PICLORAM RESIDUES IN POTATOES AND CARROTS

AND

PICLORAM PHOTODECOMPOSITION

by

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ABSTRACT

Residues of picloram in four varieties of potatoes, given pre-emergence treatments with picloram at 2 oz per acre and picloram at 2 oz + linuron at 24 oz per acre were determined by electron capture gas chromatography. Average residue levels of 3.9 and 2.7 ppb (fresh weight) were obtained for the picloram and picloram + linuron treatments. This difference was significant at the 1% level. Tuber injury, ranging from formation of corky tissues on the surface to splitting, was observed with the picloram treatments. No such injury was observed with the picloram + linuron treatments.

Yields were noticeably higher in the picloram + linuron treatments. The reduced picloram residues could thus be associated with the diluting effect of higher yields which resulted from the addition of linuron.

A similar experiment using four varieties of carrots given either pre-emergence or post-emergence treatments with picloram at $\frac{1}{2}$ and 2 oz per acre respectively gave no detectable picloram residues. Using ^{14}C carboxyl labeled picloram this finding was further investigated and it was shown that picloram was absorbed by the foliage and roots and translocated throughout the whole plant. The leaves accumulated four times more radioactivity than the taproots. The radioactivity in the leaves and taproots was in the form of the parent picloram molecule. The picloram present in the taproot was located mainly in the

xylem.

A study of the stability of picloram, its potassium salt and its methyl ester under short wave ultraviolet light (253.7 nm) revealed that all three compounds were degraded into several photoproducts. The methyl ester was the least stable, being 85% degraded after one hour exposure. Picloram and its potassium salt were more stable, each being 50% decomposed after one hour exposure. Partial polymerisation of all three compounds may also have taken place.

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INTRODUCTION

A new era in chemical weed control was ushered in in 1944 with the appearance of the auxin-like, phenoxy herbicides, namely, 2,4-D, 2,4,5-T and MCPA (34). These were the first synthetic herbicides, active at rates of a few pounds per acre rather than hundreds of pounds per acre, as was the case with the older herbicides the arsenicals and borates.

The announcement of a still more potent auxin-like growth regulator, picloram (4-amino-3,5,6-trichloro-picolinic-acid) in 1963 (32) was another forward step towards getting greater kill and greater selectivity in weed control, while lowering rates of application to a few ounces per acre. Both inhibition and promotion of growth are involved with auxin-like herbicides, depending on the concentration used, the plant species and the type of application.

Apart from their plant-killing capacities, herbicides also have beneficial effects on plants. Reports on stimulation of plant growth, leading to increased yield, by application of sublethal concentrations of 2,4-D are numerous. One recent example of this beneficial effect of herbicides, was reported by Luckwill (65) when it was found that an application of 3 mg simazine per plant was equivalent to that of 570 mg nitrogen, applied as ammonium nitrate, in enhancing growth and nitrogen content of corn.

Some of these newer chemicals, though used at rates of a few ounces per acre, are very persistent and by acting at

the cellular level can induce profound changes in an organism or its progeny.

Greater sophistication in analytical methods coupled with instances where some of the chlorinated hydrocarbons have been found in animal tissues have led to a greater awareness that some of these chemicals, or their breakdown products, are passing on to man in his diet. The concept of "Pesticide Residues" was thus born. This awareness has been manifested in several ways, including studies of uptake by, and translocation and distribution in the organism concerned; residues and persistence in the environment at large including target and non-target organisms; and metabolism and degradation by biological and non-biological systems.

Scientific curiosity, a willingness to know and the fear of the unknown have forced scientists to aim at following these man-made molecules as closely as possible once they have been sprayed on a target organism.

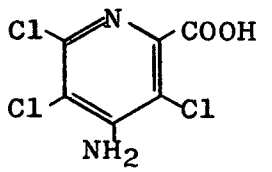
The objectives of this study were to:

1. become familiar with techniques useful for picloram residue studies.
2. find whether residues of picloram could be found in a tuber crop, potato, and a root crop, carrot.
3. determine whether picloram residue levels were affected by the presence of a second herbicide, linuron.
4. study the stability of picloram in acid, salt and ester form when subjected to ultraviolet radiation.

REVIEW OF LITERATURE

Physical and Chemical Properties of Picloram

Picloram has the molecular structure:



(4-amino-3,5,6-trichloropicolinic acid; M.W.= 241.5) and is a white crystalline powder, with a faint chlorine-like odor (32,92). Its vapor pressure at 35°C is 6.16×10^{-7} mm Hg. It is very slightly soluble in the non-polar organic solvents and quite soluble in the polar ones, as shown below:

Solvent	Solubility in ppm
Acetone	19,800
Ethanol	10,500
Acetonitrile	1,600
Diethyl ether	1,200
Water	430
Benzene	200
Carbon disulfide	50
Kerosene	10
Hexane	insoluble

It is commercially available for weed control, under the trade name of Tordon (Dow Chemical Co.).

Uses as a Herbicide

Picloram was first used to control weeds on non-crop areas like highways, railroads and around farm buildings and fences. Presently it is used for the control of brush (103,107), woody range species (23) and deep- rooted perennial weeds

(57,89,102). Rates of $\frac{1}{4}$ to 1 ounce per acre are effective against seedling broad-leaf weeds.

Perennial weeds may be controlled by 1 - 4 lb per acre. Soil applications of 6 - 8 lb per acre control most woody plants. It has a relatively long residual life in soil, estimated at about 13 months (29).

Because of its good translocation properties, it is effective both via foliar and soil applications. Mixtures of picloram and other herbicides, particularly 2,4-D and 2,4,5-T are currently used in weed control (3,4,60).

Herbicide mixtures may not only broaden the spectrum of weeds to be controlled but also reduce the rates of application, by making use of their synergistic properties. Chuma (14) reported that a mixture of picloram and 2,4-D each at 0.01 and 0.1 lb per acre respectively killed tops of field bind weed faster than 2,4-D alone or picloram alone at the same rates. This enhanced activity is due to the greater translocation rate when applied in combination than when applied singly. Bovey et al (11) reported the antagonistic properties of picloram and other herbicides. They found that the addition of paraquat to picloram decreased the effectiveness of picloram on huisache, (Acacia farnesiana (L.) and mesquite plants, Prosopis juliflora (Swartz).

Davis et al (17) showed that in mesquite, huisache and bean plants, the transport of picloram was reduced, when used in combination with paraquat. Whether differences in translocation alone can explain synergistic and antagonistic properties of picloram, when used with other herbicides, cannot be answered yet.

Picloram has a long residual life in soil and has been removed from the United Kingdom market for this reason. However it is licensed for use in British Columbia.

Herbicidal Action

General

Van Overbeck (100) defined a herbicide as "a chemical that deranges the physiology of a plant over a period long enough to kill it". A few of these physiological processes are: photosynthesis, respiration, cell division and nucleic acid metabolism. Each of these processes is the overall expression of a multitude of sub-processes which can be inhibited by herbicides. A few of these sub-processes are:

(a) Inhibition of light system I, (b) inhibition of light system II, (c) inhibition of photophosphorylation.	Photosynthesis
(d) Uncoupling oxidative phosphorylation, (e) inhibiting oxidative phosphorylation, (f) inhibiting oxygen uptake.	Respiration
(g) Inhibition of spindle formation, (h) clumping of chromosomes.	Cell division
(i) Interference with nucleic acid metabolism, (j) interference with protein synthesis.	

Picloram

The physiological basis for the herbicidal action of picloram is essentially unknown. Several workers have delved into a few aspects of its mode of action. A major advance was made when picloram was shown to possess auxinic properties (32, 49). This was an aid to understanding its mechanism of action because the physiological activity of another auxin-like herbicide

namely 2,4-D has already benefited from two decades of research. The various aspects of the mode of action of picloram are dealt with in the succeeding pages.

Picloram as a Substitue for Auxin

Picloram at low concentration can be substitued for indole-3-acetic acid (IAA) and 2,4-D as an auxin source in synthetic media. Goodwin (28) reported that 1 - 2 mg picloram per liter of synthetic medium gave optimum callus growth. In our laboratory root elongation of cress seedlings was obtained with concentrations of 0.001 - 0.005 ppm while growth retardation was observed at about 0.05 ppm concentration and above. Kefford et al (49) did a comprehensive study to test the potency of picloram as an auxin. The biological systems tested and the results are given in toto:

Tests

1. Extension of wheat and oat coleoptile sections.
2. Extension of pea stem sections.
3. Extension of sectioned pea root tips.
4. Growth of intact roots from γ-irradiated and non-irradiated wheat grain. (γ-irradiation prevents cell division but not growth).
5. Development of parthenocarpic tomato fruits.
6. Induction of cell division in tobacco stem pith.

Results (in same order as tests):

1. On the basis of the concentrations producing half maximal extension, picloram had about one third the activity of IAA in

wheat and oat coleoptile sections, but the maximum section elongation obtained with picloram and IAA were the same.

2. For the extension of pea internode sections picloram was about 100 times more active than IAA; 2,4-D had activity intermediate between picloram and IAA.
3. The growth of pea root tips was stimulated by low concentration and inhibited by high concentration of picloram. Least growth was obtained with picloram followed by IAA and 2,4-D.
4. As inhibitors of root elongation on non-irradiated seedlings IAA, 2,4-D and picloram showed approximately equal efficiency; but for γ-irradiated seeds, picloram was less inhibitory than IAA or 2,4-D.
5. When compared to 2-naphthoxyacetic acid in the induction of growth of fruits from emasculated tomato flowers, picloram was much less active, but nonetheless it showed some activity.
6. In absence of a kinin source neither IAA nor picloram induced cell division. In presence of kinin, picloram at 10^{-6}M was as effective as IAA at 10^{-5}M, the IAA concentration found optimum for this test.

Absorption as Undissociated Molecules

Baur et al (8) showed that pH had a significant effect on the uptake of picloram by potato tuber discs. The molecule is absorbed mostly in the undissociated form but uptake is not dependent on the concentration of the undissociated molecule. No report, however, has been made as to whether there

is a difference in the phytotoxicities of the dissociated and undissociated picloram molecule.

Vapors of the potassium salt of picloram have been found to be herbicidally active on bean plants (Phaseolus vulgaris) (25). Since the salt was at ambient temperature, it can be reasonably assumed that the herbicide molecule was in the undissociated state.

Translocation and distribution

Horton et al (44) using agar blocks containing ^{14}C carboxyl labeled picloram, showed that transport through petioles of bean and coleus plants and stems of pea plants is basipetal. Again they showed that picloram is transported faster then 2,4-D, under the same conditions. Bovey et al (12) showed that translocation of picloram from foliage to roots, in huisache plants, was increased by the presence of leaves. Chuma et al (14) clarified the situation better by showing that older leaves appear to play a more significant role in the absorption and subsequent movement of both picloram and 2,4-D, than the young leaves. He found no difference in the amount of picloram absorbed by intact plants and plants in which the upper leaves and growing tips were removed. However the removal of lower leaves or complete defoliation reduced the absorption of picloram. In field bind weed the presence of leaves is important in the distribution of picloram, in huisache however the distribution is the same with defoliated and undefoliated plants.

In huisache, in presence of the leaves, more picloram

was translocated to the roots than to the shoots. Removal of all the leaves, except the treated leaf, reversed the direction of foliar applied picloram.

Excretion by Roots

Hurtt et al (46) showed that picloram was excreted by roots of bean plants, after application of the herbicide to the foliage. It was released from the roots of donar plants, into the nutrient solution, within 24 hours; the excreted picloram was taken in by the roots of untreated plants and since the latter developed symptoms similar to that of picloram toxicity, it was assumed that the molecule was excreted unchanged. Excretion of picloram or other herbicides from the roots is not considered a major pathway of the detoxification mechanism of plants.

Histological Abnormalities

Kreps (53) studied the histological abnormalities induced by picloram in Canada thistle roots. Swelling and splitting were observed throughout the entire root system. Some cells in the exodermis and subexodermis areas had deteriorated, leaving air spaces in the cortex. Parenchyma cells, found in the centre of the cortex, were partially or completely destroyed. The cambium layer had disintegrated leaving the xylem still intact but detached from the rest of the root tissue. The greatest damage observed was complete destruction of cortex, phloem, cambium while xylem and periderm remained intact.

