SELECTIVITY AND MODE OF ACTION OF NITROFEN AMONG ECHO RAPE, REDROOT PIGWEED AND GREEN FOXTAIL

A thesis

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ABSTRACT

The selectivity of 2,4-dichlorophenyl p-nitrophenyl ether (nitrofen) between rape (Brassica campestris L., var. Echo) and two weed species, redroot pigweed (Amaranthus retroflexus L.) and green foxtail (Setaria viridis (L.) Beauv.), was determined quantitatively by a replicated dosage-response experiment. On an $E.D._{50}$ basis foxtail and pigweed were found to be, respectively, 5.7 and 63 times more susceptible than rape.

Selectivity was divided into three parameters, viz. differential spray retention, differential penetration and differential effects within the plant. Differences in retention were measured with a water soluble dye, while differences in penetration were determined with ^{14}C labelled nitrofen. There was less retention (.7) on the foxtail than on the rape and less penetration (.6) in the pigweed than in the rape. Under the conditions of these tests it was estimated that, on an internal basis, foxtail and pigweed were, respectively, 9 and 98 times more susceptible to nitrofen than rape.

Only limited amounts of the label of ^{14}C-nitrofen were translocated in all three species. Plants treated with ^{14}C-nitrofen under high light conditions produced several labelled compounds of different molecular size and chromatographic properties. The time at which these compounds were first detectable depended on light intensity.

It is suggested that at least two of these compounds are lipid-nitrofen conjugates or nitrofen polymers and that others are formed by cleavage of nitrofen at the ether linkage. It is also suggested that the formation of these compounds is not the prime cause of the herbicidal effect of nitrofen on foliage.

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INTRODUCTION

As early as 1960 the first of the diphenyl ether herbicides, viz. 2,4-dichlorophenyl p-nitrophenyl ether (nitrofen) was synthesized. Since that date other diphenyl ether herbicides have been developed but nitrofen (otherwise known as TOK, NIP and F.W. 925) remains the most important.

In Western Canada nitrofen is used as a post-emergence herbicide primarily in rape and mustard crops. The major weakness of nitrofen when used as a post-emergence spray is the rapidity with which weeds become resistant, creating a need for fairly precise timing if application within the economical 1.2 to 2 lb. active ingredient (ai)/acre range is to be successful.

Although there is a multitude of data on the performance of nitrofen as a herbicide in field trials, both on its own or mixed with other herbicides, there is very little information available on the reason for its selectivity between certain different genera. This selectivity does not seem to follow the usual division between monocotyledonous and dicotyledonous species.

Nitrofen shows selectivity between the brassicas and many other genera. The aim of this study is to determine the cause or causes of this selectivity.

Previous workers have shown that selectivity of a post-emergence herbicide between species is due to one or more of the following factors:

1. Differential retention.
2. Differential penetration.
3. Differential translocation.
4. Differential biochemical effects within the plant.

In order to establish which factors were responsible for the selectivity exhibited by nitrofen, each was looked at systematically.

Rape (Brassica campestris L. var. Echo) was selected as the crop species and was compared with redroot pigweed (Amaranthus retroflexus L.) and green foxtail (Setaria viridis (L.) Beauv.) as typical examples of dicotyledonous and monocotyledonous weeds, respectively.

LITERATURE REVIEW

Introduction

The herbicidal properties of 2,4-dichlorophenyl p-nitrophenyl ether (nitrofen) were first noted by Wilson and McRae in 1960. In this work a formulated aqueous dispersion was applied to cultivated soil at the rate of 1 lb. active ingredient (ai)/acre. At this level the chemical prevented the germination of seeds of curled dock (Rumex crispus L.) by 90 percent and prevented the germination of pigweed (Amaranthus retroflexus L.), crabgrass (Digitaria sp.), millet (Sorghum halepense (L.) Pers.) and sorrel (Rumex acetosella L.) by 100 percent.

Workers in New Zealand were the first to discover the selectivity of nitrofen between brassica crops and weeds. Independent work by Mason (1963) and Thompson (1963) showed that when nitrofen was used as a post-emergence spray, rates of 2 lb. ai/acre gave good control of many weed species when applied at the seedling stage. Later, both Mason (1964) and Thompson (1964) found that the addition of small quantities of 4-amino-3, 5,6, trichloropicolinic acid (picloram) to the spray formulation reduced the amount of nitrofen necessary for weed control from 2 to 1 lb. ai/acre.

Nitrofen shows selectivity between various other crop and weed species. Furuya and Arai (1966) showed that several diphenyl ether herbicides, including nitrofen, were effective in inhibiting shoot emergence of barnyard grass (Echinochloa crusgalli (L.) Beauv.) in transplanted rice (Oryza sativa L.). The selectivity of nitrofen has also been successfully employed in many horticultural crops. Marcelli (1966) used nitrofen to control Artemissa vulgaris L. in tobacco (Nicotiana tabacum L.). Noll

(1966) used nitrofen for pre-emergence weed control in carrots (Daucus carota L.), celery (Apium graveolens L.) and parsley (Petroselinum crispum Nym.) while Kempen et al. (1968) used the herbicide as a post-emergence treatment on the same crops.

Except in the high value cash crops it is not economical to use nitrofen as a pre-emergence herbicide as rates of from 4 to 8 lb. ai/acre are too costly.

The performance of nitrofen in field trials has been extensively investigated but there is a lack of information on the quantitative aspects of its selectivity. Because of this lack of information it is necessary to review the techniques developed for other herbicides in the assessment of selectivity.

Selectivity of Herbicides

Blackman (1952) was the first to make a precise assessment of the relative toxicity of herbicides. He suggested that when herbicides were applied to plants as foliar sprays, retention by and penetration into the shoot, transport and localized accumulation were all factors which determined differences in response either between compounds or between species. He proposed that the precise assessment of relative toxicity must include studies of the effects on whole plants and at cell level. He found that the percentage mortality of plants bore a sigmoid relationship to the concentration of toxicant in the spray solution. He suggested that accurate comparisons of relative toxicity can only be obtained when variation in response is measured at several dosages and the data treated by probit analysis.

Spray Retention

It has long been recognized that both the physical and chemical characteristics of the spray and the nature and aspect of leaf surfaces play an important role in determining retention by the foliage. Åslander (1927) concluded that the effectiveness of sulphuric acid as a selective herbicide for cereal crops was dependent on the differential retention of the spray droplets due to differences in leaf arrangement and waxiness. Later, Blackman and Templeman (1936) showed that the addition of a surfactant to the spray solution enhanced retention on waxy surfaces. Smith (1946) demonstrated that growth depression of kidney beans (Phaseolus vulgaris L.), induced by 2,4-dichlorophenoxyacetic acid (2,4-D), varied with droplet size and the spray volume applied and that these factors were related to retention. Brunskill (1956) showed that droplet rebound was related to droplet size, velocity and surface tension and the advancing contact angle of the spray liquid on the target surface.

The water soluble dye tartrazine was used by Blackman, Bruce and Holly (1958) to estimate the spray retention on five species. The volume of spray retained by the foliage was estimated by measuring the optical density of foliar washings. These workers found that the volume of spray retained per grm. dry weight of foliage was dependent on the growth stage of a given species, the volume of spray applied and the surface tension of the spray solution. They found that differences in retention, either between species or between growth stages, were linked to the ratio of leaf area to shoot weight, the nature of the leaf surfaces, the angle of incidence at which the droplets strike the leaf and the localized accumulation.

Retention was found to be maximal when the leaf area ratio was high, the surfaces wetted to the point of run-off and the laminae arranged horizontally. Furmidge (1962a) found that for any given surface the maximum retention (i.e. at the point of incipient run-off) was related to the theoretical retention factor which he calculated from the dynamic advancing and receding contact angles and from the surface tension of the spray liquid. However, he found this to be true only for perfectly smooth surfaces. The imperfections present on all leaf surfaces resulted in either premature run-off or increased retention. These factors combined to produce more than the expected retention at low spray volumes and less than that expected at high spray volumes. Furmidge (1962b) showed that on artificial surfaces retention increased when droplet size was reduced. He also demonstrated that retention decreased as droplet impact velocity increased and that this was particularly true for solutions with low surface tensions.

The above mentioned experiments show that there are many factors, determined both by the plant and by the spray solution, which influence spray retention. However, many workers (Blackman et al, 1958; Davies et al., 1967; Hibbitt, 1969; Schafer, 1970) have shown that when plants of different species, at the same growth stage, were subjected to the same spraying conditions, differences in retention played a major role in herbicide selectivity.

Herbicide Penetration

The aspects of herbicide penetration into foliage are extremely complex and their importance has been reviewed several times (Currier and Dybing, 1959; Foy, 1964; Robertson and Kirkwood, 1969). Penetration of

herbicides may occur both through the stomata and through the cuticle (Currier and Dybing, 1959). The penetration of the stomatal pore depends on the size of the opening (Dybing and Currier, 1961) and the surface tension of the spray solution (Van Overbeek, 1956). The entry of herbicides through the stomata is rapid and by mass flow while cuticular penetration is slow, continuous and occurs by diffusion (Foy, 1964).

