils and is retained primarily in the upper 2 inches of soil. Movement is dependent on the organic matter content of soils.
3. Disappearance of pyrazon in the field is enhanced by evenly distributed rainfall following herbicide application. The rate of disappearance is dependent on rainfall intensity and distribution. Pyrazon is persistent in soils with organic matter contents in excess of 10%.
4. Soil bacteria functioning between 4.5 and 29.5 C are capable of utilizing pyrazon. Five to 10 weeks are required for maximum proliferation of bacteria in soils at 21.0 C. Antinomycete populations are not affected by pyrazon.

5. The loss of pyrazon in soil probably occurs by bacterial degradation. Degradation of pyrazon in soil is initiated by the loss of the phenyl substituent. The breakdown product was confirmed as 5-amino-4-chloro-3-pyridazinone. After 10 weeks of incubation the amount of the breakdown product in the soil is low (less than 10%) compared to the amount of pyrazon present. The initial breakdown product is subject to additional degradation.
6. Plant growth may be stimulated or enhanced by low concentrations of pyrazon. Fresh weight of mustard increased 10 to 70% by the addition of 0.2 to 1.0 µg/g of soil.

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# APPENDIX

TABLE I.--Summary of significance in analyses of variance and interactions in pyrazon bioassays in 1965 field samples.[1]

| Source | Soil 1 | | Soil 2 | | Soil 3 | |
|---|---|---|---|---|---|---|
| | Injury | Weight | Injury | Weight | Injury | Weight |
| Rate of application | .00 | .25 | .00 | .00 | .04 | .99 |
| Time of sampling | .00 | .00 | .00 | .00 | .10 | .02 |
| R X T | .10 | .19 | .06 | .31 | .67 | .80 |
| Depth of sampling | .00 | .00 | .00 | .00 | .00 | .19 |
| R X D | .00 | .06 | .00 | .00 | .17 | .96 |
| T X D | .00 | .00 | .00 | .01 | .02 | .52 |
| H X T X D | .09 | .53 | .05 | .09 | .77 | .83 |

[1]Determined by visual injury ratings and fresh weight of mustard.

TABLE II.--Summary of significance in analyses of variance and interactions in pyrazon bioassays in 1966 field samples.[1]

| Source | Soil 4 | | Soil 5 | | Soil 6 | | Soil 7 | | Soil 8 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Injury | Weight | Injury | Weight | Injury | Weight | Injury | Weight | Injury | Weight |
| Rate | .00 | .00 | .02 | .00 | .00 | .00 | .19 | .23 | .01 | .00 |
| Time | .87 | .92 | .00 | .07 | .00 | .00 | .75 | .11 | .01 | .67 |
| R X T | .62 | .80 | .01 | .01 | .06 | .10 | .03 | .18 | .57 | .16 |

[1]Determined by visual injury ratings and fresh weight of mustard.

TABLE III.--Bioassay injury ratings in seven bioassay standards.[1]

| Pyrazon Applied | 1965 Soils | | 1966 Soils | | | | |
|---|---|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| µg/g | | | | | | | |
| 0.0 | 0.3a | 0.2a | 0.3e | 0.0cd | 0.0e | 1.0c | 1.0c |
| 0.25[2] | 1.0a | | | 1.3cd | 0.3e | 1.0c | 3.3c |
| 0.5[2] | 1.0a | 0.5a | 1.0de | 1.6cd | 1.0de | 1.0c | 9.3ab |
| 1.0[2] | 1.3a | 1.2a | 2.6d | 3.3c | 1.0de | 1.0c | 5.6bc |
| 1.5[2] | 3.3b | 1.5ab | 5.3c | 7.0b | 1.6de | 2.6c | 7.6ab |
| 2.0 | 6.0c | 2.5abc | 9.3a | 8.3ab | 2.3cde | 3.3c | 9.0ab |
| 2.5 | 6.3c | 3.7bcd | 7.0b | 9.3ab | 4.3bcd | 3.0c | 9.3ab |
| 3.0 | 9.0d | 4.5cd | 9.0a | 9.6a | 5.6bc | 7.0ab | 10.0a |
| 3.5 | 9.0d | 4.5cd | 9.3a | 7.6ab | 6.0b | 6.0b | 10.0a |
| 4.0 | 9.6d | 4.2cd | 9.6a | 9.6a | 7.3ab | 7.3ab | 10.0a |
| 4.5 | | 5.5d | 9.6a | 9.3ab | 9.6a | 9.3a | 10.0a |

[1]Injury ratings (IR), where 0 indicated no injury and 10 indicated that all plants were killed. Means in the same column followed by the same letter are not significantly different ($P < 0.05$).