Ethylene Production

Baur et al (7) found a tenfold increase in ethylene

production after treatment of huisache and mesquite seedlings with picloram. There is some controversy in the literature as to whether auxin-like herbicides induce ethylene production in resistant species, susceptible species or both. Morgan et al (79) showed that 2,4-D promoted ethylene production from susceptible dicotyledonous plants and not from the resistant monocotyledons. Abeles (2) however showed that 2,4-D increased ethylene production in both resistant (corn) and susceptible (soybean) plants. The rate of ethylene production was greater in soybean than in corn.

Ethylene itself is a growth regulator and auxin-induced ethylene production is believed to be mediated through the production of specific enzymes, which in turn enhance the sythesis of ethylene.

Carbohydrate Synthesis

Increases in total carbohydrates and reducing sugars have been shown (56). Root exudates from corn seedlings treated with 50 - 500 ppm picloram gave increases in total carbohydrates ranging from 170 to 430%, while the increase in reducing sugars ranged from 190 to 270%. This increase in carbohydrate exudation is detrimental to the plant by causing increased root rot in cereal seedlings (95). No explanation for this observation has yet been postulated.

Lipid Synthesis

The effect of picloram on lipid synthesis has been reported (69). Lipid synthesis was followed by incubating sesbania hypocotyls (Sesbania punicea) with malonic acid-2-^{14}C,

the lipids were extracted and the incorporation measured by amount of radioactive lipids produced. An average increase of about 45% above the control was obtained with concentrations of 1 - 20 mg of picloram per liter. However there was no correlation between increase in lipid synthesis and concentration of picloram used.

Respiration and the Tricarboxylic Acid Cycle

Mitochondria are heavily involved in respiration and isolated mitochondria have proved useful in studying inhibitors of respiration. Foy et al (22) tested the effect of several herbicides on the substrates of the tricarboxylic acid cycle of isolated cucumber mitochondria. Picloram at 10^{-3}M strongly inhibited the oxidation of succinate and moderately inhibited the oxidation of α-keto-glutarate. The inhibition was measured by the amount of oxygen consumed. For succinate and α-keto-glutarate the oxygen consumptions, above the contro, were 10 and 43% respectively after 1 hour.

Enzyme Synthesis

During germination the de novo synthesis of proteolytic enzymes, leading to an increase in the proteolytic activity, in squash cotyledons has been reported. Ashton et al (6) tested the effect of several herbicides on the proteolytic activity of squash cotyledons. Picloram at 10^{-3} and 10^{-5}M concentrations inhibited proteolytic activity by 38% and 78% respectively. At 10^{-3}M concentration the inhibition for 2,4-D was 45%. On the other hand West et al (105) have shown that inhibition of

proteolytic activity by 2,4-D is not responsible for inhibition of growth. Moreland (78) reported an hibition in α-amylase induction of 60% in barley seeds as a result ot treatment with picloram.

Nucleic Acid Metabolism and Protein Synthesis

Malhotra et al (68) investigated the relationship between species sensitivity to picloram and changes in nucleic acid metabolism. For the 5 species of graded sensitivity used - barley (very resistant), wheat, corn, cucumber and soybean (very sensitive) - he found a positive correlation between induction of RNA synthesis and herbicidal sensitivity. The sensitive plant species responded to picloram treatment with an increase in RNA content whereas the RNA content of the resistant species changed very little. Since the sensitive plant species have high DNA and RNA contents, compared to the resistant ones, it was thought that the resistant species may have some kind of "block" which prevents accumulation of nuclear material. One such block might be in the level of deoxyribonuclease and ribonuclease. Malhotra also showed that the endogenous level of deoxyribonuclease and ribonuclease in the resistant species was much higher than in the sensitive ones. The specific activities of bound ribonuclease correlated inversely with herbicidal sensitivity. Resistant plants were higher in bound nucleases than sensitive ones. No such correlation for the free enzyme was found. It is possible that picloram may have a similar effect in promoting nucleic acid synthesis in both

resistant and sensitive species. However the presence of higher levels of native bound nucleases in the resistant species prevent accumulation of nucleic acids. The nucleic acids may be degraded as soon as they are synthesised. The role of nucleases, however, in the metabolism of the cell is still largely unknown.

Key (50) showed that ribonucleic acid synthesis and protein synthesis are essential processes for cell elongation. Working with 2,4-D, Key et al (51) showed that nucleic acid metabolism was suppressed when growth was suppressed and was accelerated when growth was accelerated. No such work has yet been reported for picloram.

Moreland et al (78) investigated the incorporation of a few precursors into protein and RNA and found that picloram had:

1. no effect on incorporation of ATP-8-^{14}C into RNA.
2. no effect on incorporation of l-leucine-1-^{14}C into protein.
3. an inhibitory effect on the incorporation of orotic acid-6-^{14}C into RNA.

Animal Toxicity

Picloram is of low toxicity to humans, livestock, wildlife and fish. Jackson (47) reported that single oral doses of 540 and 720 mg per kg body weight were not toxic to cattle and sheep respectively.

The following oral LD_{50} for small animals was reported by Lynne (62):

Animal	LD_{50}* (mg per kg)
Rat	8,200
Mouse	2,000 - 4,000
Guinea pig	3,000
Rabbit	2,000
Chicken	6,000

Hardy (37) found that the presence of 1 ppm picloram in water did not affect the development, behaviour and reproduction of daphnia, guppies and algae.

Fate in Animals

Fisher et al (21) analysed the feces, milk and urine of dairy cows, fed with 5 ppm picloram-treated fodder. He found that no residue was present in the milk and feces and most of it was eliminated intact in the urine. On the other hand Kutschinski (54) found that the average residue levels of picloram in cow's milk were 0.05 and 0.2 ppm when cattle were fed with 300 and 1,000 ppm picloram respectively in forage. He also found that the residues disappeared within 58 hours after withdrawal of the picloram from the diet.

Decomposition by Microorganisms

Picloram is apparently neither an important substrate for, nor an inhibitor of growth of microorganisms. Youngson et al (110) found that only small amounts of picloram, ranging from 0.24 to 1.21% were decomposed in cultures of bacteria

*LD_{50}: The statistic used to indicate the degree of toxicity. It is expressed as the number of milligrams of a toxicant per kilogram of body weight of an animal, sufficient to kill fifty per cent of such animals.

and fungi. The decomposition was followed by evolution of CO_2.

Goring et al (30) showed that concentrations of picloram as high as 1,000 ppm did not inhibit the growth of microorganisms.

Fate in Soil

Picloram persists in all types of soil, under different temperature and moisture levels for more than one growing season. Studies by Herr (40) and Herr et al (41) indicated that soil organic matter is most influential in preventing the leaching of picloram. Hamaker et al (33) reached the same conclusion, adding that "the sorption of picloram is primarily caused by organic matter and hydrated metal oxides, with clays probably playing a minor role". In spite of being sparingly soluble in water, picloram is leached relatively easily in all types of soil, though more so in sandy soils, (74). Hartley (39) suggested that hydrolysis and oxidation reactions might be accelerated by the adsorption of herbicides by soil colloids. Armstrong et al (5) and Harris (38) have published evidence supporting this non-biological breakdown in the case of the chlorotriazine herbicides.

Hance (35,36) investigated this process for picloram, He found that for a constant amount of air-dried soil, the rate of breakdown of picloram was not significantly affected by the amount of water added during the incubation period.

This eliminated the assumption that the extent of adsorption on soil particles could influence the decomposition. Using different amounts of soil, he showed that the rate of breakdown of the herbicide was dependent on the amount of soil present. Since the rate of decomposition is related to the amount of soil and not to the extent of adsorption it would appear that the decomposition occurs at specific sites in the soil. Whether these specific sites are soil colloids as suggested by Hartley or on carbon and/or hydrated oxides of iron and aluminium as suggested by Hamaker is not known.

Fate in Plants

Radioautography of wheat seedlings, grown in soil containing ^{14}C carboxyl labeled picloram, showed the presence of radioactive residues in all the organs. Redemann (87) showed that in mature wheat grain, most of the residues existed as the parent molecule, with small amounts of breakdown products. The latter have been identified as:

oxalic acid	8%
4-amino-3,5-dichlorohydroxypicolinic acid	5%
4-amino-2,3,5-trichloropyridine	4%
unchanged picloram	83%

In cotton plants, however, intact picloram with no other metabolite was found. Meikle et al (71) found that 95% of the radioactivity was distributed in the leaves and stems and 5% in the roots.

Residues of Picloram

Most of the literature on "Residues of Picloram" deal with methodology. Only a limited amount of information is available on residues *per se* found in treated crops and dairy products.

Crops

Samples of wheat and barley treated with picloram at rates of 0.2 to 1 ounce per acre, applied as post-emergence sprays, were analysed by Bjerke *et al* (10). Seventy-five per cent of the samples were reported to contain residues less than 0.05 ppm. Residues as high as 0.22 ppm in wheat grain, 0.44 ppm in wheat straw and 0.64 ppm in barley grain were also reported.

Dairy Products

Kutschinski *et al* (55) reported that steers fed daily, over a period of two weeks, with 200 - 1,600 ppm picloram in their diet had the following amounts of picloram residues in their tissues:

Fat and muscle	less than 0.05 - 0.5 ppm
blood and liver	0.12 - 2.0 ppm
kidney	2.0 - 18.0 ppm

Analytical Methods

The choice of analytical method depends on several factors, such as the amount of compound present, the level of detection (whether milligram or nanogram range), the nature of biological or other materials from which the compound under investigation is to be isolated and lastly the availability of

equipment. Broadly two types of analyses are encountered: formulation and residue.

In formulation analysis all of the following methods can be applied:

1. Colorimetric
2. Ultraviolet spectroscopy (UV)
3. Infrared spectroscopy (IR)
4. Bio-assay
5. Thin layer chromatography (TLC)
6. Gas liquid chromatography (GLC)

The first five methods are preferable because of rapidity of measurement. In residue analysis, where very small amounts of the compound are to be stripped from large amounts of biological or other materials, TLC and GLC are the standard techniques used.

Colorimetric Method

A colorimetric method suitable for the analysis of picloram in commercial formulations or in soils containing high residue levels, has been described (13). The method consists of extracting the sample with solutions of 1M ammonium acetate or 2M potassium chloride containing sufficient potassium hydroxide to give an extract of pH 7.0.

The picloram, as the potassium salt, is treated with 3.5M sulfuric acid and 0.1M sodium nitrite. The color of the diazo compound is allowed to develop at room temperature in the dark, as it is unstable to ultraviolet light. The absorbance at

405 nm* is measured on a spectrophotometer. This method is preferred where speed rather than sensitivity is the criterion.

Ultraviolet Spectroscopy

Hummel (45) has used the UV absorbance of picloram in its estimation. The material is dissolved in 6M hydrochloric acid and the absorbance at 285 nm measured.

Infrared Spectroscopy

This method was developed by Melcher (72). The sample is extracted with acidified acetone, the solvent evaporated to dryness and the residue dissolved in dimethyl formamide and transferred into an infrared cell. The absorbance at 11.15 μ** is due to picloram.

Bio-assay

The susceptibility of certain plants to extremely small quantities of picloram has made possible the use of bio-assay methods for its determination. Bio-assay methods may detect quantities less than 1 ppm, however they are not qualitative.

A bean bio-assay test had been described by Leasure (59). The limits of detection were reported to be 0.5 ppb*** and 1 ppb in sand and soil respectively. Goodwin (27) claimed that bio-assay with sesbania seedlings could detect picloram in presence of phenoxy herbicides, as sesbania is insensitive to be latter. The lowest limit of detection was reported to be 0.004 ppm.

* 1 nanometer (nm)= 1×10^{-9} meter
** 1 micron (μ)= 1×10^{-6} meter
*** 1 part per billion (ppb)= 1 part in 10^{9} parts

Thin Layer Chromatography

The thin layer chromatography of picloram had been described by several workers. Whitenberg (106) reported the detection of picloram in the 1 to 0.1 µg range on silica gel and alumina plates, developed with the solvent system: petroleum ether + chloroform + 95% ethanol, 7 + 2 + 1 (v/v) and sprayed successively with: (a) 1% tertiary butyl hypochlorite in cyclohexane and (b) 1% potassium iodide + 1% soluble starch in distilled water. No R_f values were reported.