The structure of the cuticle has been studied by many workers (Frey-Wyssling, 1953; Van Overbeek, 1956; Frey-Wyssling and Muhlethaler, 1965; Martin, 1966). The cuticle consists of an outer lipoidal layer of waxes and cutin and an inner hydrophilic layer of pectin and cellulose. No clear distinction exists between these layers (Sargent, 1965), and it appears that penetrants either follow an aqueous or a lipoidal route through the cuticle (Crafts, 1956; Crafts and Foy, 1962). Cutin hydrates and swells when wet and this is thought to increase its permeability. Hydrocarbons and surfactants are believed to solubilize into the cuticle and/or the plasmalemma thus displacing lipid molecules and increasing permeability (Foy, 1964). Foy suggested that the waxy and fatty constituents of the cuticle may constitute an important pool for holding fat soluble compounds, thus retarding partition into the symplast. Franke (1967) suggested that the hydrophilic nature of the epidermal cell walls allowed entry of water soluble compounds but inhibited movement of lipophilic substances. He suggested that these latter compounds must enter via the waxy cuticle and then through the ectodesmata.

Various environmental factors influence penetration. Currier and Dybing (1959) suggested that by stimulating photosynthesis light may affect penetration. Photosynthesis of the guard cell chloroplasts causes stomatal opening and facilitates herbicide entry. Increase in photosynthesis also

stimulates translocation of solutes, including herbicide molecules. Removal of the herbicide from the proximity of the cuticle increases the concentration gradient across the cuticle and probably enhances penetration. Kylin (1960) demonstrated that light increased penetration by enhancing permeability of the plasmalemma. Further evidence suggests that light may control the active uptake of foliar applied compounds (Sargent and Blackman, 1965).

Sargent and Blackman (1962) showed that penetration was increased with a rise in temperature. Sargent (1965) suggested that this increase in penetration was partly due to the decreased cytoplasmic viscosity and increased translocation which occurred at high temperatures.

Prasad, Foy and Crafts (1967) found that penetration was increased with a rise in relative humidity. They suggested that a high relative humidity, or the addition of a humectant to the spray solution, allowed the herbicide to remain in solution for longer periods, thus enhancing penetration.

The addition of surfactants increases absorption, often to the extent of reducing selectivity between species. Freed and Montgomery (1958) found that the type of surfactant, and not just the reduction in surface tension, may be important. By reducing the surface tension of the spray solution surfactants allow more complete wetting of waxy surfaces and facilitate entry of the spray into the substomatal cavity.

In studies on the effect of pH on penetration Van Overbeek (1956) demonstrated that a low pH suppresses ionization of the cuticle and the herbicide and facilitates the entry of readily ionizable compounds.

Crafts (1957) considered the effect of internal pH on leaf absorption and translocation of a solution (pH 3.3) of 2,4-D acid. Undissociated 2,4-D molecules entering the aqueous phase of the epidermis encountered a medium of pH 5.5, became partially dissociated and diffused into the cytoplasm and vacuoles.

Dybing and Currier (1959) used fluorescent dyes to assess penetration. Sargent and Blackman (1962) used ^{14}C labelled 2,4-D to study penetration into the foliage of Phaseolus vulgaris L. and Coleus Blumei Benth. Drops of labelled herbicide were applied within sections of plastic tubing placed on leaf discs. The treatments were kept in an environment of high relative humidity. At the termination of the experiment the leaf discs were washed to remove surface activity. The activity within the dried discs was determined with a Geiger Muller detector. Using the same technique Kirkwood et al. (1968) demonstrated differential absorption between 2-methyl, 4-chlorophenoxyacetic acid (MCPA) and 2-methyl, 4-chlorophenoxybutyric acid (MCPB) by washing treated leaves with isotonic sucrose solution and counting the residual activity.

Schafer (1970) using 3,5-dibromo-4-hydroxybenzonitrile-^{14}C (bromoxynil), a lipophilic herbicide, washed treated leaves for different periods with 25 percent ethanol in toluene to determine if washing leached label from the leaf. He found no significant difference in activity between recovery times and concluded that activity was only being removed from the leaf surface.

Several studies (Holly, 1956; Sargent and Blackman, 1962; Schafer, 1970) have indicated that penetration does not play a major role in determining selectivity between species. On the other hand, the selectivity

of 2,4-D (Repp, 1958) and 3-amino-1,2,4-triazole (amitrole) (Herrett and Linck, 1961) between species has been explained on the basis of differential cuticle structure and thickness.

Herbicide Translocation

Once in the leaf the herbicide molecules may move through the apoplast (non-living region) or symplast (living region) or both (Robertson and Kirkwood, 1969). Crafts (1961) suggested that a compound moving by the apoplastic route traverses the anticlinal walls of epidermal and mesophyll cells into bundle sheath extensions. From there the herbicide moves to the xylem elements and is subsequently transported to the bundle endings within the treated leaf. Little is known of the symplastic route. Crafts believed that cell to cell movement may be activated by protoplasmic streaming. Woodford (1957) suggested that this initial cell to cell transport determines whether or not a herbicide is capable of symplastic translocation. Franke (1967) suggested that movement within the mesophyll is a three stage process, involving diffusion into free space, adsorption to the surface of the plasmalemma by physico-chemical binding and movement into the cytoplasm by an energy requiring process. On reaching the sieve tubes the herbicide is transported more rapidly than through the mesophyll cells. The mechanism controlling movement in the phloem is unknown. Thaine (1969) suggested that movement of solutes occurs in microscopic tubules which have contractile walls and are continuous between sieve tube cells. He postulated a peristalsis-like mechanism for the walls of these tubules.

Various factors may effect translocation. Prasad et al. (1967) showed that high relative humidity and high temperature favour trans-

location. Agbakoba and Goodin (1969) found that younger plants translocate herbicides more readily than older ones.

Autoradiography has generally been employed to gain qualitative information on the movement of radioactive labelled compounds within the plant. A labelled herbicide may be applied to any part of the plant and its movement with time studied by sampling at intervals (Weaver and DeRose, 1946; Wood, Mitchell and Irving, 1947). Crafts (1965) stated that:

> "A properly labelled tracer applied to lower leaves penetrates and moves downward; applied to median leaves it moves both basipetally and acropetally; applied to upper leaves it moves upward. In short-time experiments such movement bypasses other mature leaves. Such movement must be by mass flow of food materials in the phloem; herbicides that penetrate the phloem apparently move with the foods."

There are a number of problems associated with making autoradiographs of pressed plant material. Leonard and Hull (1965) used Saran Wrap[1] as a separator to prevent the production of compression marks caused by the direct contact between the X-ray film and the plant. They found that Saran Wrap excluded approximately 50 percent of the soft beta radiation produced by ^{14}C. In order to reduce the absorption of radiation by plant water the material is usually dried prior to exposure of the film. Hot air drying may cause movement of the label. To avoid this movement the plants are often sectioned first (Vostral and Buchholtz, 1966; Agbakoba and Goodin, 1969) but the best method is to freeze dry the plants before exposure of the film (Crafts and Yamaguchi, 1964).

[1]A product of Dow Chemical Co.

To obtain a quantitative assessment of translocation Agbakoba and Goodin (1969) and Schafer (1970) sectioned and extracted plants after treatment. The activities of the extracts were determined and used as a measure of translocation.

Translocation of the label is not necessarily evidence of movement of the intact herbicide. Prasad et al. (1967) used ^{14}C and ^{36}Cl labelled 2,2-dichloropropionic acid (dalapon) to overcome this problem.

Hogue and Warren (1968) showed that differential translocation between tomato (Lycopersicum esculentum Mill.) and parsnip (Pastinaca sativa L.) was a factor involved in the selectivity of 3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea (linuron) between these two species.

Mode of Action and Metabolism of Nitrofen

Very little work has been done on the mode and mechanism of action of nitrofen within the plant. The herbicide is contact in action (Herbicide Handbk. Weed Soc. Amer.) and therefore significant translocation is unlikely. Also nitrofen darkens on exposure to air and ultraviolet light (ibid.). Gutenmann and Lisk (1967) looked for nitrofen and its residues in the dairy cow. No trace of nitrofen was found in the milk, urine or faeces and it was observed that the herbicide readily disappeared in the presence of fresh rumen fluid in vitro., a metabolite, 2,4-dichlorophenyl, p-aminophenyl ether, being produced. This metabolite was also absent from the milk, urine and faeces. No reports were found in the literature of nitrofen metabolites being formed in plants.

Takehisa et al. (1966) showed that 0.0001 to 0.1 M. solutions of nitrofen had no effect on cell division in Vicia faba L.

Furuya and Arai (1966) and Matsunaka (1969a) showed that diphenyl ethers substituted in the ortho position (e.g. nitrofen) unlike those substituted in the meta position required light for activation.

Matsunaka (1969b) used carotenoid and chlorophyll deficient mutants in studying the effect of nitrofen on rice seedlings. Yellow mutants were found to possess primarily xanthophyll pigments. He found that in all xanthophyll-rich plants (i.e. both green and yellow) 5 p.p.m. nitrofen reduced growth by approximately 50 percent. In albino plants, containing no xanthophylls, 5 p.p.m. nitrofen gave little or no reduction in growth. Pre-illuminated nitrofen solution did not show any significant activity on rice plants in the dark. From these results Matsunaka postulated that:

> "The light energy absorbed by the xanthophylls (or chlorophylls) may be used for the activation of the diphenyl ether compounds having 2,4- or 2,4,6- substituents on one benzene ring, which will be converted into toxic compounds and exhibit toxic action."

Matsunaka also postulated that:

> "An alternative mechanism of action could be that light acts upon the regulation of the hormonal level in higher plants (Briggs, 1963). The plants which have a hormonal level affected by light energy absorbed by pigments, e.g. xanthophylls, and which have different hormonal levels from those of plants in the dark may be very susceptible to the ortho-substituted diphenyl ether herbicides. In this case no conversion of the herbicide into another toxic form need occur."