[2]In soils 2 and 3 pyrazon applied at 0.25, 0.4, 0.8, and 1.2 µg/g, respectively.

TABLE IV.--Pyrazon residues in three soils during 1965 as related to rate of application, as accessed by bioassay injury ratings.[1]

| Herbicide Treatment[2] | | Soil 1 | Soil 2 | Soil 3 |
|---|---|---|---|---|
| PCA | TCA | Injury | Injury | Injury |
| lbs/A | lbs/A | IR | IR | IR |
| 0 | 0 | 0.11c | 0.41c | 0.08c |
| 0 | 8 | 0.06c | 0.07d | 0.14bc |
| 2 | 8 | 1.14b | 0.59c | 0.42a |
| 3 | 0 | 1.38b | 0.89bc | 0.25ab |
| 3 | 8 | 1.11b | 0.85bc | 0.36ab |
| 4 | 0 | 1.64b | 1.33b | 0.47a |
| 4 | 8 | 1.50b | 1.40b | 0.28ab |
| 6 | 0 | | 2.56a | |
| 6 | 8 | 2.53a | 3.33a | 0.39a |
| 10 | 0 | | 3.56a | |

[1]Injury ratings (IR), where 0 indicated no injury and 10 indicated that all plants were killed. Means in the same column followed by the same letter are not significantly different ($P < 0.05$).

TABLE V.--Fresh weight of mustard with several rates of pyrazon added to three soils in 1966.[1]

| Pyrazon Applied | Soil 4 | Soil 5 | Soil 8 |
|---|---|---|---|
| µg/g | g/cup | g/cup | g/cup |
| 0.0 | 1.60a | 2.02a | 0.53a |
| 0.25 | | 1.40b | 0.81a |
| 0.5 | 1.18b | 1.41b | 0.09a |
| 1.0 | 0.77c | 0.63c | 0.37a |
| 1.5 | 0.33d | 0.60c | 0.24a |
| 2.0 | 0.09d | 0.14d | 0.13a |
| 2.5 | 0.16d | 0.27d | 0.10a |
| 3.0 | 0.05d | 0.09d | 0.01a |
| 3.5 | 0.06d | 0.04d | 0.00a |
| 4.0 | 0.02d | 0.09d | 0.00a |
| 4.5 | 0.05d | 0.00d | 0.00a |

[1]Means with the same letter in the same column are not significantly different ($P < 0.05$).

TABLE VI.--Pyrazon concentrations and disappearance in seven soils as related to soil properties and rainfall, 6 pounds per acre applied.[1]

| Soil No. | Clay | Organic Matter | Rainfall/Month | | Pyrazon Residues | | Rate of Disappearance |
|---|---|---|---|---|---|---|---|
| | | | Prior to 1st Sample | Bet. 1st and Last Sample | 2 to 2.5 Mo. After Appl. | After 4 Mo. | |
| | % | % | in/mo | in/mo | µg/g | µg/g | µg/g/mo |
| 2 | 39 | 6.8 | 1.8 | 4.8 | 3.3 | 2.2 | 0.70 |
| 3 | 9 | 13.7 | 1.5 | 3.5 | 0.4 | 0.4 | 0.00 |
| 4 | 24 | 4.2 | 1.6 | 2.0 | 2.1 | 1.3 | 0.13 |
| 5 | 28 | 4.3 | 2.3 | 2.7 | 2.4 | 1.4 | 0.59 |
| 6 | 28 | 4.2 | 2.9 | 3.4 | 4.0 | 1.8 | 0.97 |
| 7 | 26 | 12.9 | 1.8 | 2.8 | 2.8 | 2.3 | 0.23 |
| 8 | 10 | 2.9 | 1.8 | 2.8 | 2.5 | 0.2 | 1.04 |

[1]Concentrations determined by comparison of bioassay injury ratings to standards in the same soil.