Abbot et al (1) reported the separation of twelve herbicides, including picloram by TLC. The supports consisted both of single and mixed absorbents of silica gel and keiselguhr and out of the eight solvent systems tried, only one was successful in moving picloram, namely: chloroform + acetic acid, 19 + 1 (v/v) with an R_f of 0.21; the support was silica gel.

Meikle (71) described four solvent systems for the paper chromatography of picloram. The solvent systems are listed below:

Solvent system	R_f picloram
1. 1-butanol saturated with 1.5M ammonium hydroxide	0.47
2. tert-amyl alcohol + 15M ammonium hydroxide + water, 10 + 1 + 5 (v/v)	0.25
3. benzene + propionic acid + water, 2 + 2 + 1 (v/v)	0.39
4. toluene + propionic acid + water, 2 + 2 + 1 (v/v)	0.35

Paper chromatography has now been largely replaced by thin layer chromatography on cellulose support, as it gives better resolution, lower limits of detection and a reduction in the development time.

Gas Chromatography

The presence of three chlorine atoms and two nitrogen atoms in the picloram molecule makes its detection by gas chromatography, equipped with an electron capture detector, possible. Organic acids when gas chromatographed as such give large tailing peaks with long retention times, (21). When esterified however, the esters give sharp peaks, making quantitization possible.

In the residue analysis of pesticides by gas chromatography three broad steps are to be considered:

(a) extraction or "stripping" of the residue from the sample
(b) clean-up or isolation of the pesticides from the contaminants and
(c) qualitative and quantitative measurement of the residue by gas chromatography.

Extraction

Extraction is necessary in any analysis where the compound under investigation is present in extraneous materials. Depending on the material from which the pesticide is to be stripped off, two extraction procedures have been currently used, namely:

1. blending with suitable solvents
2. soxhlet extraction with appropriate solvent, with or without thimble.

The need for a "universal" or broad spectrum extracting solvent in pesticide residue analysis has led to the development of the solvent propylene carbonate (93). Both polar and non-polar compounds can be extracted with it.

The extraction procedures described for picloram divide into two main groups viz:

1. extraction with dilute alkali, (10,58)
2. extraction with acidified polar organic solvents, (21,73,92).

In the first method of extraction, the material is blended with ice cold 0.1M potassium hydroxide in a 10% potassium chloride solution. After filtration, the extract is acidified with mineral acid and the picloram partitioned into diethyl ether. The principle of this method is that potassium hydroxide converts picloram into its highly water soluble potassium salt. Addition of potassium chloride decreases the amount of coextractives. When acidified, the potassium salt is converted into picloram acid. Since the solubility of picloram in diethyl ether is 1,200 ppm compared to 430 ppm in water, it goes preferentially into the ether layer.

In the present study this method of extraction was not found to be convenient. When fresh carrots and potatoes are extracted with dilute potassium hydroxide solution a colloidal suspension is obtained, which immediately clogs a filter paper

and takes a considerable time to filter over glass wool. When this filtered solution is acidified a gelatinous like precipitate is obtained which again presents filtration difficulties. On extraction with ether an emulsion is obtained which can only be broken down by centrifugation. All these problems make an alkaline extract inconvenient for fresh carrots and potatoes.

In the second extraction procedure the material is blended with acidified acetone or acidified diethyl ether. Acetone is more currently used because of the higher solubility of picloram in this solvent. (Solubility in acetone 19,800 ppm, in ether 1,200 ppm).

In the present study extraction with acidified acetone of both carrots and potatoes gave a clear solution which did not present any filtration problem, and was subsequently adopted.

Clean-up

After solvent extraction the compound or compounds under investigation are in the presence of coextractives and a clean-up step is often essential if gas chromatography is to be the final step in the analysis. Several workers have reported that this step may not be necessary in the extraction of pesticides from soil (10,92).

The following clean-up procedures are currently used:

1. Column chromatography, with alumina, silica gel, florisil or activated charcoal as adsorbent.
2. Gel filtration using sephadex (LH 20) or other ion exchange resins.

3. Thin layer chromatography (TLC) or another variation of this technique, channel layer chromatography (CLC). Both single and mixed layers of alumina, silica gel, florisil and cellulose have been used.
4. Cosweep distillation.
5. Thin layer electrophoresis (TLE) or high voltage paper electrophoresis (HVE).

The thin layer electrophoresis and high voltage paper electrophoresis of ionisable herbicides have been described (86,101). The principles are essentially the same: the material is applied to a support, either a cellulose thin layer plate or filter paper, the support is buffered and a high voltage (about 100 volts per cm) applied to the two ends. An efficient cooling system is necessary to prevent drying of the support. Purkayastha (86) showed that the following herbicides can be separated from one another by thin layer electrophoresis: 2,4-D, 2,4,5-T, MCPA, 2,4-DB, fenoprop, dicamba, amiben and picloram.

For picloram, two spots described as strong and medium, with opposing polarities (cationic and anionic) and different migration rates were obtained. No further work was carried out to find out if either one was the unchanged picloram molecule.

Bjerke's (10) method of clean-up of picloram residues present in small grain and straw has been adopted by other workers. The method consists of passing an ethereal extract of grain or straw containing picloram through an alumina column,

and eluting the picloram with 0.25M sodium bicarbonate solution. Picloram (acid) is obtained by acidifying the bicarbonate extract.

In the present study this method was not adopted because of the unavailability of a sufficient number of chromatographic columns equipped with teflon stop cocks. Improvised chromatographic columns drawn from glass tubing with tygon tubing at the ends gave spurious peaks on the chromatogram and were rejected. A clean-up method, described later, using a mixture of 15% silica gel + 85% cellulose was used.

Methylation

Organic acids are polar and difficult to chromatograph. Conversion into their esters, which are less polar, removes this difficulty. Methylation is also desirable to decrease the retention time in the column, to decrease tailing and to increase the sensitivity of the detection. This increase in sensitivity is very desirable when dealing with trace amounts of the pesticide.

Most esterification procedures were initially developed for fatty acids, (24,75,84,91). Some of these procedures have been successfully applied to herbicides containing the -COOH group. The phenyl acetic acid herbicides have been esterified by diazomethane (94). Marquardt et al (70) used diazomethane and boron trifluoride-methanol mixture for the methylation of 2,4-D.

Woolson et al (108) compared the efficiency of methylation of several herbicides with the following methylation reagents:

1. Thionyl chloride ($SOCl_2$)
2. Diazomethane (CH_2N_2)
3. Boron trifluoride-methanol (BF_3-MeOH)
4. Sodium nitrite-sulfuric acid ($NaNO_2$-H_2SO_4)
5. Perchloric acid ($HClO_4$)
6. Dimethyl sulfate $(CH_3)_2SO_4$

In all cases diazomethane gave the best yields of esters. This reaction is ideal because the co-product is gaseous nitrogen which offers no separation problem. The reaction can be written as:

$$RCOOH + CH_2N_2 \longrightarrow RCOOCH_3 + N_2$$

In the present study dimethyl sulfate, boron trifluoride-methanol and diazomethane were tried. Dimethyl sulfate gave a brown viscous liquid when warmed with the carrot and potato extracts. This viscous liquid formed a gummy layer and did not dry easily when streaked on a TLC plate. Boron trifluoride-methanol mixture when used with Bjerke's method of clean-up and electron capture gas chromatograph did not prove to be satisfactory because of the relatively large amount of solvent required to wash all the fluoride ions away. Otherwise the fluoride ions give interfering peaks on the chromatogram. Moreover boron trifluoride etches glass vessels slowly, giving them a frosty appearance. Diazomethane, though highly toxic and

needing to be freshly prepared, was found to be most convenient and was adopted. The advantages are that the reaction is almost instantaneous, takes place at room temperature and gives a quantitative yield.

Detection and Estimation

Gas chromatography is a useful and powerful technique for the qualitative and quantitative analysis of trace amounts of organic compounds, both natural and synthetic. It is especially suitable for residue work because of the relatively large number of samples that can be analysed, once the laborious processes of extraction, clean-up and concentration have been achieved.

A gas chromatographic retention time, as a single parameter for qualitative and quantitative analysis, can introduce serious errors, but coupled with one or more other methods, it can give positive identification of a compound. Confirmation of a compound is obtained by finding the retention times on at least two columns with liquid phases of differing polarity or by correlating the gas chromatographic data with data obtained from other physical methods like: TLC, UV, IR, NMR and MS.

The detection of picloram by gas chromatography has been described by several workers. Since the column is the heart of a gas chromatograph a few recorded column packings are given below:

1. Fisher et al (21)

   10% carbowax 20M on Anakrom ABS, 90-100 mesh

2. Merkle et al (73)

   1.5% SE 30 on Chromasorb W, 80-100 mesh

3. Bjerke et al (10)

   1% LAC-2R-466 + 0.5% H_3PO_4 (w/w) on Gas Chrom Z, 80-100 mesh

4. Leahy et al (58)

   2.5% Neopentyl glycol adipate on Chromasorb W, 80-100 mesh

5. Saha et al (92)

   2% Versamid 900 on Chromasorb W, 80-100 mesh

A few of the above liquid phases have been tried as substrates in the GLC of the methyl ester of picloram in the presence of natural products from carrots and potatoes that cannot be separated by the clean-up method used. Versamid 900 is reported to be a high temperature liquid phase, but conditioning of the column at 150°C under a flow of nitrogen gas, produced a browning of the liquid phase. Several trials were made with the same result and the Versamid column was rejected. Because of long retention times, 23 and 20 minutes, both carbowax 20M and neopentyl glycol adipate are not suitable when a large number of analyses are to be performed. The best liquid phase was found to be OV-1, which is a methyl silicone similar to SE-30 but having greater thermal stability.

Artifacts in Gas Chromatography

It has been suggested that the term artifact be confined to interference which arises from the technique itself and to use the term "naturally interfering component" for such components arising form the sample per se, (90). In the literature, however, peaks arising from naturally interfering components are still being termed "artifacts". Most of these artifacts were reported in the insecticide analysis of green plants (26), wild life (42) and soil (83). Polychlorinated biphenyls were reported as artifacts interfering with the detection of the organochlorine insecticides such as DDT (52), Aldrin, and BHC (61). To date no artifact, of analytical or sample origin, has been reported in the gas chromatographic detection of herbicides.

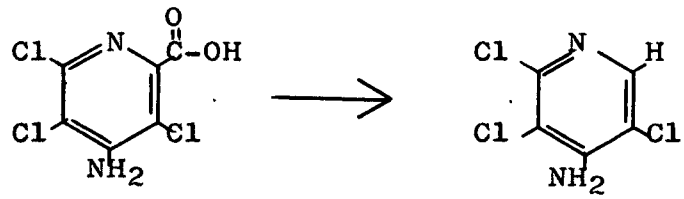
Newer Trends in the Analysis of Picloram

On-column decarboxylation

Several of the most promising herbicides have free acidic groups and their detection by GLC has several disadvantages, including cumbersome extraction procedures, handling of toxic reagents for methylation and predominance of interfering peaks.

Hall (31) described an on-column decarboxylation of picloram. This makes use of a pyrolysis column connected to the main column in such a way that after injection the components pass first through the pyrolysis column then into the main column. Pyrolysis of the acid (I) leads to the decarboxylated

product (II) which is then detected.



(I) (II)

The efficiency of the decarboxylation reaction was reported to be about 95%. Two other advantages of this method, in the case of picloram are that the compound (II) can be detected at a lower temperature than the methyl ester, and impurity peaks obtained as a result of esterification are no longer present in the pyrolysis method.

The thermal decaroxylation method can be used in the analysis of other herbicides (31). This decarboxylation technique has been described to be superior to the available methods for the analysis of picloram residues.

Alkaline pre-column

The alkaline pre-column described by Miller et al (76) has been devised to remove "troublesome crop peaks" in the analysis of pesticide residues, from crops. The alkaline pre-column, as the name suggests, is an alkaline coumn made up of 25% KOH/NaOH on Gas Chrom Q connceted prior to the main column. During operation, the pre-column is heated just as is the main column. The advantage of this alkaline pre-column is that no lengthy clean-up steps are required. Its potential for

removing interfering peaks in carrots was clearly shown (76). The disadvantage of this method is that some pesticides are destroyed completely by it.