SECTION 1

Selectivity of Nitrofen among Echo Rape, Redroot Pigweed and Green Foxtail

Abstract. The selectivity of 2,4-dichlorophenyl p-nitrophenyl ether (nitrofen) between rape (Brassica campestris L., var. Echo) and two weed species, redroot pigweed (Amaranthus retroflexus L.) and green foxtail (Setaria viridis L. Beauv.), was determined quantitatively by a replicated dosage-response experiment. On an $E.D._{50}$ basis foxtail and pigweed were found to be, respectively, 5.7 and 63 times more susceptible than rape.

Selectivity was divided into three parameters, viz. differential spray retention, differential penetration, and differential effects within the plant. Differences in retention were measured with the use of a water soluble dye, while differences in penetration were determined with ^{14}C labelled nitrofen. There was less retention (2/3) on the foxtail than on the rape and less penetration (3/5) in the pigweed than in the rape. Under the conditions of these tests it was estimated that, on an internal basis, foxtail and pigweed were, respectively, 9 and 98 times more susceptible to nitrofen than rape.

Introduction

The herbicide, 2,4-dichlorophenyl p-nitrophenyl ether (nitrofen) is used in Western Canada for the postemergence control of weeds in brassica crops. Mason (8), working in New Zealand, first established the selectivity of nitrofen between brassicas and other species.

Comparisons of relative toxicity between species can be obtained when the variation in response is measured at several dosages and the data treated by probit analysis (1). Then comparisons may be made at the E.D.$_{50}$ level for each species. Having established the magnitude of the selectivity it is then of interest to analyse its causes. Blackman, Bruce and Holly (2) found that differences in retention and penetration of the herbicide are the first factors that should be investigated. With the use of a dye these workers showed that differences in retention can play a major role in selectivity.

Working with (2,4-dichlorophenoxy)acetic acid (2,4-D), Holly (6) found cuticle resistance to penetration to be of little importance, while Repp (9) explained species selectivity on the basis of cuticle structure and thickness.

The herbicide may show further selectivity due to differences in various internal factors, such as translocation and metabolism. The experiments reported here were conducted to investigate the selectivity that occurs from the time the herbicide leaves the spray nozzle until it enters the foliage. Rape (Brassica campestris L., var. Echo) was selected as the crop species and compared with redroot pigweed (Amaranthus retroflexus L.) and green foxtail (Setaria viridis L. Beauv.) as typical examples of broadleaf and grass weeds, respectively.

Materials and Methods

The plants used in these experiments were all at the five leaf stage and grown in the greenhouse. A day length of 16 hr was maintained using supplementary light with an intensity of 1000 ft-c at the plant surface. There were three plants per pot and the pots were subirrigated.

Dosage-response. This experiment consisted of a completely randomised design. There were eight treatments, including a control, with five replicates of each.

The spray formulation consisted of nitrofen emulsified with ethyl acetate,

water and Advawet #10[3] (20:78:2). The nitrofen used in this experiment was reclaimed from the emulsifiable concentrate by evaporation and recrystallisation from 95% ethanol. The spray was delivered at the rate of 112.25 L/ha (10 gpa) at a pressure of 2.64 kg/ sq cm (37.5 psi) using a 65015 TeeJet nozzle.

The foliar parts were harvested after five days. At this time regrowth was just visible on plants receiving the lowest dosages. The foliage was dried and weighed and the relative growth (R.G.) determined for each treatment by the formula:

$$R.G. = \frac{W_T - W_o}{W_c - W_o}$$

Where: W_o = mean dry weight of plants at the time of spraying.

W_c = mean dry weight of control plants (sprayed with ethyl acetate: water:Advawet #10 (20:78:2)).

and W_T = mean dry weight of treatment.

Spray retention: In this experiment the spray parameters were the same as those used in the above experiment. Four pots of each species were used. Three pots were treated and the fourth was used as a control. The treated plants were sprayed with a water soluble dye (Niagara Sky Blue 6B)[4] made up in the control emulsion. When the plants had dried the dye was washed off in 10 ml of water in cuvettes. The absorbance of suitable dilutions of the washings and dye concentrate was read in a Bausch and Lomb Spectronic 20 spectrophotometer at 630 mμ. The spray retention on each species was determined as μl of spray per g dry weight of foliage.

[3] A surfactant - product of the Carlisle Chemical Co.

[4] A product of the Allied Chemical Co.

The surface tensions of the dye, the control, and herbicide emulsions were determined by the capillary method and all found to be 26 dynes per cm at 21 C.

Penetration of nitrofen. The plants used in this study were transferred from the greenhouse to a growth chamber three days prior to the commencement of the experiment. Controlled growth conditions in the chamber were as follows: light period, 16 hr of 2500 ft-c at the plant surface, with a constant temperature of 21 C.

Treatments consisted of three species by three dates of harvest with 10 replicates of each. The treatments consisted of 5 µl of an emulsion of ring labelled nitrofen ^{14}C (approximately 1700 dpm/µl), in ethyl acetate: water:Advawet #10 (20:78:2), placed on the third leaf of each plant. The microdrops were placed within 3 mm internal diameter, lanolin rings located on the leaf surface away from main veins (except in the case of foxtail where this was not possible).

After 2, 4 and 8 days small squares of leaf tissue, containing the treated area, were harvested and washed in petroleum ether (bp 63-68 F) for 10 seconds. Washing for periods up to 30 seconds did not affect the recovery of nitrofen. However, at least seven seconds of similar washing were required to remove a lanolin ring from the leaf surface and for this reason a washing time of 10 seconds was employed.

After washing, the squares of leaf tissue were extracted with 10 ml of p-dioxane:methyl cellosolve (5:1) plus 10.2 g of omnifluor[5] and 50 g naphthalene per liter. The extractions and washings were then counted in a Nuclear-Chicago 720 series liquid scintillation counter. Corrections were made for background and colour quenching; the latter from tables calculated for the instrument used.

[5]98% PPO and 2% Bis-MSB - product of the New England Nuclear Chemical Co.

The activity of the extractions was taken as a measure of the herbicide that had penetrated while the sum of the activities of both the extraction and washing solutions was taken as the total applied.

Results and Discussion

All differences between means were tested using lsd. Only differences significant at the 5% level are reported. The results of the dosage-response experiment are presented in Figure 1. The $E.D._{50}$ values and selectivity ratios are presented in Table 1. At the five leaf stage it was impossible to depress the growth of rape much below 50%. These results demonstrated the extreme tolerance of rape to nitrofen. On the other hand, the low value of 10.44 g/ha required to give an $E.D._{50}$ for pigweed showed this species' extreme susceptability.

It was shown (Table 2) that the dye retention on rape was greater than on foxtail but no different from pigweed. Since the addition of either Niagara Sky Blue 6B dye or nitrofen to the spray emulsion did not alter surface tension, dye retention can be equated to herbicide retention. Many factors influence the efficiency of retention, including the orientation, area, position, age, and pubescence of the leaf (7). Variation in retention between species has also been attributed to differences in the hydrophobic nature of leaf waxes (4, 5, 3). The difference in spray retention between foxtail and rape can be explained by differences in projected leaf area and the nature of leaf surfaces. The horizontally arranged rape leaves intercept spray droplets readily. Foxtail, however, has only a small projected leaf area and leaves arranged at an acute angle to the path of spray droplets, resulting in fewer droplets impinging on the leaf surface and greater loss of droplets by rebounding (3).

Without the use of a surfactant in the spray emulsion the retention, especially on foxtail, would probably be much reduced, as Holly (7) found