TABLE VII.--Pyrazon residues in two clay loam soils as related to rate of pyrazon bioassay application and time and depth of sampling in 1965, as accessed by injury ratings.[1]

| Soil Depth | Soil 1 (months after application) | | | Soil 2 (months after application) | | |
|---|---|---|---|---|---|---|
| | 2.4 | 3.4 | 4.6 | 2.0 | 3.0 | 4.2 |
| in | IR | IR | IR | IR | IR | IR |
| | 4 lbs/A applied | | | 4 lbs/A applied | | |
| 0-2 | 3.75 | 3.50 | 3.25 | 5.66c | 5.00c | 0.66e |
| 2-4 | 1.25 | 1.75 | 1.25 | 0.00e | 0.66e | 0.00e |
| 4-8 | 0.00 | 0.00 | 0.00 | 0.00e | 0.00e | 0.00e |
| | 4 lbs/A + TCA applied | | | 4 lbs/A + TCA applied | | |
| 0-2 | 3.50 | 3.25 | 2.75 | 5.00c | 4.66c | 2.66d |
| 2-4 | 0.75 | 1.75 | 1.50 | 0.00e | 0.33e | 0.00e |
| 4-8 | 0.00 | 0.00 | 0.00 | 0.00e | 0.00e | 0.00e |
| | | | | 6 lbs/A applied | | |
| 0-2 | | | | 8.66ab | 8.00ab | 4.33c |
| 2-4 | | | | 0.00e | 1.00e | 0.66e |
| 4-8 | | | | 0.00e | 0.00e | 0.33e |
| | 6 lbs/A + TCA applied | | | 6 lbs/A + TCA applied | | |
| 0-2 | 7.25 | 5.50 | 3.25 | 10.00a | 9.33a | 7.66b |
| 2-4 | 2.00 | 3.25 | 1.25 | 1.00d | 1.00d | 0.33e |
| 4-8 | 0.00 | 0.25 | 0.00 | 0.00e | 0.33e | 0.33e |
| | 3 lbs/A applied | | | 10 lbs/A applied | | |
| 0-2 | 3.50 | 3.50 | 2.75 | 9.66a | 10.00a | 9.66a |
| 2-4 | 0.50 | 1.00 | 1.00 | 0.66e | 1.33de | 0.66e |
| 4-8 | 0.25 | 0.00 | 0.00 | 0.00e | 0.00e | 0.00e |
| | Mean of All Treatments[2] | | | Mean of All Treatments[2] | | |
| 0-2 | 3.03A | 2.81A | 1.94B | 4.33A | 4.76A | 3.00B |
| 2-4 | 0.65D | 1.28C | 0.78D | 0.16C | 0.66C | 0.26C |
| 4-8 | 0.03E | 0.15E | 0.00E | 0.00C | 0.13C | 0.16C |

[1]Injury ratings (IR), where 0 indicated no injury and 10 indicated that all plants were killed. Means with a soil followed by the same letter of the same case are not significantly different ($P < 0.05$).

[2]Includes pyrazon applied at 0, 2, and 3 pounds per acre with and without TCA, in addition to treatments shown.

TABLE VIII.--Pyrazon concentrations in the upper two inches of six soils in 1966.[1]

| Time After Application | Rate of Pyrazon Applied (lbs/A) | | | | Mean[2] | Rainfall Prior to Sampling[3] |
|---|---|---|---|---|---|---|
| | 0 | 4 | 6 | 6 + TCA[2] (8 lbs/A) | | |
| months | µg/g | µg/g | µg/g | µg/g | µg/g | in |
| | | | Sandy Clay Loam (Soil 4) | | | |
| 2.5 | 0.0 | 1.2 | 2.1 | 1.4 | 1.2A | 4.1 |
| 4.6 | 0.2 | 1.8 | 1.2 | 2.1 | 1.2A | 4.8 |
| 5.8 | 0.2 | 1.8 | 1.7 | 1.7 | 1.2A | 1.8 |
| Mean | 0.1a | 1.6b | 1.7b | 1.7b | | |
| | | | Sandy Clay Loam (Soil 5) | | | |
| 2.5 | 0.1c | 1.5a | 2.4a | 1.9a | 1.4A | 5.9 |
| 4.6 | 0.1c | 1.0a | 1.0a | 2.3a | 1.4A | 6.6 |
| 6.2 | 0.0c | 0.4c | 0.2c | 0.0c | 0.2B | 3.6 |
| Mean | 0.1a | 1.0b | 1.2b | 1.4b | | |
| | | | Sandy Clay Loam (Soil 6) | | | |
| 1.7 | 0.0e | 3.5ab | 4.2a | 2.8b | 2.5A | 5.1 |
| 4.9 | 0.0e | 0.4de | 0.9de | 1.7cd | 0.8B | 10.7 |
| Mean | 0.0a | 1.9b | 2.5b | 2.2b | | |
| | | | Sandy Clay Loam (Soil 7) | | | |
| 1.8 | 0.6 | 0.8 | 2.8 | 1.1 | 1.4A | 3.5 |
| 4.0 | 0.1 | 1.3 | 2.3 | 2.7 | 1.4A | 6.1 |
| Mean | 0.3a | 1.0a | 2.5a | 1.9a | | |
| | | | Sandy Clay Loam (Soil 8) | | | |
| 1.8 | 0.0 | 0.5 | 2.5 | 1.7 | 0.8A | 3.5 |
| 4.0 | 0.0 | 0.1 | 0.2 | 1.2 | 0.0B | 6.1 |
| Mean | 0.0a | 0.3b | 1.3c | 1.4c | | |