Trimethyl Silyl Derivatives

Though methylation with diazomethane is a "clean reaction", the usual way of preparing it from "Diazald" (N-Methyl-N-nitroso-p-toluene sulfonamide) (18, 19) gives several peaks on a chromatogram. Trimethyl silyl ethers (TMS) of bile acids were first prepared by Makita (67). Later Horii et al (43) showed that the TMS derivatives of carboxylic acids can be easily prepared and gas chromatographed. This method may prove useful in preparing the TMS derivatives of the acidic herbicides prior to gas chromatography.

Photodecomposition

Introduction

In the past decade photochemistry has blossomed dramatically into a field of its own. After an initial breakthrough in 1952 (80), the cry of organic chemists around the world seemed to be "if you have some of it, irradiate it".

The presence of pesticides in the soil, water and air, the possibility of their undergoing a variety of reactions by UV light and the search for new methods of decreasing the pesticide burden of the environment have added another dimension to the picture.

The usefulness of short wave UV light to break down chemical bonds may be seen from the following Tables (16):

TABLE I

Energy Distribution

wavelength (nm)	Energy (Kcal/mole)
200	143
300	95
420	68

TABLE II

Bond Energies

Bond	Energy (Kcal/mole)
C-C (in ethane)	88
C-H (in methane)	98
O-H (in water)	119
C-N (in HCN)	73
C-Cl(in CCl_4)	81

Table I gives the relationship between the wavelengths of UV light and the energy associated with each wavelength. Table II gives the bond energies of a few commonly encountered bonds in organic compounds. It is seen that the 200 - 300 nm range has enough energy to break most of the commonly occurring bonds in organic pesticides.

Herbicide Photodecomposition

The photodecomposition of several herbicides has been reported. Bell (9) reported the formation of 5 degradation products of 2,4-D. One major product of 2,4-D sodium salt was shown to be 2,4-dichloro phenol (15). Jordan et al (48) studied the photolysis of the urea and triazine herbicides. Diquat and

paraquat have also been photolysed (96,97) under laboratory conditions. Mitchell (77) reported a study on the photo-decomposition of 141 pesticides (herbicides, insecticides and fungicides) spotted on chromatography paper.

Because of the complexity of the problem of elucidating molecular structure of organic molecules, only a few instances are recorded where the photoproducts were identified.

Redemann (88) reported that picloram was rapidly photolysed by UV radiation and by sunlight. The photoproducts were not identified. Plimmer et al (85) found that the methyl ester of picloram was converted into a single major product, with one chlorine atom less than the parent molecule. Merkle et al (74) reported that 60% picloram spread either on a glass surface or on a soil surface was degraded by UV light in 48 hours. Under the same experimental conditions 35% was reported to be broken down by sunlight.

Our observations on the decrease in concentration of standard solutions of methyl ester of picloram kept on a bench top, and the reported decrease in phytotoxicity of picloram in sunlight, under field conditions, led to an investigation of the photodecomposition of picloram, its potassium salt and its methyl ester. The salt form is commercially available as a herbicide and the methyl ester is used in the gas chromatographic analysis of picloram.

MATERIALS AND METHODS

Field Trial

Potatoes

Between May and August 1969 four varieties of potatoes (Solanum tuberosum L.), namely Kennebec, Netted Gem, Norland and Pontiac were grown in the field in a completely randomized design. The field 18.0 meters x 9.0 meters was divided along its length into 16 plots each 0.6 m x 9.0 m with 0.45 m spacings between the plots and a clearance of 0.82 m at the edges. The potatoes were planted 0.3 m apart, in single rows in the middle of the plots.

Picloram was applied at 2 oz per acre* alone, or in combination with 24 oz linuron per acre. Linuron was used in combination with picloram because linuron is registered for use in weed control on potatoes as well as on carrots. The required amounts of the commercial herbicides (as the active ingredients) were sprayed at the rate of 400 gallons of water carrier per acre. This high volumne spray was used to reduce drift to a minimum. All sprayings were done one week after planting i.e. as a pre-emergence treatment. After a growth period of 100 days samples of tubers were collected, by digging hills in the centre of each treatment, leaving the edges to act as guards.

Carrots

An attempt was made to study the level of residues of picloram in carrots (Daucus carota L.). Four varieties, namely:

*1 oz per acre= 70 g per hectare

Royal Chantenay, Gold Pack, Touchon and Imperator, were grown in the field between May and August 1969, in a completely randomized design similar to that described previously. Picloram was applied at the rates of ½ and 2 oz per acre as follows:

1. Picloram ½ oz per acre (post-emergence).
2. Picloram ½ oz per acre (post-emergence) + linuron 24 oz per acre (pre-emergence).
3. Picloram 2 oz per acre (pre-emergence) + linuron 24 oz per acre (pre-emergence).

Sample Preparation

The carrot taproots and potato tubers were washed thoroughly and surface dried. About 4 - 8 pounds were cut into small cubes, mixed thoroughly and 2 pound samples taken by quartering. These samples were then stored in a deep-freeze until analysed.

Extraction

A 100 g sample was blended with 100 ml methanol + 25 ml N sulfuric acid for 5 minutes. Addition of mineral acid to the extraction solvent is essential if the picloram is to be subsequently esterified (99). It has been suggested that a trace of the mineral acid enters the ether phase and catalyses the diazotisation process (10).

The extract was filtered twice in the same funnel containing a pad of glass wool, into a 500 ml ℔ round bottomed flask. This aqueous alcoholic extract was flash evaporated for 3/4 - 1 hour to remove most of the methanol. For solubility reasons, removal of methanol is imperative if the picloram is to

be partitioned into diethyl ether. The flash evaporation assembly is shown in Figure 1.

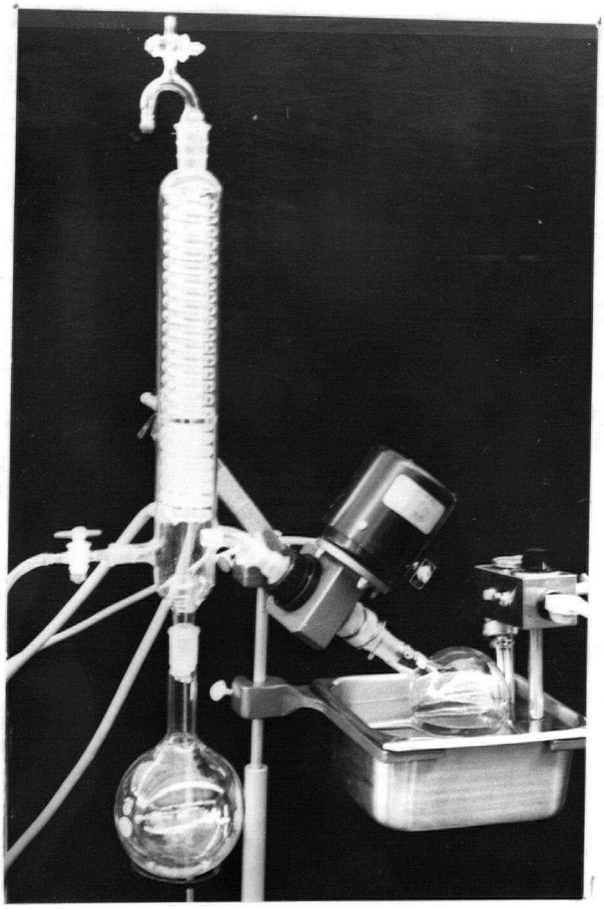


Fig. 1. Flash evaporation assembly

The aqueous concentrate was transferred into a 100 ml separatory funnel containing 10 ml saturated sodium chloride solution. This was extracted by gentle shaking with 3 x 15 ml diethyl ether. After each extraction the ether layer was dried

over anhydrous sodium sulfate and collected together into a 100 ml boiling tube. The ether extract was evaporated to dryness on a 40°C water bath. All operations were done in a fume cupboard.

The dried residue was cooled to room temperature and treated with 2 ml diazomethane-ether reagent, prepared as described by De Boer (18,19). The reagent was added down the side of the tube so as to concentrate all the product at the bottom of the tube. The reaction was allowed to proceed for 10 minutes, at room temperature, after which excess diazomethane was decomposed by returning the tube to the water bath. Care was taken not to remove any undecomposed diazomethane out of the fume cupboard at any time because of its highly toxic and potentially explosive nature (19). The reagent was freshly prepared before use because of its short life (2 - 3 hours) at room temperature. All sharp edges, like ends of glass tubings in the distilling apparatus, were fire-polished to eliminate spots where explosions are most likely to occur.

Clean-up

The residue was dissolved in a few drops of methanol and streaked on a TLC plate. This process was repeated three times. The plate consisted of a mixed layer of 85% 'avicel' cellulose and 15% silica gel G, dried at room temperature. Cellulose powder, containing a binder, when spread on a TLC plate gives a hard coating which can be streaked without damaging the surface. Silica gel does not have this property. On the other

hand silica gel gives a better separation of plant pigments than cellulose. Mixtures of various compositions of cellulose and silica gel were tried and the mixture: 85% cellulose + 15% silica gel was adopted because it gave a hard surface making repeated streaking possible and also giving good separation of the plant products (visible under UV light) from the methyl ester of picloram.

The streaker was made by drawing the small end of a pasteur pipette in a flame and attaching a short length of a very small diameter teflon tubing to it as shown in Figure 2.

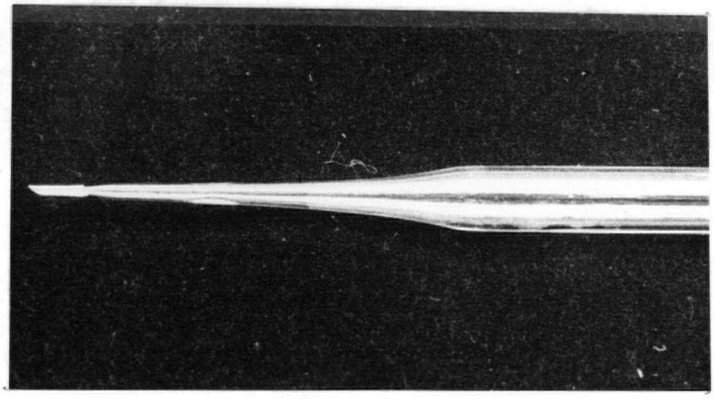


Fig. 2. Streaker showing teflon tip on left

With this apparatus it was possible to streak a band about 2 mm width.

The right hand corner of the TLC plate was spotted with a solution of methyl ester of picloram to serve as a reference. The plate was developed in the solvent system: hexane + acetone + methanol, 50 + 7 + 5 (v/v) to a previously marked

15 cm line. This solvent system was found to be the best to effect the separation. The R_f of the methyl ester was about 0.75.

Localization of the known ester was obtained by shielding the TLC plate with another glass plate and spraying only the zone containing the reference spot. The spray reagent consisted of a 0.005% solution of acridine in ethyl alcohol. Viewing the plate under short wave UV light showed the pyridine ring of picloram as a violet spot on a blueish green background. This was marked with a pencil. The TLC plate is shown in Figure 3.

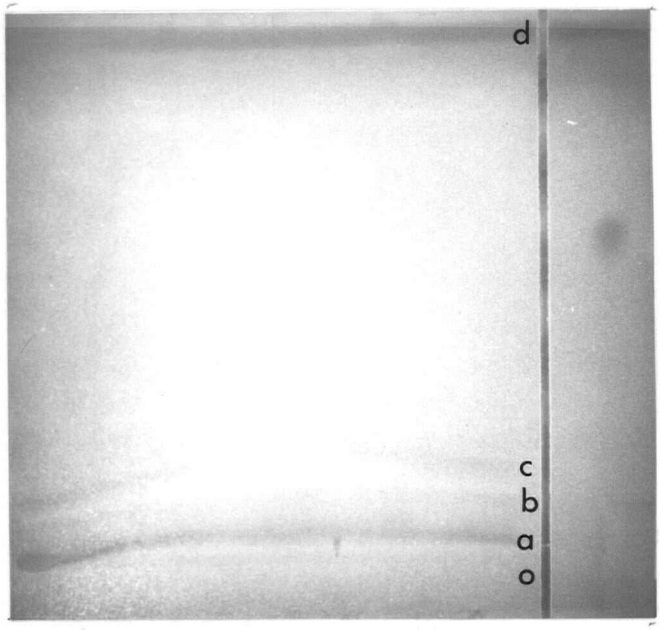


Fig. 3. TLC plate streaked with potato extract (left) and spotted with methyl ester picloram (right). The plate was photographed under short wave UV light. The natural products separated into bands: a,b,c and d. The origin is denoted by O.