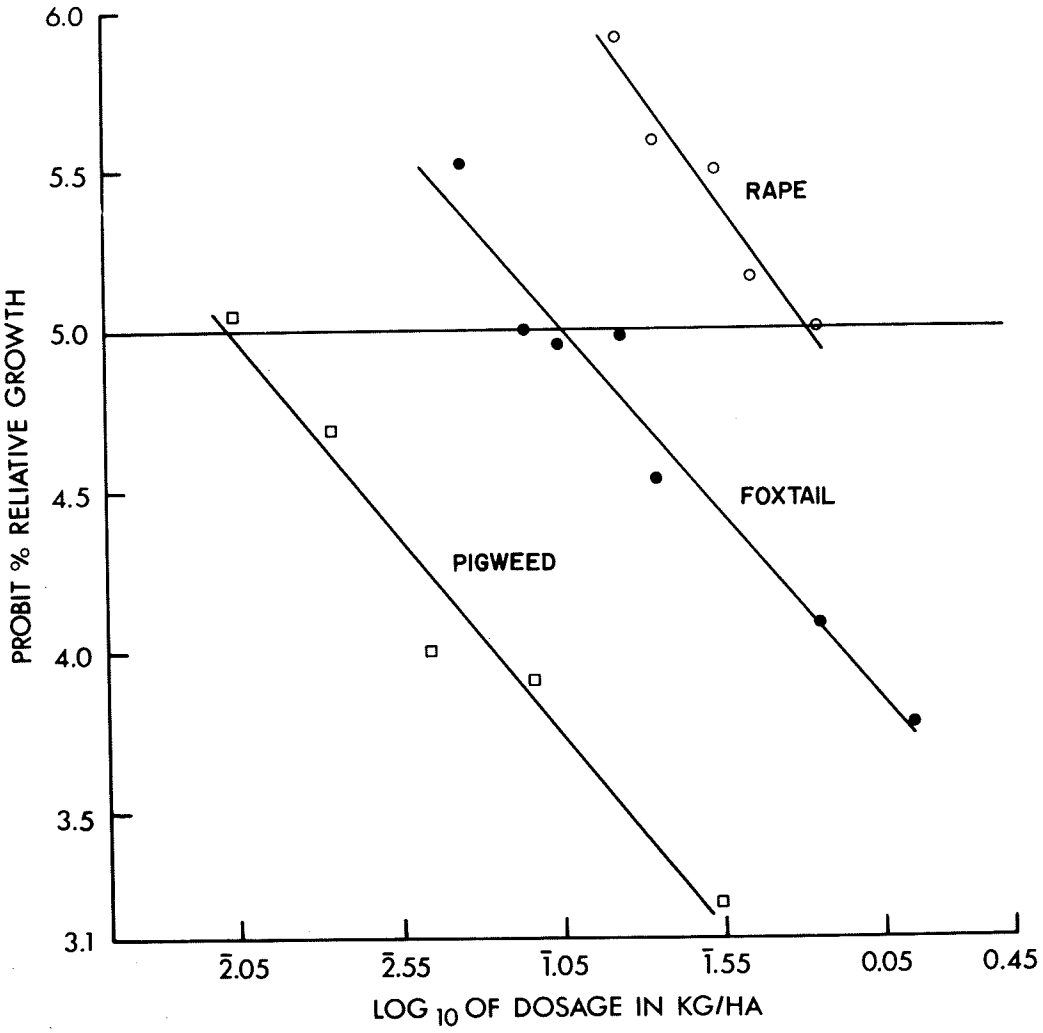

Figure 1. The relative toxicity of nitrofen to rape, foxtail, and pigweed.

Table 1. $E.D._{50}$ levels and selectivity ratios for rape, foxtail and pigweed.

Species	$E.D._{50}$[a] in g/ha	Selectivity ratio = $\frac{E.D._{50}\ \text{rape}}{E.D._{50}\ \text{weed}}$
Rape	660.91	
Foxtail	114.83	5.7
Pigweed	10.44	63.0

[a] ED_{50} represents the dose necessary to cause a 50% reduction in relative growth.

Table 2. Means of spray retention and retention ratios.

Species	Mean spray retention in µl dye/g dry foliage[a]	Retention ratio = $\frac{\text{Retention on weed}}{\text{Retention on rape}}$
Rape	491	
Foxtail	324	0.66
Pigweed	406	0.83

[a] lsd at 5% level = 128 µl/g.

to be the case in other graminaceous species. The large reduction in surface tension which results with even small additions of surfactant causes the droplets to collapse and coalesce on the leaf surface. This process is followed by the more or less complete wetting of the leaf (2, 7).

In the penetration experiment a difference between dates of harvesting was found in pigweed (Table 3) indicating that, in this species, penetration had not stopped after two days. Less penetration was found in pigweed than in the other two species, when compared at the last date of harvest. Holly (7) showed previously, for various herbicides, that there can be a lack of positive correlation between absorption and selectivity. Holly also indicated that Brassica species may absorb more herbicide than some others.

The findings of these experiments clearly indicate that retention and penetration do not account for the selectivity of nitrofen between rape and the two weed species. Conversely, reduced retention on foxtail and reduced penetration in pigweed decrease the potential herbicidal effect of nitrofen on these species.

In order to make some estimate of the importance of the different factors controlling selectivity within the leaf the relationship proposed by Schafer[6] is employed.

$$\text{viz. Ratio of selectivity, } Rs = \frac{1}{Rr} \times \frac{1}{Rp} \times \frac{1}{Ri}$$

[6]Schafer, D. E. 1970. Selectivity and Chemodynamics of 3,5-dibromo-4-hydroxybenzonitrile in winter wheat (Triticum aestivum L.) and coast fiddleneck (Amsinckia intermedia Fisch. and Mey). Ph.D. thesis. Oregon State Univ. 110p.

Table 3. Means of % ^{14}C penetrating leaf and penetration ratios.

Species	% penetration of ^{14}C after[b] 2 days	4 days	8 days	Penetration ratio = Penetration in weed after 8 days / Penetration in rape after 8 days
Rape	17.8	23.8	23.5	
Foxtail	19.6	17.7	20.3	0.87
Pigweed	10.0	13.5	15.1	0.64

[b] lsd for comparing pigweed dates = 3.0

lsd for species comparison at 2 days = 4.1

lsd for species comparison at 8 days = 4.6

$$\text{where } Rs = \frac{\text{Herbicide concn. at E.D.}_{50} \text{ in rape.}}{\text{Herbicide concn. at E.D.}_{50} \text{ in weed.}}$$

$$Rr = \frac{\text{Spray retention on rape in ul/g dry foliage.}}{\text{Spray retention on weed in ul/g dry foliage.}}$$

$$Rp = \frac{\%\ ^{14}\text{C penetrating rape leaf after 8 days.}}{\%\ ^{14}\text{C penetrating weed leaf after 8 days.}}$$

$$Ri = \frac{\text{Internal factors responsible for selectivity in rape.}}{\text{Internal factors responsible for selectivity in weed.}}$$

Thus the internal selectivity between rape and foxtail is:

$$\frac{1}{Ri} = \frac{5.7}{0.66 \times 1} = 9$$

and the internal selectivity between rape and pigweed is:

$$\frac{1}{Ri} = \frac{63}{1 \times 0.64} = 98$$

Given the same concentration of nitrofen within the foliage foxtail is 9 times, and pigweed 98 times more susceptible than rape.

Some of the factors involved in the internal selectivity will be presented in a subsequent paper, where the effect of translocation differences and the affinity of nitrofen for cell lipids will be discussed.

The recommended application rate for rape in the field is 1.34 kg/ha (1.2 lb/A), to be applied at a spray volume of 112 L/ha (10 gpa). As can be seen from Figure 1, in the greenhouse this rate reduced growth in foxtail by 90% and depressed growth completely in pigweed. Growth of rape also was reduced, initially. However, under the conditions of this experiment it was impossible to depress growth in rape below 50% because the higher concentrations of herbicide rapidly dried on the leaf surface and failed to penetrate. For this reason rape was able to recover rapidly.

Acknowledgement

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Literature Cited

1. Blackman, G. E. 1952. Studies in the principles of phytotoxicity. I. The assessment of relative toxicity. J. Exp. Bot. 3:1-27.

2. Blackman, G. E., R. S. Bruce, and K. Holly. 1958. Studies in the principles of phytotoxicity. V. Interrelationships between specific differences in spray retention and selective toxicity. J. Exp. Bot. 9:175-205.

3. Brunskill, R. T. 1956. Physical factors affecting the retention of spray droplets on leaf surfaces. Proc. Br. Weed Control Conf. 3:593-603.

4. Fogg, G. E. 1947. Quantitative studies on the wetting of leaves by water. Proc. R. Soc. Bot. 134:503-522.

5. Fogg, G. E. 1948. Adhesion of water to the external surfaces of leaves. Discuss. Faraday Soc. 3:162.

6. Holly, K. 1956. Penetration of chlorinated phenoxyacetic acids into leaves. Ann. appl. Biol. 44:195-199.

7. Holly, K. 1964. Herbicide selectivity in relation to formulation and application methods, p 423-464. In L. J. Audus (ed.). Physiology and Biochemistry of Herbicides. Academic Press, New York and London.

8. Mason, G. W. 1963. Weed control in field brassica crops. Proc. N.Z. Weed and Pest Control Conf. 16:65-70.

9. Repp, G. 1958. On the selective action of 2,4-dichlorophenoxyacetic acid. The relationship between the susceptability to growth regulators and the structure of the outer surfaces of plants. Z. Acker-u. Pfl. Bau. 107:49-66.

SECTION 2

The Fate of Nitrofen in Echo Rape, Redroot Pigweed and Green Foxtail

Abstract. The fate of 2,4-dichlorophenyl p-nitrophenyl ether (nitrofen) in the foliage of rape (Brassica campestris L., var Echo), redroot pigweed (Amaranthus retroflexus L.), and green foxtail (Setaria viridis L. Beauv.) was investigated with the aid of ^{14}C-nitrofen. Only limited amounts of the label were translocated in these species. Plants treated with ^{14}C-nitrofen under high light conditions produced several labelled compounds of different molecular size and chromatographic properties. The time at which these compounds were first detectable depended on light intensity. It is suggested that at least two of these compounds are lipid-nitrofen conjugates or nitrofen polymers and that others are formed by cleavage of nitrofen at the ether linkage. It is also suggested that the formation of these compounds is not the prime cause of the herbicidal effect of nitrofen on foliage.

Introduction

In Western Canada the herbicide 2,4-dichlorophenyl p-nitrophenyl ether (nitrofen) is used for selective, postemergence weed control in Brassica crops, while in Japan it is used as a preemergence herbicide in transplanted rice.

Matsunaka and Inada (2) showed that light is essential for the toxicity of nitrofen on germinating rice seedlings. Nitrofen applied at concentrations below 5 ppm was more toxic with increasing illumination. At nitrofen concentrations greater than 5 ppm the effect of illumination was apparent even below 1000 lux.

It was shown (2) that pre-illuminated nitrofen solution (even with the addition of riboflavine and fluorescein) had no herbicidal activity on rice plants in the dark. The addition of several known photosynthetic inhibitorsto the nitrofen solution did not affect its activity (2), indicating that the mechanism of action of nitrofen is different from that postulated for the bipyridylium herbicides (4, 5). It was also shown (2) that illumination on the third day after treatment was more effective than on the first and second days. Blue light was the most effective in producing herbicidal activity. From these studies it was concluded that the activation of nitrofen was by a photo-biochemical process.