[1]Means within a soil followed by the same letter of the same case or designation or no letter are not significantly different ($P < 0.05$).

[2]Includes pyrazon applied alone at 3 lbs/acre and at 2 and 3 lbs/acre in combination with TCA, in addition to treatments shown.

[3]Between time of application and first sampling and between sampling periods.

TABLE IX.--Bacteria and actinomycete populations in three soils amended with pyrazon, incubated at 21.0 C.[1]

| Incubation Time | Bacteria | | Actinomycetes | | Soil pH |
|---|---|---|---|---|---|
| | Control | Amended Soil[2] | Control | Amended Soil[2] | |
| weeks | $10^6$ bact/g | $10^6$ bact/g | $10^5$ actino/b | $10^5$ actino/g | |
| | | | Clay Loam | | |
| 2 | 31b | 47b | 4 | 3 | 6.8 |
| 5 | 163b | 384a | 30 | 7 | 7.0 |
| 10 | 85b | 99b | 5 | 7 | 6.8 |
| 15 | 64b | 13b | 6 | 6 | 6.7 |
| Treatment Mean | 85b | 135b | 11 | 7 | |
| | | | Sandy Loam | | |
| 2 | 10 | 12 | 4 | 3 | 6.7 |
| 5 | 18 | 50 | 6 | 13 | 7.2 |
| 10 | 20 | 29 | 2 | 6 | 7.0 |
| 15 | 64 | 46 | 4 | 33 | 7.1 |
| Treatment Mean | 28 | 34 | 4 | 14 | |
| | | | Sandy Clay Loam | | |
| 2 | 43 | 10 | 7 | 8 | 7.4 |
| 5 | 17 | 164 | 4 | 2 | 7.4 |
| 10 | 138 | 282 | 11 | 9 | 7.5 |
| 15 | 45 | 60 | 7 | 4 | 7.5 |
| 20 | 105 | 43 | 1 | 0 | 7.3 |
| Treatment Mean | 83 | 112 | 6 | 4 | |

[1]Means followed by no letter or the same letter are not significantly different ($P < 0.05$).

[2]3 µg/g added to clay loam and sandy loam, 4 µg/g added to sandy clay loam.

TABLE X.--Bacteria and actinomycete populations in a sandy clay loam amended with pyrazon, incubated at four temperatures.[1]

| Incubation Time | Bacteria | | Actinomycetes | |
|---|---|---|---|---|
| | Control | Amended Soil | Control | Amended Soil |
| weeks | $10^6$ bact/gm | $10^6$ bact/gm | $10^5$ actino/gm | $10^5$ actino/gm |
| | | 4.5 C | | |
| 5 | 218 | 267 | 6 | 1 |
| 10 | 36 | 68 | 3 | 9 |
| 20 | 31 | 169 | 1 | 2 |
| Treatment Mean | 75 | 165 | 3 | 4 |
| | | 12.0 C | | |
| 5 | 28 | 22 | 4 | 2 |
| 10 | 152 | 101 | 7 | 14 |
| 15 | 46 | 140 | 3 | 4 |
| 20 | 10 | 15 | 1 | 0 |
| Treatment Mean | 59 | 74 | 4 | 5 |
| | | 21.0 C (see Table IX) | | |
| | | 29.5 C | | |
| 5 | 150 | 419 | 70 | 60 |
| 10 | 279 | 177 | 6 | 5 |
| 15 | 151 | 79 | 48 | 29 |
| 20 | 33 | 65 | 0 | 2 |
| Treatment Mean | 153 | 185 | 31 | 24 |

[1] 4 µg/gm added, means are not significantly different ($P < 0.05$).