Scraping and Elution

A band 2 cm above and 2 cm below the reference spot was scraped off. A pasteur pipette, containing a plug of glass wool, was used both as a collecting and eluting device. A vacuum line connected to the small end was used to suck the cellulose-silica gel powder into the pipette. Elution was accomplished by disconnecting the vacuum line, clamping the pipette vertically, and eluting with 3 x 10 ml methanol, at the rate of about 1 ml per minute. This rate was obtained by gently compressing the glass wool plug.

Liquid-liquid Partitioning

The methanol extract was transferred into a separatory funnel, diluted with 200 ml distilled water and extracted successively with 15 and 5 ml benzene. The benzene extracts were dried over anhydrous sodium sulfate, collected into a 25 ml volumetric flask and made up to volume. Liquid-liquid partitioning was necessary because benzene, which could be injected directly into the gas chromatograph, failed to elute the methyl ester from the TLC substrate. A more polar solvent like methanol was required. Since the methyl ester is more soluble in methanol (22.34 g/100 ml) than in benzene (12.60 g/100 ml) (92), addition of water was necessary for efficient partitioning.

Gas Chromatography

Column Preparation

0.45 g of the liquid phase, OV-1, was dissolved in 75 ml of chloroform in a 500 ml ℔ round bottomed flask, 15 g of Chromasorb W was added and the slurry refluxed for 1 hour. The chloroform was evaporated to dryness on a rotary evaporator, under vacuum. This was then packed into a 1.8 m long and 6 mm od spiral pyrex tube by means of a vacuum pump. Uniform packing was obtained by repeatedly tapping the tube firmly along its entire length until no further settling accurred. The column was then conditioned at 240°C for 48 hours under a nitrogen gas flow of 60 ml/min. The column was not connected to the detector during this conditioning phase.

Gas Chromatograph

The gas chromatograph used was a PYE SERIES 104 CHROMATOGRAPH, equipped with a radioactive (10 millicurie) ^{63}Ni electron capture detector. The output was fed into a Honeywell 12-inch recorder.

The assembly is shown in Figures 4A and B.

Fig. 4. Gas Chromatography Assembly

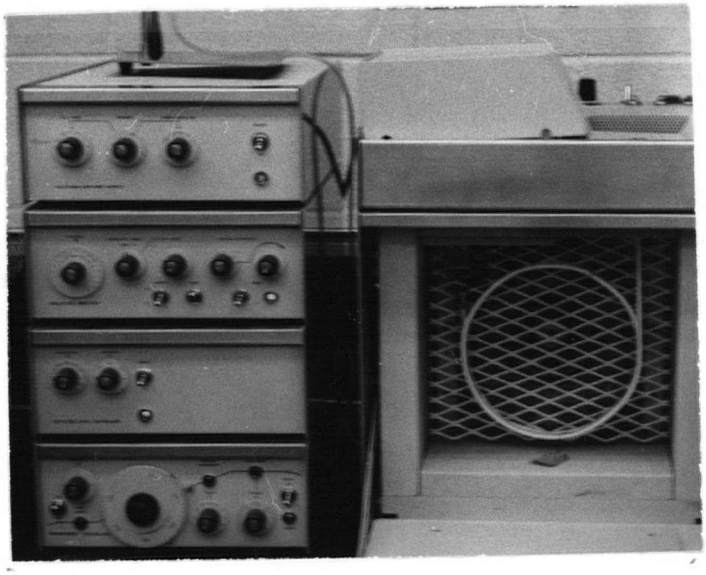


Fig. 4A. Gas Chromatograph, showing circular column

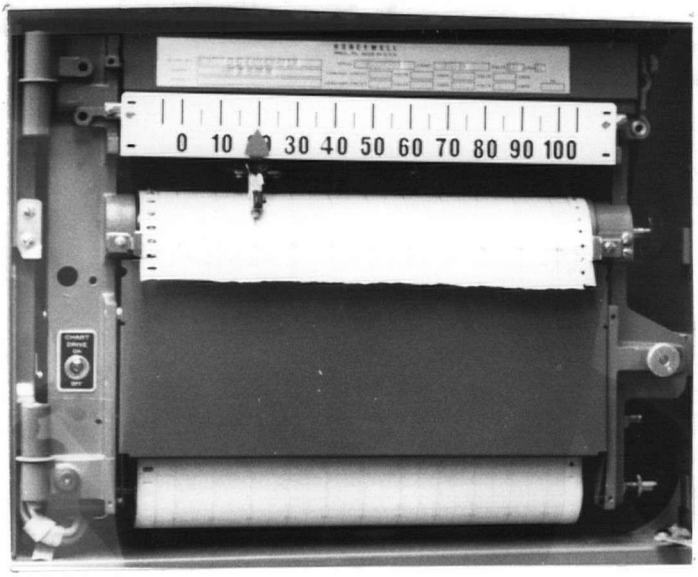


Fig. 4B. Honeywell Recorder

Operating Conditions

Column temperature	225°C
Injector block temperature	250°C
Detector temperature	265°C

Carrier gas, purified nitrogen at a flow rate of 60 ml per minute.

Chart speed 30 inches per hour.

5 µl samples injected with a Hamilton 701 N, 10 µl syringe.

Under these operating conditions the methyl ester appeared as a sharp peak with a retention time of 1 minute.

Greenhouse Experiments

Absorption and Translocation

Three week-old plants of Royal Chantenay carrots of uniform size were removed from vermiculite and transferred to nutrient solution (see Appendix I), contained in 100 ml test tubes, at the rate of one plant per tube, by the technique of Crafts and Yamaguchi (109). A gentle stream of air was bubbled continuously through the solution by means of a capillary tube. Transpirational losses were made up daily with distilled water. After two days, when the plants had recovered from traumatic effects, they were treated with the labeled herbicide.

Three experiments, each replicated twice, were conducted. The first was a control. In the second and third the herbicide was applied to the oldest leaf and to the nutrient solution respectively. The rate was 1 µCi (1 µM) per treatment. To prevent

run-off from the leaf, the solution (50 µl) was applied with a micro syringe. For the root treatment the 1 µCi (50 µl) was injected into the nutrient solution.

The plants were removed at intervals of 1 and 3 days. The roots and treated leaves were washed with 0.01 N ammonium hydroxide solution, followed by distilled water. The water was blotted off and the plants were pressed flat under sheets of blotting paper and quickly frozen under a layer of dry ice. The plants were then freeze dried, mounted and radioautographed. A three week exposure gave satisfactory images.

Distribution, Metabolism and Localization

Two month-old carrot plants, with taproots about 2 cm in diameter and 8 - 10 cm in length were removed from vermiculite and transferred to nutrient solution contained in 1 liter beakers, at the rate of 5 plants per beaker. Care was taken to preserve the secondary roots during the uprooting and washing processes. Aeration and other procedures were similar to that described perviously. After 2 days stabilisation 5 mg radioactive picloram dissolved in 5 ml of 50% ethanol was added to the nutrient solution in each beaker. After a feeding period of 5 days the plants were harvested and the storage and secondary roots washed thoroughly with 0.01 N ammonium hydroxide solution, followed by distilled water. The different parts of the plants were sectioned and the leaves, taproots and secondary roots collected together and weighed separately. This provided the materials needed for the distribution, metabolism and

localization studies.

Distribution of Radioactive Picloram within the Whole Plant

Two grams of leaves and 5 grams each of the tap and secondary roots were ground with silica sand in 10 ml acetone - methanol (1:1) mixture in a mortar. The extracts were centrifuged at 2,000 revolutions per minute. The supernatants were collected, and the pellets extracted twice with 3 ml aliquots of the solvent mixture. The supernatants for each tissue were pooled together and made up to 20 ml. One ml of each extract was mixed with 10 ml of liquid scintillation fluid (see Appendix II) in a vial, and the counts per minute recorded on a liquid scintillation counter (Model Mark I, Nuclear Chicago).

The experiment was replicated twice. The average counts per minute per gram of tissue and the total cpm for the leaves, taproots and secondary roots were computed.

Determination of Metabolites in Leaves and Taproots

Twenty-five grams of leaves or 100 grams of taproots were blended for 5 minutes with 100 ml acetone - methanol (1:1) mixture and centrifuged. The extract was flash evaporated to about 10 ml and this was extracted with 3 x 5 ml ether. The ether layers were pooled together and concentrated to about 5 ml. An aliquot of this extract (10 - 40 µl) was spotted on a cellulose TLC plate. Solutions of picloram and its methyl ester (only other derivative of picloram available) were also spotted on the same plate and developed in the solvent system hexane + acetone + methanol + acetic acid, 50 + 7 + 5 + 2 (v/v).

Radioautograms of the developed TLC plates were made.

Localization of Picloram in Carrot Longitudinal Section

Longitudinal sections, about 1 mm thick, were obtained by shaving the carrot with a metal planer. The sections were selected for uniformity, washed and freeze dried. The dried sections were mounted on blotting paper and radioautographed.

Photodecomposition Studies

Stock Solutions

1. Picloram	^{14}C carboxyl labeled, activity 1 µCi/uM, 2 mg dissolved in 2 ml methanol.
2. Potassium salt picloram	2 mg picloram dissolved in 2 ml 0.005M KOH in methanol.
3. Methyl picolinate	2 mg picloram methylated with 1 ml diazomethane-ether reagent. The ether evaporated to dryness and the residue dissolved in 2 ml methanol.
4. Ultraviolet source	short wave length, 253.7 nm.

Batches of three 5 µl aliquot each of the solution 1,2 and 3 were spotted separately on three TLC plates. The solvent was evaporated to dryness and the three spots on each plate exposed to 0,24 and 48 hours respectively, to the UV source, enclosed in a "Chromatovue" box. The plates were then developed in solvent system: water + methanol + acetic acid, 80 + 20 + 2 (v/v), to a previously marked 15 cm line. They were then dried at low temperature (40 - 50°C) with a continuous current of air to remove the acetic acid. The plates were then taken to the dark room, where each one was pressed in contact with an X-ray film.

The film was kept in position by another glass plate and the edges taped together. The whole operation was done under safety light conditions. The plates were stored in a light proof drawer, lined with lead sheets, to prevent stray radiation reaching them. After one week exposure the films were removed and processed in the standard way for ordinary negatives. The R_f values of the unexposed and exposed compounds and their degradation products were computed. The extent of degradation of each compound was obtained by carefully scratching the corresponding spot from the TLC plate, transferring the cellulose into a vial containing 10 ml of the scintillation fluid, (see Appendix II). Radioactive counts were obtained by the use of a liquid scintillation counter. The extent of decomposition was computed from the counts per minute.

RESULTS

Potatoes

All four varieties of potatoes contained detectable amounts of picloram. For each variety the picloram + linuron treatments consistently gave lower amounts of picloram residues than the picloram alone treatments. No picloram residue was found in the controls.

The means of the picloram alone treatments ranged from 3.5 to 4.2 ppb with a grand mean of 3.9 ppb of picloram residues. The means of picloram + linuron treatments ranged from 2.4 to 2.9 ppb with an overall mean of 2.7 ppb of picloram residues. The difference of 1.2 ppb between these two overall means was significant at the 1% level.

Since there was no significant variety or variety x treatment interaction, there is little evidence that the varieties responded differently to the treatments.

An average recovery of 73% was obtained when untreated potatoes were fortified with known amounts of picloram.

The picloram peaks (as methyl ester) are shown in Figures 5A, B and C. The residues of picloram in the treated potatoes (in parts per billion fresh weight) are given in Table III.