Matsunaka (3) studied the effect of nitrofen on xanthophyll and chlorophyll deficient rice mutants. He concluded that the energy transferred by xanthophylls and possibly chlorophylls from absorbed light was involved in the direct activation of nitrofen.

No information was found on the fate of nitrofen in the foliage of Brassica crops. Nitrofen is selective among rape (Brassica campestris L., var. Echo), redroot pigweed (Amaranthus retroflexus L.), and green foxtail (Setaria viridis L. Beauv.) (1). The fate of nitrofen in these species is outlined in the present report.

Materials and Methods

Plants used in these experiments were grown in the greenhouse and treated at the 5-leaf stage. A day length of 16 hr was maintained using supplementary light of 1000ft-c at the plant surface. Mean day and night temperatures were 21 C and 15 C, respectively. There were three plants per pot and the pots were subirrigated.

Leaf sections. Plants were sprayed with 1500 ppm technical nitrofen made up in ethyl acetate:water:Advawet #10[3] (20:78:2) at 112.3 L/ha (10 gpa) at a pressure of 2.64 kg/cm^2 (37.5 psi) using a 65015 TeeJet Nozzle. Foliage was harvested after 2 days and fixed in 37% formaldehyde:glacial acetic acid:ethanol:water (2:1:10:7) for 24 hr. The leaves, mounted in carrot tissue, were sectioned into 30% ethanol with a microtome, mounted in glycerol, and photographed.

In the following experiments all ^{14}C-nitrofen (ring labelled) emulsions used were made up in ethyl acetate:water:Advawet #10 (20:78:2) and applied as 5 uliter drops within 3 mm, internal diameter lanolin rings, located on the adaxial surface of the leaf, away from main veins (except in the case of foxtail where this was not possible). Radioactivity was determined by liquid scintillation counting. Corrections were made for background and colour quenching.

Translocation of ^{14}C-nitrofen. The plants used in this experiment were grown in silica sand and watered on alternate days with a complete nutrient solution. Drops of ^{14}C-nitrofen emulsion (12400 dpm/uliter) were applied to the third, fourth and fifth leaves of separate plants

[3] A surfactant-product of the Carlisle Chemical Co.

of each species. After 1, 2, 4, 8, and 16 days the treated plants, together with one untreated control, were collected, washed free of sand, and mounted on cardboard under Saran Wrap[4]. These plants were pressed at -40 C for 2 days prior to exposure of Kodak No Screen X-ray film for 5 weeks.

After exposure of the film the plants from the 16 day treatments were divided into: treated spot, remainder of the treated leaf, foliage above the treated leaf, foliage below the treated leaf, and the root system. The treated spots were washed in petroleum ether (bp 17-20 C) for 10 sec to remove surface activity and the plant parts were freeze dried. After weighing, the samples were combusted in a modified Schoniger flask in an atmosphere of oxygen. The $^{14}CO_2$ was collected in 5 ml of ethanolamine:methyl cellosolve (1:1) scrubber. The activity of 1 ml of scrubber was determined using 10 ml of toluene:methyl cellosolve (2:1) plus 4.08g omnifluor[5] per liter. Combustion of standard samples showed there was a 93% recovery using this method.

Biochemical reactions of ^{14}C-nitrofen. All treatments used in these experiments consisted of two drops of ^{14}C-nitrofen emulsion (1800 dpm/µliter and 1500 ppm), applied to the third and fourth leaves of each plant. At harvest, squares of leaf tissue containing the treatments were washed in petroleum ether to remove surface activity prior to extraction. Unless otherwise indicated, 10 ml of p-dioxane:methyl cellosolve (5:1) (here after referred to as dicell) plus 10.2 g omnifluor and 50g naphthalene per liter was used as the fluor in scintillation counting.

[4]A product of Dow Chemical Co.

[5]98% PPO and 2% Bis-MSB-product of the New England Nuclear Chemical Co.

Leaf squares were harvested after 1, 4, 8, and 16 days and extracted with 3 ml of dicell and centrifuged at 1700g for 15 min. Two ml of supernatant was applied to a 34 by 2.6 cm Sephadex LH-20 column (60 ml void volume) and eluted with dicell at a flow rate of 0.4 ml/min. Two ml fractions were collected and counted. The ether washings (leaf surface activity) were also run on the column using dicell as an eluant.

This experiment was repeated using controlled growing conditions. Four days prior to treatment plants were transferred to a growth chamber. Conditions in the chamber were as follows: a 14 hr day with a light intensity of 1000 ft-c at the plant surface and a constant temperature of 30 C. Leaf squares were harvested after 12, 24, and 48 hr.

To make a quantitative assessment of the amount of radioactivity associated with compounds other than ^{14}C-nitrofen thin layer chromatographic (here after referred to as T.L.C.) techniques were employed. Four days prior to treatment plants were transferred to a growth chamber maintained at: a 14 hr day with a light intensity of 1700 ft-c at the plant surface and with day and night temperatures of 21 C and 15 C, respectively. Leaf squares were harvested after 1, 2, and 4 days and extracted directly (without washing) in 0.3 ml dicell.

T.L.C. plates with a 250μ absorbant layer were prepared from a slurry of cellulose powder MN 300[6] in 7% olive oil in p-dioxane. Dicell extracts were applied as 10 applications of 5 μliters and run in methanol: acetone:water (15:5:1). Radioautographs were made from the T.L.C. plates

[6]Product of the Macherey and Nagel Co.

using a 5 week exposure period. The areas of radioactivity were scraped from the plates and counted in 10 ml of toluene plus 6.12g omnifluor per liter.

Results and Discussion

Visible effects of nitrofen within the leaf. Complete collapse of all cells occurred when 1500 ppm nitrofen was sprayed on to the leaves of foxtail and pigweed (Figure 1). Only the upper epidermis and some palisade mesophyll cells were affected when rape was similarly treated. These symptoms are consistent with the rapid wilting visible in leaves of foxtail and pigweed, but not in rape, that occurs after spraying.

Translocation of ^{14}C-nitrofen. The radioautographs showed only slight translocation of foliarly applied ^{14}C-nitrofen and are not presented here. The results of a quantitative assessment of translocation supported these observations (Table 1).

Translocation was first detected in foxtail 2 days after treatment. Movement of the label was primarily towards the leaf tip suggesting xylem translocation. Some diffusion of the label was also found in the third and fifth leaves of rape and pigweed. There was no appreciable increase in translocation after 4 days in any species.

Biochemical reactions of ^{14}C-nitrofen. All the radioactivity applied to plants grown in the greenhouse could be extracted with dicell and eluted from LH-20 columns. The radioactivity in the eluate was present as four distinct peaks (Figure 2). These peaks were found one day after treatment and increased in size with time.

The positions of the main chlorophyll and carotenoid peaks were used as an indication of the molecular size of the radioactive compounds.

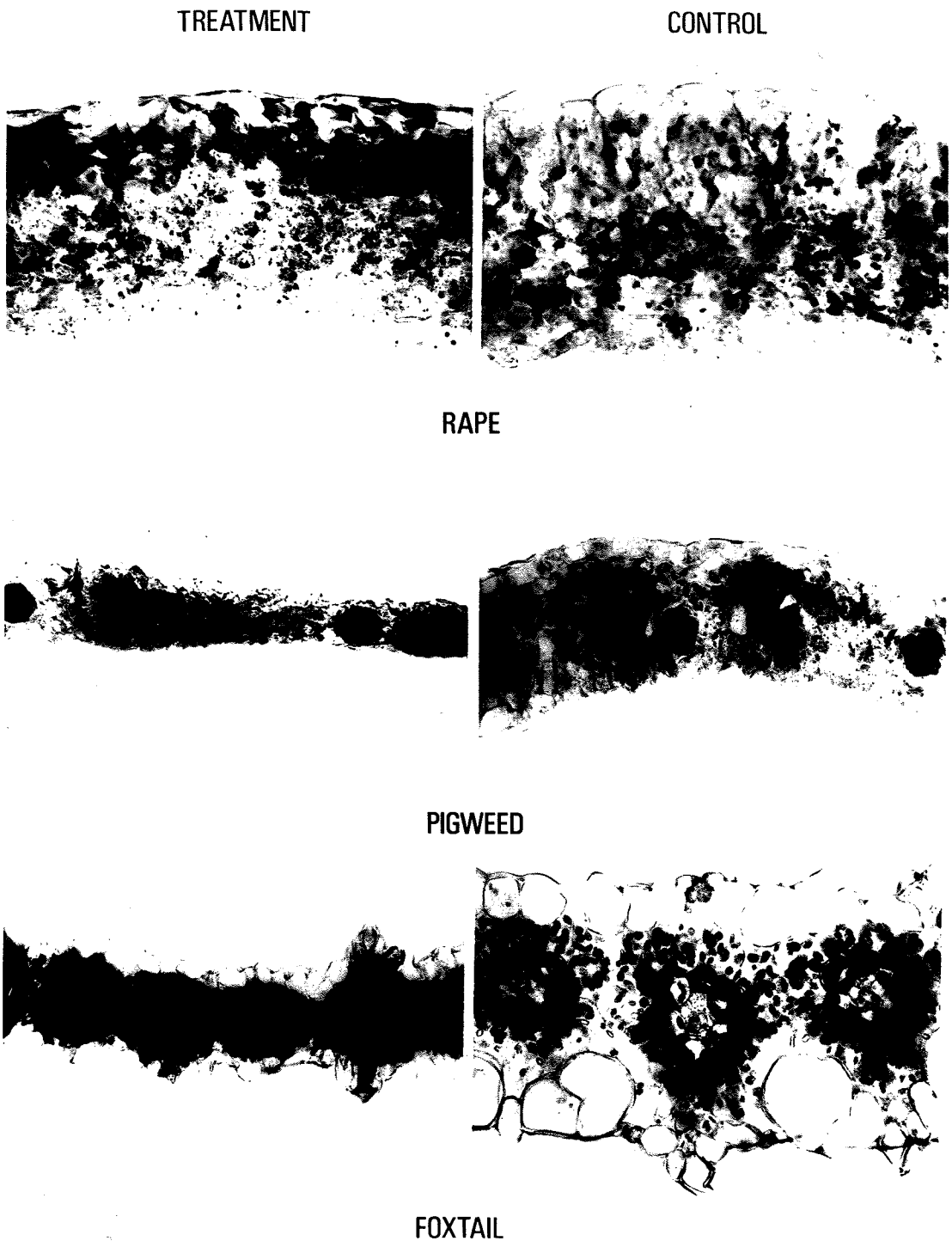

Figure 1. Photomicrographs of leaf transverse sections showing the effect of 1500 ppm nitrofen on rape, pigweed, and foxtail 2 days after application. X256.