TABLE III

Residues of Picloram in ppb Fresh Weight Potatoes

Variety	Treatment			
	Picloram @ 2 oz		Picloram @ 2 oz + Linuron @ 24 oz	
Netted Gem	4.0	4.0 ± 0.2	2.4	2.8 ± 0.8
"	4.2		2.6	
"	3.8		4.0	
"	4.2		2.4	
Kennebec	3.3	3.5 ± 0.2	2.8	2.4 ± 0.5
"	3.6		2.9	
"	3.6		1.9	
"	3.5		2.1	
Norland	4.9	4.2 ± 1.0	2.7	2.9 ± 0.7
"	4.5		3.6	
"	2.7		2.1	
"	4.9		3.3	
Pontiac	3.3	3.8 ± 1.0	2.9	2.6 ± 0.3
"	4.7		2.8	
"	4.7		2.1	
"	2.7		2.7	

Grand mean picloram treatment for all varieties	3.9
Grand mean picloram + linuron treatment for all varieties	2.7
Difference of means	1.2**

** significant at the 1% level

Table IV gives the percentage recovery of picloram from untreated potatoes, fortified with known amounts of the herbicide.

TABLE IV

Recovery of Picloram from Potatoes

ppm picloram added	ppm picloram found	% recovery	average recovery
0.02	0.017	85	
0.02	0.015	75	
0.02	0.015	75	73%
0.04	0.031	75	
0.04	0.026	65	
0.04	0.028	70	

Carrots

No residues of picloram were detected in either the pre-emergence or the post-emergence treatments, though an average recovery of 70% was obtained with carrots spiked with picloram.

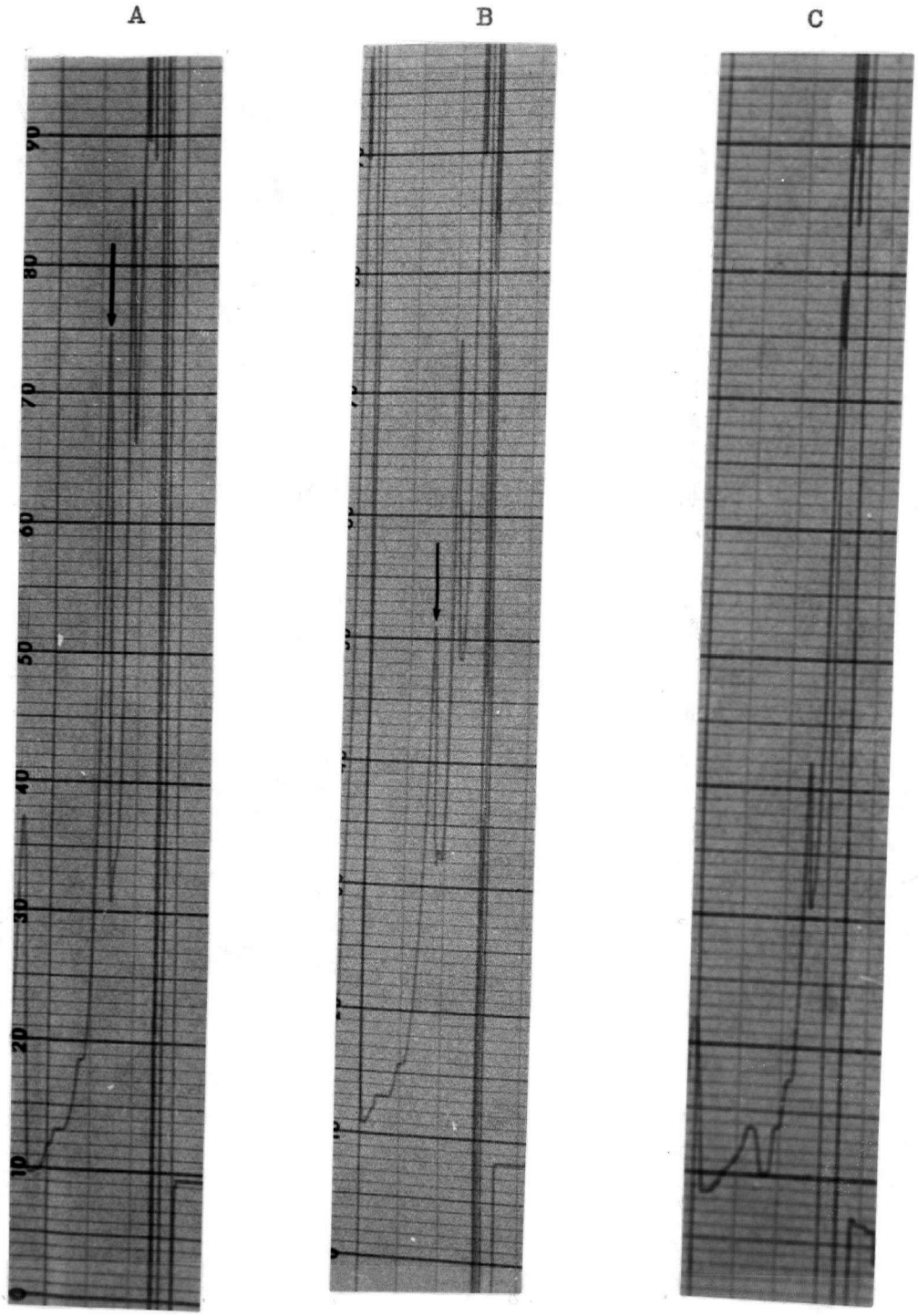


Fig. 5. Gas Chromatographic peaks of potato tuber extracts
(A) Control spiked with picloram before analysis
(B) Pre-emergence treatment with picloram @ 2 oz/acre
(C) Control

Vertical arrows show peaks given by methyl ester of picloram.

Absorption and Translocation

Leaf Treatment

Radioautographs for the leaf applications are shown in Figures 6A and B. These clearly demonstrate that basipetal translocation of picloram and redistribution throughout the whole carrot plant had taken place. An increase in the amount of radioactivity in the leaves was noticed from day 1 to day 3. No such increase was noticed in the roots.

Fig. 6A. Results of treatment of 3-week old carrot plants with labeled picloram. Radioautographs (right) and mounted plants (left). Dosage 1 µCi applied on compound leaf (shown by arrow). Treatment time: 1 day.

Fig. 6B. Results of treatment of 3-week old carrot plants with labeled picloram. Radioautographs (right) and mounted plants (left). Dosage 1 µCi applied on compound leaf (shown by arrow). Treatment time: 3 days.

Root Treatment

Radioautographs for the root applications are shown in Figures 7A and B. These demonstrate that acropetal translocation in the carrot plant had taken place. On day 1 more radioactivity was concentrated in one leaf. On day 3 the radioactivity was distributed throughout the entire plant. The absence of secondary roots in this treatment was very prominent. Untreated plants did not show any darkening on the X-ray film.

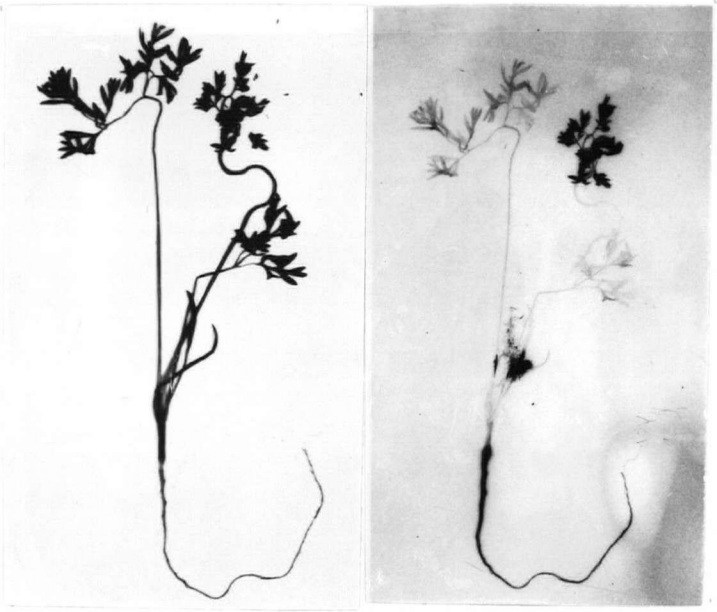


Fig. 7A

1 day

Fig. 7. Results of treatment of 3-week old carrot plants with labeled picloram. Radioautographs (right) and mounted plants (left). Dosage 1 µCi applied to nutrient solution.
Treatment times: (A) 1 day, (B) 3 days.

Fig. 7B

3 days

Distribution of Picloram in 2 month-old Carrot Plants

The distribution of radioactivity in the plants is shown in Table V.

TABLE V

Distribution of Radioactivity

Tissue	cpm/g fresh tissue	Total cpm	% Distribution/g fresh tissue	% Distribution within whole plant
Leaves	15,500	558,000	75	54
Storage roots	3,800	456,000	19	44
Secondary roots	1,280	20,480	6	2

On a fresh weight basis the leaves accumulated four times more radioactivity than did the storage roots. The weight of the storage roots was about four times that of the leaves. Thus on a whole plant basis there was roughly the same amount of radioactivity in the leaves as in the storage organs. Very little radioactivity was found in the secondary roots.

Metabolism of Picloram in Carrot Leaf and Taproot

The radioautogram of the leaf and carrot extracts is shown in Figure 8.

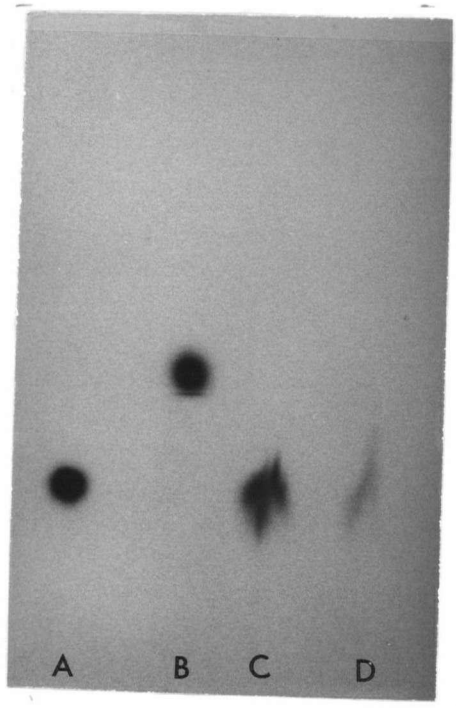


Fig. 8. Radioautogram of carrot taproot and leaf extracts.
(A) picloram, (B) methyl ester picloram, (C) carrot taproot, (D) carrot leaf.

Because of the low level of radioactivity in the tissues it was necessary to spot the maximum amount of extracts on the TLC plates. This maximum amount was limited by the fact that after a few microliters had been spotted (different for leaf and root extracts), the spot became greasy and dried out with great difficulty. For this reason the amount of radioactivity in the taproot and leaf extracts is qualitative and not quantitative. The spreading observed on the radioautogram

was due to overloading. The centre of the spots given by both extracts corresponded to the parent picloram.

Localization of Picloram in Carrots Sections

Radioautograms of the longitudinal carrot sections are shown in Figure 9.

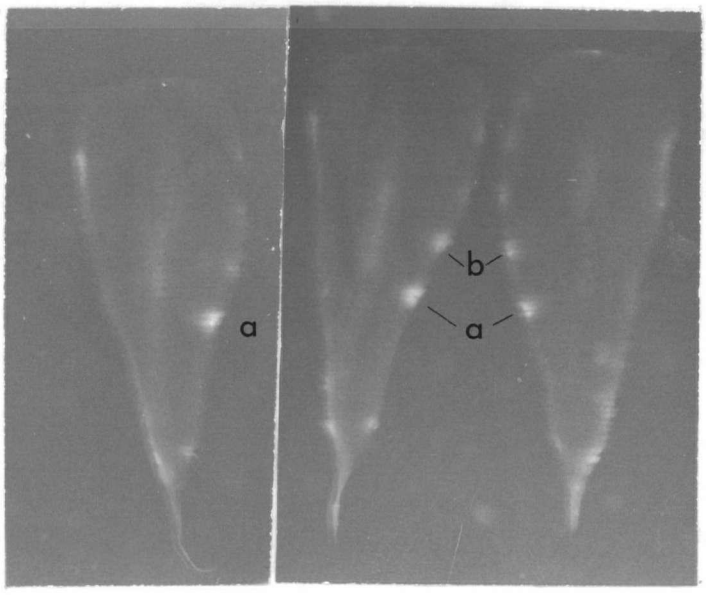


Fig. 9. Negative radioautograms of longitudinal carrot sections. The radioautograms from left to right correspond respectively to the 5th, 6th and 7th sections in the mount shown in Figure 10.

The radioactivity is localised mostly in the xylem tissue. The radioactivity in the cortex might be due to accumulation of the radioactive solution in the holes, cracks and folds present in the cortex. Some of these radioactive spots (a and b in Figure 9) are located where the secondary roots joined to the taproot. It has been shown however (Table V)

that the secondary roots did not accumulate more radioactivity than the taproot.

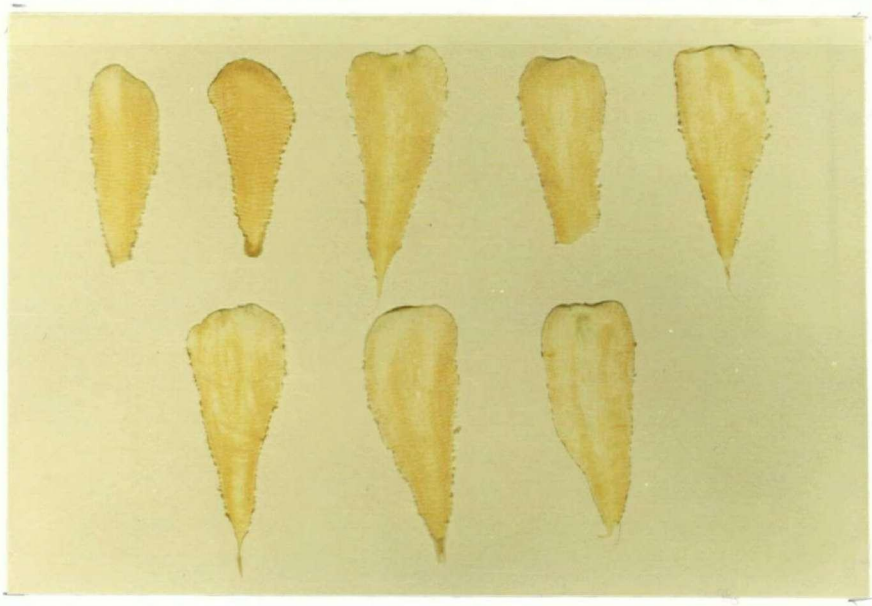


Fig. 10. Mounted longitudinal sections of carrot. The sections from top left to bottom right are from the outside to the middle of one carrot. The negative radioautograms of the 5th, 6th and 7th section, from top left, are shown in Figure 9.

PHOTODECOMPOSITION

The R_f values of the photoproducts of picloram, its potassium salt and its methyl ester are given in Table VI.

TABLE VI

R_f Values of Control and Irradiated Compounds

Compound	R_f (control)	R_f (irradiated)
Picloram	0.79	r* 0.53 0.60 0.69 0.80
Potassium salt picloram	0.76	r* 0.53 0.60 0.69 0.77
Methyl ester picloram	0.58 (major) 0.69 0.82 (very weak)	r* 0.58 0.69 0.85

r* residual spot at origin of chromatogram.

The extent of photodecomposition after 24 and 48 hours is given in Table VII.

TABLE VII

Extent of Decomposition after 24 and 48 hours Exposure to UV light

Compound	Amount decomposed (%)	
	24 hours	48 hours
Picloram	50	65
Potassium salt	50	55
Methyl ester	85	90

For all three compounds, both the 24 and 48 hours exposure gave "residual spots" at the origin of the chromatogram. The unexposed picloram and its potassium salt gave only one spot each when chromatographed. The methyl ester, however, gave three spots, one of which (R_f 0.82) is very weak and barely visible as shown in Figure 14. Each of the three compounds gave several radioactive products on exposure to UV light. The molecular structures of these have not been identified, but compounds with identical R_f values are designated by identical letters. Picloram and the potassium salt gave three photoproducts each, and all three have the same R_f values (0.53, 0.60, 0.69). The methyl ester gave two photoproducts one of which (R_f 0.69) corresponds to one of the above mentioned three compounds.

Table VII shows that picloram and its potassium salt are 50% decomposed within the first hour, whereas the methyl ester is 85% decomposed in the same time.

The radioautograms of the thin layer chromatograms are shown in Figures 11A, B and C.

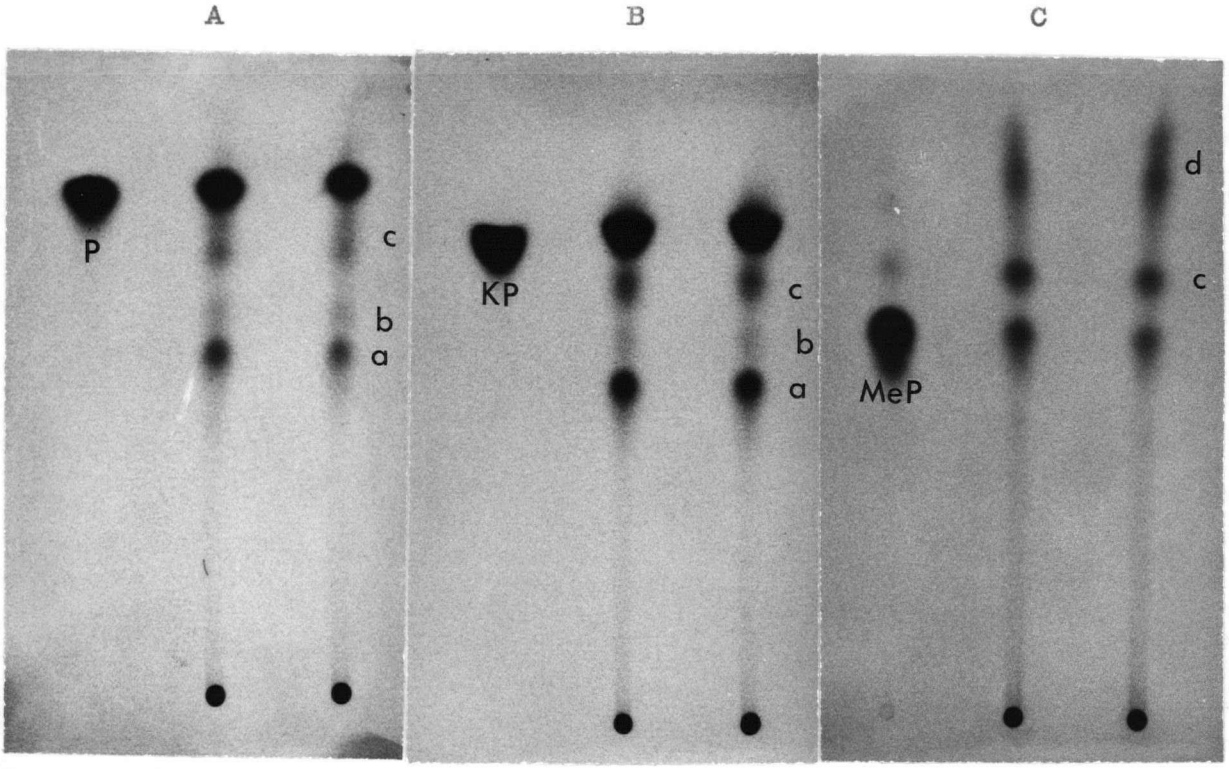


Fig. 11. Radioautograms of thin layer chromatograms spotted with: picloram (A), potassium salt picloram (B), and methyl ester picloram (C). Each plate was exposed to UV light (253.7 nm), for 0, 24 and 48 hours (left to right) and developed in the solvent system: water + methanol + acetic acid, 80 + 20 + 2 (v/v). Spots given by the unexposed compounds are designated by P (picloram) KP (potassium salt picloram) and MeP (methyl ester picloram). Residual spots at the origin were obtained for all three compounds, for both 24 and 48 hour exposures. Photoproducts having the same R_f values are indicated by identical letters.

DISCUSSION

Potatoes

The main effect of picloram was significant at the 1% level, but there was no significant variety or variety x treatment interaction. Thus there is little evidence that the varieties responded differently to the treatments.

The picloram treatment consistently gave higher amounts of residues, in all four potato varieties, compared to the picloram + linuron treatment. The ratio of residues in the picloram to the picloram + linuron treatment is about 1.5 : 1. Yield data were not recorded but were markedly higher in the picloram + linuron treatments. The reduced picloram residues could thus be associated with the diluting effect of higher yields which resulted from the addition of linuron. Dilution of pesticides in plants as a result of plant growth has been suggested (20). The yields of the treatments were not actually measured because the rate of 2 oz picloram per acre, when applied alone, killed about 25% of the plants. This amount is evidently approaching the lethal rate for potatoes. When the higher yield of the picloram + linuron treatment is considered it would appear that, on a plant basis, more picloram is accumulated in the picloram + linuron treatment than in the picloram alone treatment. This could be due to there being more physiologically active plants resulting from the former treatment.

At harvest time the plants in the picloram + linuron treatment had a normal root system, and had no visible injury symptom either on the foliage or on the tubers. On the other hand the plants in the picloram treatment were stunted, and had little foliage. Most tubers were small, and developed a corky tissue on the surface. Splitting of some tubers was also observed. These injuries are shown in Figures 12A nd B.

A B

Fig. 12. Injury on surface of potato tubers due to picloram. (A) Formation of corky tissues. (B) Splitting of tuber in addition to formation of corky tissues.

The only residue study of picloram in crops under field conditions was described by Bjerke et al (10) where he obtained about 0.05 ppm picloram in wheat grain. In the present study the residues for the picloram and picloram + linuron treatments, for all four varieties, averaged 3.9 and 2.7 ppb respectively.

Assuming an 80% moisture content in fresh potatoes (104), residue levels of 0.02 and 0.014 ppm on a dry matter basis are obtained for the two treatments. No tolerance* has yet been set for picloram in food. On the basis of Jackson's (47) and Hardy's (37) toxicological studies of picloram it may be concluded that this level is not injurious to any of the organisms tested.

The level of picloram residues present in the tubers at harvest may be so small as not to cause any acute toxicities to humans and animals. However the picloram residue level present in the soil, may be significant to the point of adversely affecting a subsequent crop. No picloram residues were determined in the soil in the present study. Figure 13 shows a leaf of a potato plant growing in the experimental plot, in the spring following treatment. The leaf clearly shows the deformation typical of auxin herbicides. Since the plants were growing from tubers left over from the previous season, it is not possible to say whether the deformation is due to the picloram residue present in the soil alone or to picloram residues present in the tubers alone or to a combination of these two factors.

*Tolerance: the permitted concentration of a residue in or on a food.

Fig. 13. Potato leaf deformation, resulting from residual effect of picloram.

Studying the effect of linuron on the uptake of inorganic ions (81), it has been shown that when linuron was incorporated into the nutrient solution it increased the uptake of Ca^{++}, Mg^{++}, NO_3^-, SO_4^{--} ions and there is a decrease in the uptake of water. It was suggested that this could be brought about by changes in membrane permeability. Whether incorporation of linuron increases membrane permeability leading to any change in picloram uptake cannot be answered yet. On the other hand, Davis et al (17) showed that in mesquite, huisache and bean plants, the transport of picloram was reduced when used in combination with paraquat.

Onsager et al (82) showed that the residues of organo chlorine insecticides in mature sugar beets were directly proportional to the residues in the soil at the time of planting. In the present study, whether picloram was sprayed alone or mixed with linuron, it was applied at a constant rate of 2 oz per acre, giving two different residue levels. Therefore, for this herbicide, it seems that the proportionality factor differs depending whether the herbicide is applied alone or in combination with linuron.