Table 1. Quantitative assessment of movement of the ^{14}C of labelled nitrofen after 16 days in rape, pigweed, and foxtail.

Plant Part	Rape		Pigweed		Foxtail	
	activity in dpm[a]	% total dpm	activity in dpm	% total dpm	activity in dpm	% total dpm
treated spot	43,030	95.8	39.130	92.4	46,830	97.8
treated leaf	1,340	3.0	2,580	6.1	850	1.8
upper foliage	80	0.2	230	0.5	110	0.2
lower foliage	0	0.0	350	0.8	30	0.1
roots	450	1.2	80	0.2	50	0.1

[a]Corrected for the control plants.

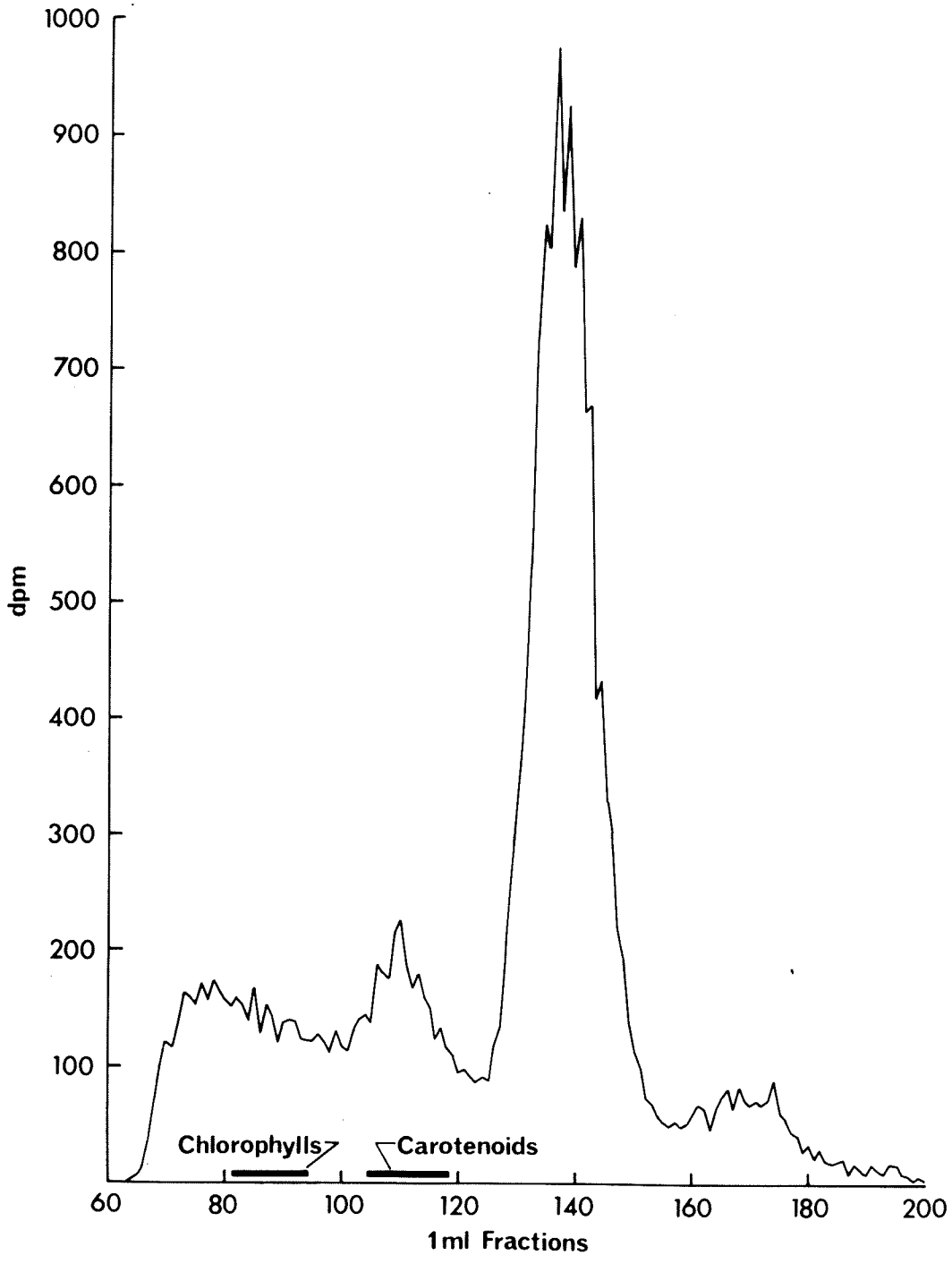

Figure 2. Peaks of radioactivity found in foliar extracts of pigweed separated on a Sephadex LH-20 column. Plants were grown under high light intensity for 16 days after treatment with ^{14}C-nitrofen.

It was estimated that the first peak had a mol wt of 900 to 1100 and the second a mol wt of 500 to 600. The compound forming the major peak was assumed to be ^{14}C-nitrofen because it co-chromatographed (T.L.C.) with a standard both on silica gel GF254 (run in chloroform) and on cellulose MN 300 impregnated with olive oil (run in methanol: acetone:water (15:5:1)).

The ether washing (leaf surface activity) possessed only a single peak of radioactivity which had the same elution time as the ^{14}C-nitrofen peak of the dicell extracts. This peak was assumed to be ^{14}C-nitrofen.

From its position the fourth peak was assumed to be half the nitrofen molecule (i.e. split at the ether linkage). It was tentatively concluded that there were four major compounds or groups of compounds with ca. mol wts of 1100, 550, 300, and 150 respectively. It was not established whether the radioactivity in the 1100 and 550 mol wt ranges was produced by polymerization of ^{14}C-nitrofen or by conjugation of the chemical with cell lipids, or both.

All the radioactivity recovered from plants grown in the growth chamber under conditions of low light intensity and high temperature was found in the ^{14}C-nitrofen peak. With higher light intensity and lower temperatures two spots of radioactivity, in addition to ^{14}C-nitrofen, were detectable in plant extracts 2 days after treatment, run on T.L.C. plates. These compounds were easily detectable 4 days after treatment (Figure 3). Thus, light intensity and/or quality is correlated with the rate of formation of nitrofen derivatives.

Twice as much of the radioactivity was present as nitrofen derivatives in pigweed as compared to the other species (Table 2). This fact may partially account for pigweed's high susceptibility to nitrofen.

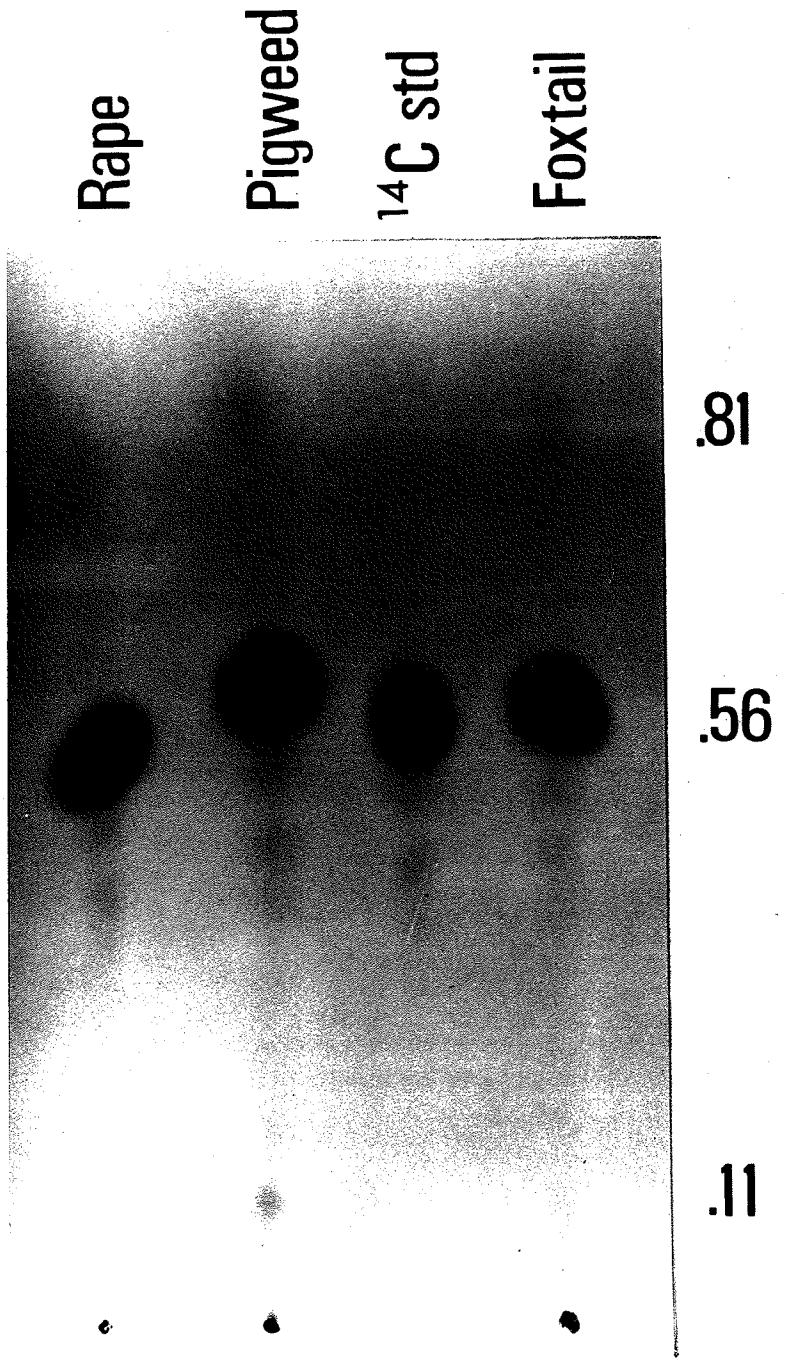

Figure 3. Radioautograph of T. L. C. plate showing the position of ^{14}C-nitrofen and its labelled derivatives.

Table 2. Radioactivity of the spots shown in the T. L. C. radioautograph in Figure 3.

	Rape			Pigweed			Foxtail		
R.F.	dpm	% total[a]	% in leaf[b]	dpm	% total	% in leaf	dpm	% total	% in leaf
.81	156	4.65	19.52	161	5.64	41.80	64	3.07	17.36
.56	3106	92.52	68.58	2595	90.02	33.01	1967	94.44	68.53
.11	95	2.83	11.89	97	3.40	25.19	52	2.49	14.11
.11+.81	251	7.48	31.42	258	9.04	66.99	116	5.57	31.46

[a]Spot activity as a percentage of the total applied to the plate.

[b]Spot activity as a percentage of that estimated to be in the leaf. Percent penetration was taken as 23.8 for rape, 17.7 for foxtail and 13.5 for pigweed. These figures are estimates of the % penetration after 4 days reported elsewhere (1).

A light controlled biochemical process apparently causes ^{14}C-nitrofen to polymerize or enables it to combine with certain unidentified cell lipids. At the same time a light controlled process may be responsible for splitting the nitrofen molecule at the ether linkage. The action of light on xanthophylls, reported by Matsunaka (3), may well be the cause of the formation of xanthophyll-nitrofen conjugates. Such a conjugate would have a mol wt of ca. 850 and may be included in the first radioactive peak found in the LH-20 experiments. Matsunaka (2) showed that illumination on the third day after treatment caused the most phytotoxicity. However, nitrofen derivatives were not easily detectable until 4 days after treatment.

The mechanism of action of nitrofen derivatives is unclear. They may be toxic or if lipid-nitrofen conjugation occurs the function of the lipid partner may be impaired (e.g. the chlorophyll protection thought to be provided by carotenoids).

The toxic action of nitrofen is apparently more rapid than the formation of nitrofen derivatives. The formation of these products does not satisfactorily explain the rapid wilting that occurs in pigweed and foxtail after spraying the rapid action of nitrofen on these two species might be explained by a physical disruption of the plasmalemma as suggested by Van Overbeek and Blondeau (6) in their studies on the phytotoxicity of oils. The resistance of rape might be explained by this species' different membrane characteristics.

The use of electron microscopy to study the effect of nitrofen on the plasmalemma and other cell membranes and the identification of the nitrofen derivatives found in these experiments would allow a clearer understanding of the mechamism of action of nitrofen.

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Literature Cited

1. Hawton, D. and E. H. Stobbe. 1970. Selectivity of nitrofen among Echo rape, redroot pigweed and green foxtail. Weed Sci. (submitted for publication).

2. Matsunaka, S. 1969. Activation and inactivation of herbicides by higher plants. Residue Rev. 25:45-58.

3. Matsunaka, S. 1969. Acceptor of light energy in photoactivation of diphenylether herbicides. J. Agr. Food Chem. 17:171-175.

4. Mees, G. C. 1960. Experiments on the herbicidal action of 1, 1′-ethylene -2, 2′-dipyridylium dibromide. Ann. Appl Biol. 48: 601-612.

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

6. Van Overbeek, J. and R. Blondeau. 1954. Mode of action of phytotoxic oils. Weeds. 3:55-65.

GENERAL DISCUSSION

The selectivity of nitrofen among rape, pigweed, and foxtail is presented and discussed in the first section of this study. The regression correlation coefficients of the dosage-response experiment and the analyses of variance for the retention and penetration studies are given in Appendix Tables 1, 2 and 3. These results clearly indicate that the selectivity of nitrofen among the three species is controlled by internal factors within the foliage and not by retention and penetration differences.

Some of the factors involved in the difference in internal response to the toxic action of nitrofen are presented in the second section of this study. Investigations showed that translocation of the herbicide was not a factor involved in the toxicity and selectivity of nitrofen. (Autoradiographs presented in Appendix Figure 1).

Experiments with water and 0.1 M NaCl extraction of ^{14}C-nitrofen treated leaf tissue and elution on a Sephadex G25 column showed that there was a trace of radioactivity associated with the high molecular weight protein fraction.

It was shown that at high light intensities lipophilic ^{14}C-nitrofen derivatives of molecular sizes different from that of nitrofen were produced. The compounds with a molecular size greater than that of nitrofen were considered to be either nitrofen polymers or lipid-nitrofen conjugates produced photo-biochemically. Matsunaka and Inada (1967) showed that light is essential for nitrofen activation in germinating rice seedlings. Matsunaka (1969b) showed that germinating rice seedlings possessing xanthophyll and chlorophyll pigments were more susceptible to nitrofen than plants lacking these pigments. He suggested that

the light energy trapped by xanthophylls and possibly chlorophylls was used in the activation of nitrofen. The results of the present study indicate that this activation could take the form of nitrofen polymerization or lipid-nitrofen conjugation. If lipid-nitrofen conjugation occurs it is possible that the chloroplast lipids themselves are involved.

It was observed that at higher temperatures and lower light intensities necrosis of ^{14}C-nitrofen treated foliage was enhanced without the accompanying production of ^{14}C-nitrofen derivatives. For this reason, and because of the rapid wilting and darkening of foliage that occurs in foxtail and pigweed after spraying, it is suggested that the formation of nitrofen derivatives is not the prime factor associated with nitrofen toxicity and selectivity.

The work of Crafts and Reiber (1948), Currier (1951) and Van Overbeek and Blondeau (1954) showed that the phytotoxicity of oils, particularly aromatics, might well be due to a physical disruption of the plasmalemma and tonoplast. Such a disruption would cause leakage of vacuolar fluids into the intercellular spaces with an accompanying darkening and wilting of the foliage. Van Overbeek and Blondeau (1954) found that aromatics with between eight and fourteen carbon atoms were the most toxic to foliage and it may well be relevant that the nitrofen molecule has a total of twelve such atoms. These workers suggest that the toxic action of oils is by the solubilization of molecules into the membrane thus disrupting it and causing a loss of semi-permeability. They further suggest that entry of foreign molecules into the membrane is controlled by the Brownian movement of its constituent molecules. Brownian movement increases with rise in temperature allowing easier penetration of large molecules and causing enhanced toxicity.

If physical disruption of cell membranes is the prime cause of toxicity it may well be that differences in membrane structure account for the selectivity of nitrofen in the foliage of rape, pigweed and foxtail.

SUGGESTIONS FOR FURTHER WORK

A more comprehensive study of the relationship of the effects of light and temperature on nitrofen toxicity is needed. The identification of the light produced compounds and the determination of the action spectrum of their formation would be the first step in this study.

Electron microscope studies of the effects of nitrofen on cell membranes may help to explain the internal selectivity that the chemical has among rape, pigweed and foxtail.

BIBLIOGRAPHY

Agbakoba, C. S. O., and J. R. Goodin. 1969. Effect of stage of growth of field bindweed on absorption and translocation of ^{14}C-labelled 2,4-D and picloram. Weed Sci. 17:436-438.

Aslander, A. 1927. Sulfuric acid as a weed spray. J. Agr. Res. 31: 1065-1091.

Blackman, G. E. 1952. Studies in the principles of phytotoxicity. I. The assessment of relative toxicity. J. Expt. Bot. 3:1-27.

Blackman, G. E., R. S. Bruce and K. Holly. 1958. Studies in the principles of phytotoxicity. V. Inter-relationships between specific differences in spray retention and selective toxicity. J. Expt. Bot. 9:175-205.

Blackman, G. E., and W. G. Templeman. 1936. The eradication of weeds in cereal crops by sulphuric acid and other compounds. J. Agr. Sci. 26:368-390.

Briggs, W. R. 1963. The phototropic responses of higher plants. Ann. Rev. Pl. Physiol. 14:311-352.

Brunskill, R. T. 1956. Physical factors affecting the retention of spray droplets on leaf surfaces. Proc. Br. Weed Control Conf. 3:593-603.

Crafts, A. S. 1956. Weed control: applied botany. Amer. J. Bot. 43: 548-556.