Lichtenstein et al (64) showed that even different varieties of the same plant (carrot) could absorb different amounts of insecticides from soil. In our study it was shown that all four varieties of potatoes had the same capacity for accumulating picloram from soil. It should be mentioned that the residue accumulated by the tuber at harvest does not depend only on one process, namely uptake from soil. It is the amount that has been absorbed by the roots, translocated into the plant and later transported into the tuber after metabolism by the leaves and shoots (if any) and leakage by the roots into the soil have taken place. Excretion of foliage applied picloram by roots of bean plants has been confirmed (46). Picloram may not be metabolised by potato plants because they are very susceptible to the herbicide, as are bean plants.

One gas chromatographic peak as such, in the qualitative and quantitative determination of pesticide residues extracted from plants or animals, may lead to serious errors if not

confirmed by an independent method. In residue analysis the method of choice is thin layer chromatography. In this study two independent methods were used, namely thin layer chromatography and gas chromatography. The purpose of the TLC was twofold:

1. to remove interfering plant products and
2. to isolate the methyl ester by making use of its R_f value.

The ensuing gas chromatography then provided a confirmatory test as well as a quantitative evaluation.

Carrots

The observation that no residues of picloram were found in carrots was interesting because, throughout the literature on pesticide residues, root crops are mentioned as the greatest absorbers of organochlorine compounds. Lichtenstein et al (63,64) found that carrot translocated and accumulated more residues of organochlorine insecticides than any other root crop, with the exception of parsnip.

As the absorption and translocation, distribution, metabolism and localization studies were all done to cast some light on the absence of picloram in the carrot taproots, they are all discussed together here.

Absorption and translocation of picloram in a wide spectrum of plants have been shown by several workers (12,14, 17,44,46,49). However it has not been reported whether the carrot plant can absorb and translocate picloram. The results

of the absorption and translocation experiments clearly show that young carrot plants can absorb and translocate picloram, when applied both to the foliage and to the roots. This would indicate that both pre-emergence and post-emergence treatments would be effective in getting the picloram into the young carrot plant. Further it was shown that 2-month old carrot plants fed with radioactive picloram in the nutrient solution absorbed and translocated varying amounts of radio-activity in all of its tissues. Since picloram has a half-life of the order of 13 months in soil (29), and since there was enough picloram in the soil to cause leaf deformation in the succeeding year, it may be reasonably assumed that the carrot plant could absorb piclorm present in the soil over its entire growth period.

Since picloram was not detected in the treatments and since about 70% of the added picloram was detected in recovery studies, it may be suggested that the picloram was broken down in the carrot plant. The taproot and leaf metabolism experiment may not have been sensitive enough to detect it because only one carbon atom in the picloram molecule, namely the -COOH group, was labeled. If partial decarboxylation as a mechanism of detoxification was operating, radioactivity would not be detected by radioautography as the labeled CO_2 would be lost through the stomata.

Redemann (87) found that 83% of the radioactivity in mature wheat grain was found as the unchanged picloram.

Meikle (71), however, found that in cotton plants all the radioactivity was present as the parent picloram. Different plant species and even different varieties of the same plant species can degrade a foreign molecule to different extents depending on whether the plant is resistant or susceptible to the foreign molecule (66). To what extent the picloram molecule is degraded in the carrot plant cannot be answered yet.

From Table V it is seen that, either on a weight basis or on the whole plant basis, more picloram was translocated and probably accumulated into the leaves than into the roots. This could probably be one mechanism by which the carrot plant overcomes the herbicidal effect of picloram in the taproot. Carrots must also be more resistant to the chemical than are potatoes as no symptoms of picloram injury were noticed on the treated carrots.

The localization study indicated that within the carrot taproot some radioactivity was found in the xylem. This could represent that amount of radioactive material being transported through the xylem at harvesting time. Lack of radioactivity in the other tissues of the taproot may indicate that the xylem is transporting the material into the leaves which may be acting as a sink.

Photodecomposition

This experiment was not performed under the ideal conditions of a photolysis experiment. However it approaches more closely the conditions that exist when herbicides are present on or around soil particles. Again on soil or plant surfaces, the presence of trace amounts of metallic oxides, serving as catalysts, may greatly accelerate the rate of photochemical reactions. The pH of the medium also influences the breakdown of organic molecules (9).

Exposure to UV light of all three compounds under investigation produced an intense residual radioactive spot at the origin of the developed chromatogram. A similar phenomenon was obtained by Smith (98), working with diquat. Further Smith showed that his residual spot was not diquat and had no phytotoxic properties. Whether these residual spots are due to polymerisation of the compound, by the action of UV light, has not been investigated yet. Again it is not known whether they have phytotoxic properties.

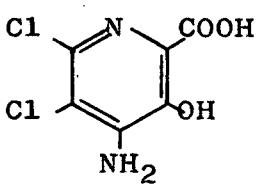
The chromatography of the unexposed methyl ester gave more than one spot but, to date, no isomer of this compound has been reported. On the other hand the chromatography of the unexposed picloram gave only one spot. Purkayastha (86) reported that the thin layer electrophoresis of picloram produced a main anionic and an additional cationic spot.

The identical R_f values produced by the three compounds indicate that they may all have common photoproducts; probably the

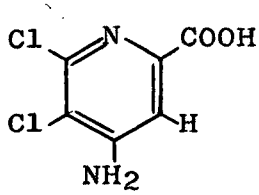
same pathway of degradation may be involved. Identity of a few of these products is essential in suggesting any degradation pathway.

The methyl ester is more rapidly degraded than either picloram or the potassium salt, and the latter two compounds are degraded at nearly the same rates. A probable explanation is that the methyl ester absorbs UV light more strongly than either picloram or its potassium salt. Picloram and its potassium salt behave very much alike in their decomposition rates as well as in the pattern of photoproducts formed.

Plimmer and Hummer (85) reported that the methyl ester is converted into a single major product by the loss of one chlorine atom. He postulated that either the 3-hydroxy-4-amino-5,6-dichloro-2-picolinic acid (I) and/or the 4-amino-5,6-dichloro-2-picolinic acid (II) may be formed:



(I)



(II)

The present experiment suggests the formation of at least two products marked "c" and "d" in Figure 11C. Again the spot "d" could be due to two compounds, unresolved by the solvent system. The formation of several photoproducts is

also supported by the chromatogram shown in Figure 14. The photoproducts are shown by arrows. Here also more than one photoproducts is observed.

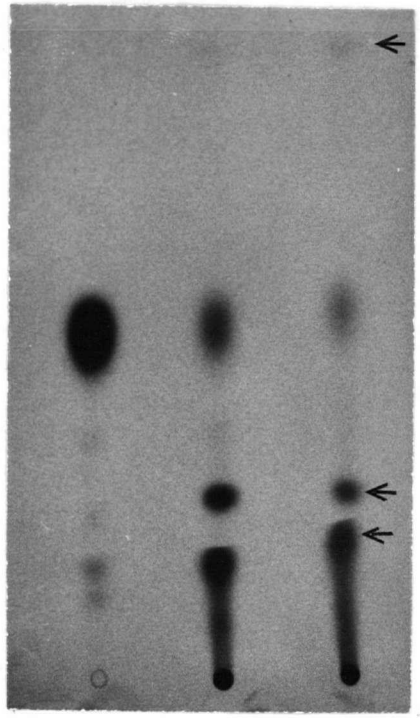


Fig. 14. Radioautogram of thin layer chromatogram spotted with methyl ester picloram and exposed to UV light (253.7 nm) for 0, 24 and 48 hours (left to right). Solvent system: hexane + acetone + methanol + acetic acid, 50 + 7 + 5 + 2, (v/v). The unexposed compound (left) gave four weak spots below the main spot. The photoproducts for the 48 hour exposure are indicated by arrows. Note progressive decrease in intensity of main spot with increased exposure.

Visual comparison of the total intensities of all spots for the 48 hour exposure and 0 hour exposure may indicate that some radioactivity is lost. Since there is only one labeled carbon ($^{14}COOH$) on the molecule, it may be concluded that degradation by a decarboxylation mechanism may be involved.

Photodecomposition may be a significant process by which the picloram herbicide, both in the form of acid and potassium salt, may be broken down on soil surfaces. Ultraviolet radiation may, however, be a very important factor in degrading the methyl ester and may have to be watched for when standard solutions of it are stored for some time.

SUMMARY

Picloram used as a pre-emergence spray at a sub-lethal rate on four varieties of potatoes was found to leave residues of the herbicide in the tubers. A picloram + linuron mixture was found to leave lower amounts of picloram residues than the picloram alone treatments. A dilution factor, resulting from higher yields with the herbicide combination, may be involved. The difference between the means of the picloram and picloram + linuron treatments was significant at the 1% level. However there was no significant variety or variety x treatment interaction.

Pre-emergence and post-emergence treatments of picloram on four varieties of carrots were found to leave no detectable amounts of picloram in the taproots of all varieties. This was investigated further and it was found that young carrot plants can absorb and translocate picloram, when it is applied both to the foliage and to the roots.

Following the distribution of radioactive picloram in 2-month old carrot plants it was found that, on a weight basis, the leaves accumulated about four times more radioactivity than the taproots.

Chromatographic evidence showed that the residual radioactivity, both in the foliage and in the taproots, was probably in the form of the parent molecule, with no other metabolite.

When picloram is present in carrot taproot it is more likely to be present in the xylem than in any other tissue.

Investigation of the stability of radioactive picloram, its potassium salt and its methyl ester under short wave ultraviolet light (253.7 nm) showed that the methyl ester is quite unstable, being 85% degraded into several photoproducts after one hour exposure. Picloram and its potassium salt are somewhat more stable, each being 50% decomposed into several photoproducts after one hour exposure. One common feature of all three compounds is the formation of an intense radioactive spot, at the base of the chromatogram. This was not resolved by the solvent system and may have been due to a polymerisation product.

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Appendix I

COMPOSITION OF NUTRIENT SOLUTION

The following amounts of stock solutions are added to 1,500 ml distilled water and the volume brought up to 2,000 ml.

Stock solution	Volume (ml)
1 M $Ca(NO_3)_2$	10
1 M KNO_3	10
1 M $MgSO_4$	4
1 M KH_2PO_4	2
FeEDTA*	2
Micronutrients**	2

* 1 ml stock solution contains 5 mg of Fe.

** Micronutrient stock solution contains 2,86 g of H_3BO_3 (boric acid), 1.81 g of $MnCl_2.4H_2O$ (manganese chloride), 0.11 g of $ZnCl_2$ (zinc chloride), 0.05 g of $CuCl_2.2H_2O$ (copper chloride), and 0.025 g of $Na_2MoO_4.2H_2O$ (sodium molybdate) per liter.

Appendix II

COMPOSITION OF SCINTILLATION FLUID

Dioaxane	800 ml
Toluene	200 ml
Ethanol	30 ml
PPO (2,5-diphenyloxazole)	7 g
POPOP (2,2'-paraphenylene bis-5-phenyloxazole)	200 mg
Naphthalene	50 g
Cab-O-Sil	36 g

Appendix III

CHEMICAL NAMES OF PESTICIDES USED IN TEXT

1.	Aldrin	1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo, exo-5, 8-dimethanonaphthalene
2.	Amiben	3-amino-2, 5-dichlorobenzoic acid
3.	BHC	1,2,3,4,5,6-hexachlorocyclohexane
4.	2,4-D	2,4-dichlorophenoxyacetic acid
5.	2,4,5-T	2,4,5-trichlorophenoxyacetic acid
6.	2,4-DB	4-(2,4-dichlorophenoxy) butyric acid
7.	DDT	1,1,1-trichloro-2, 2 bis (p-chlorophenyl) ethane
8.	Dicamba	3,6-dichloro-2-methoxybenzoic acid
9.	Diquat	1,1'-ethylene-2, 2'-dipyridylium dibromide
10.	Fenoprop	2-(2,4,5-trichlorophenoxy) propionic acid
11.	Linuron	3-(3,4-dichlorphenyl)-1-methoxy-1-methyl urea
12.	MCPA	2-methyl-4-chlorophenoxyacetic acid
13.	Paraquat	4,4'-bipyridylium-1, 1'-dimethyl dichloride
14.	Picloram	4-amino-3,5,6-trichloropicolinic acid
15.	Simazine	2-chloro-4, 6-bis (ethylamino)s-triazine
16.	Tordon	4-amino-3,5,6-trichloropicolinic acid (picloram)