Crafts, A. S. 1957. The chemistry and mode of action of herbicides. Adv. Pest Control Res. 1:39-81.

Crafts, A. S. 1961. The chemistry and mode of action of herbicides. 269 p. Interscience, New York.

Crafts, A. S. 1965. Relation between food and herbicide transport. In. Isotopes in Weed Research. p. 3-7 I.A.E.A. Austria.

Crafts, A. S., and C. L. Foy. 1962. The chemical and physical nature of plant surfaces in relation to the use of pesticides and their residues. Residue Rev. 1:112-139.

Crafts, A. S., and H. G. Reiber. 1948. Herbicidal properties of oils Hilgardia. 18:77-156.

Crafts, A. S., and S. Yamaguchi. 1964. The autoradiography of plant materials. Man. Calif. Agric. Exp. Stn. Ext. Serv. 35:1-143.

Currier, H. B. 1951. Herbicidal properties of benzene and certain methyl derivatives. Hilgardia. 20:383-406.

Currier, H. B., and C. D. Dybing. 1959. Foliar penetration of herbicides - review and present status. Weeds. 7:195-213.

Davies, P. J., D. S. Drennan, J. D. Fryer, and K. Holly. 1967. The basis of the differential phytotoxicity of 4-hydroxy-3,5-di-iodobenzonitrile. I. The influence of spray retention and plant morphology. Weed Res. 7:220-233.

Dybing, C. D., and H. B. Currier. 1959. A fluorescent dye method for foliar penetration studies. Weeds. 7:214-222.

Dybing, C. D., and H. B. Currier. 1961. Foliar penetration of chemicals. Pl. Physiol. 36:169-174.

Foy, C. L. 1964. Review of herbicide penetration through plant surfaces. J. Agric. Fd. Chem. 12:473-477.

Franke, W. 1967. Mechanisms of foliar penetration of solutions. Ann. Rev. Pl. Physiol. 18:281-301.

Freed, V. H., and M. Montgomery. 1958. The effect of surfactants on foliar absorption of 3-amino-1,2,4-triazole. Weeds. 6:386-389.

Frey-Wyssling, A. 1953. The submicroscopic morphology of protoplasm. 411 p. Elsevier, Amsterdam.

Frey-Wyssling, A., and K. Muehlethaler. 1965. Ultrastructural plant cytology. 377 p. Elsevier, New York.

Furmidge, C. G. L. 1962a. Physico-chemical studies on agricultural sprays. IV. The retention of spray liquids on leaf surfaces. J. Sci. Fd. Agric. 13:127-140.

Furmidge, C. G. L. 1962b. Studies at phase interphases. I. The sliding of liquid drops on solid surfaces and a theory for spray retention. J. Colloid Sci. 17:309-324.

Furuya, S., and M. Arai. 1966. Studies on some properties of diphenyl ether type herbicides. Zasso Ken Kyu. 5:99-104.

Gutenmann, W. H., and D. J. Lisk. 1967. Metabolism of TOK herbicide in the dairy cow. J. Dairy Sci. 50:1516-1518.

Herbicide Handbook., Weed Soc. of Amer. 1967. 293 p.

Herrett, R. A., and A. J. Linck. 1961. Penetration of 3-amino-1,2,4-triazole in Canada thistle and field bindweed. Weeds. 9:224-230.

Hibbitt, C. J. (1969) Growth and spray retention of wild oat and flax in relation to herbicidal selectivity. Weed Res. 9:95-107.

Hogue, E. J., and G. F. Warren. 1968. Selectivity of linuron on tomato and parsnip. Weed Sci. 16:51-54.

Holly, K. 1956. Penetration of chlorinated phenoxyacetic acids into leaves. Ann. appl. Biol. 44:195-199.

Kempen, H., H. Agamalian, A. H. Lange and R. Brendler. 1968. Weed Control in Carrots, Celery and Parsley. Calif. Agr. 22:2-3.

Kirkwood, R. C., J. Dalziel, A. Matlib and L. Somerville. 1968. Some factors influencing the activity of foliage-applied M.C.P.A. and M.C.P.B. in Vicia faba. Proc. Br. Weed Control Conf. 9:650-659.

Kylin, A. 1960. The accumulation of sulphate in isolated leaves as affected by light and darkness. Bot. Notiser. 113:49-81.

Leonard, O. A., and R. J. Hull. 1965. Translocation of ^{14}C-labelled substances and $^{32}PO_4$ in mistletoe-infected and uninfected conifers and dicotyledonous trees. In. Isotopes in Weed Research. p. 31-46. I.A.E.A. Austria.

Marcelli, E., A. Vardabasso and F. Fantechi. 1966. Experimental weed control in tobacco in southern Italy. Tabacco. 70:133-169.

Martin, J. T. 1966. The cuticles of plants. N.A.A.S. q. Rev. 72: 139-144.

Mason, G. W. 1963. Weed control in field brassica crops. Proc. N.Z. Weed Control Conf. 16:65-70.

Mason, G. W. 1964. Further developments in field brassica weed control. Proc. N.Z. Weed Control Conf. 17:85-90.

Matsunaka, S. 1969a. Activation and inactivation of herbicides by higher plants. Residue Revs. 25:45-58.

Matsunaka, S. 1969b. Acceptor of light energy in photoactivation of diphenyl ether herbicides. J. Agr. Food Chem. 17:171-175.

Matsunaka, S., and K. Inada. 1967. Mode of action of diphenyl ether herbicides. I. Light for herbicidal activity. Abstr. Ann. Meeting Agr. Chem. Soc. Japan. p. 47.

Noll, C. J., 1966. Chemical weeding of carrots. Proc. Northeast. Weed Control Conf. 20:24-28.

Prasad, R., C. L. Foy and A. S. Crafts. 1967. Effects of relative humidity on absorption and translocation of foliarly applied dalapon. Weeds. 15:149-156.

Repp, G. 1958. On the selective action of 2,4-D. The relationship between the susceptibility to growth regulators and the structure of the outer surfaces of plants. Z. Acker-u. PflBau. 107:49-66.

Robertson, M. M., and R. C. Kirkwood. 1969. The mode of action of foliage-applied translocated herbicides with particular reference to the phenoxy-acid compounds. I. The mechamism and factors influencing herbicide absorption. Weed Res. 9:224-240.

Sargent, J. A. 1965. The penetration of growth regulators into leaves. Ann. Rev. Pl. Physiol. 16:1-12.

Sargent, J. A., and G. E. Blackman. 1962. Studies on foliar penetration. I. Factors controlling the entry of 2,4-dichlorophenoxyacetic acid. J. Exp. Bot. 13:348-368.

Sargent, J. A., and G. E. Blackman. 1965. Studies on foliar penetration. II. The role of light in determining the penetration of 2,4-dichlorophenoxyacetic acid. J. Exp. Bot. 16:24-47.

Schafer, D. E. 1970. Selectivity and chemodynamics of 3,5-dibromo-4-hydroxybenzonitrile in winter wheat (Triticum aestivum L.) and coast fiddleneck (Amsinckia intermedia Fisch. and Mey.). Ph.D. Thesis. Oregon State Univ. 110p.

Smith, H. H. 1946. Quantitative aspects of aqueous-spray applications of 2,4-dichlorophenoxyacetic acid for herbicidal purposes. Bot. Gaz. 107:544-551.

Takehisa, S., H. Ishikawa, S. Fujimaki, T. Noguchi. 1966. Effects of 3,4-dichloropropionanilide, 2,4-dichloro- p-nitrodiphenyl ether, and 3-amino-1,2,4-triazole on mitosis and chromosomes in Vicia faba roots. Zasso Ken Kyu. 5:154-156.

Thaine, R. 1969. Movement of sugars through plants by cytoplasmic pumping. Nature. 222:873-875.

Thompson, A. 1963. Weed control in brassica, fodder beet and mangold crops. Proc. N.Z. Weed Control Conf. 16:60-64.

Thompson, A. 1964. Weed control in field brassicas. Proc. N.Z. Weed Control Conf. 17:91-96.

Van Overbeek, J. 1956. Absorption and translocation of plant growth regulators. Ann. Rev. Pl. Physiol. 7:355-372.

Van Overbeek, J., and R. Blondeau. 1954. Mode of action of phytotoxic oils. Weeds. 3:55-65.

Vostral, H. J., and K. P. Buchholtz. 1966. Translocation and residue studies with M.C.P.B. in peas. Weeds. 14:244-247.

Weaver, R. J., and H. R. DeRose. 1946. Absorption and translocation of 2,4-dichlorophenoxyacetic acid. Bot. Gaz. 107:509-521.

Wilson, H. F., and D. H. McRae. 1960. U. S. patent #3,080,225. approved Mar. 5, 1963. 8 p.

Woodford, E. K. 1957. The toxic action of herbicides. Outl. Agric. 1: 145-154.

Wood, J. W., J. W. Mitchell and G. W. Irving, Jr. 1947. Translocation of a radioactive plant-growth regulator in bean and barley plants. Science. 105:337-339.

APPENDIX

Appendix Table 1. Correlation coefficients for regression lines shown in Figure 1 (Section 1). Dosage-response experiment.

Species	Correlation coefficient	Value for 5% significance
Rape	0.966	0.878
Pigweed	0.981	0.878
Foxtail	0.977	0.754

Appendix Table 2. Analysis of variance for the retention of Niagara Sky Blue dye on rape, pigweed and foxtail.

Source of variation	df	mean square	'F'
Treatments	2	21105.61	6.59*
Error	6	3203.28	

* significant at the 5% level.

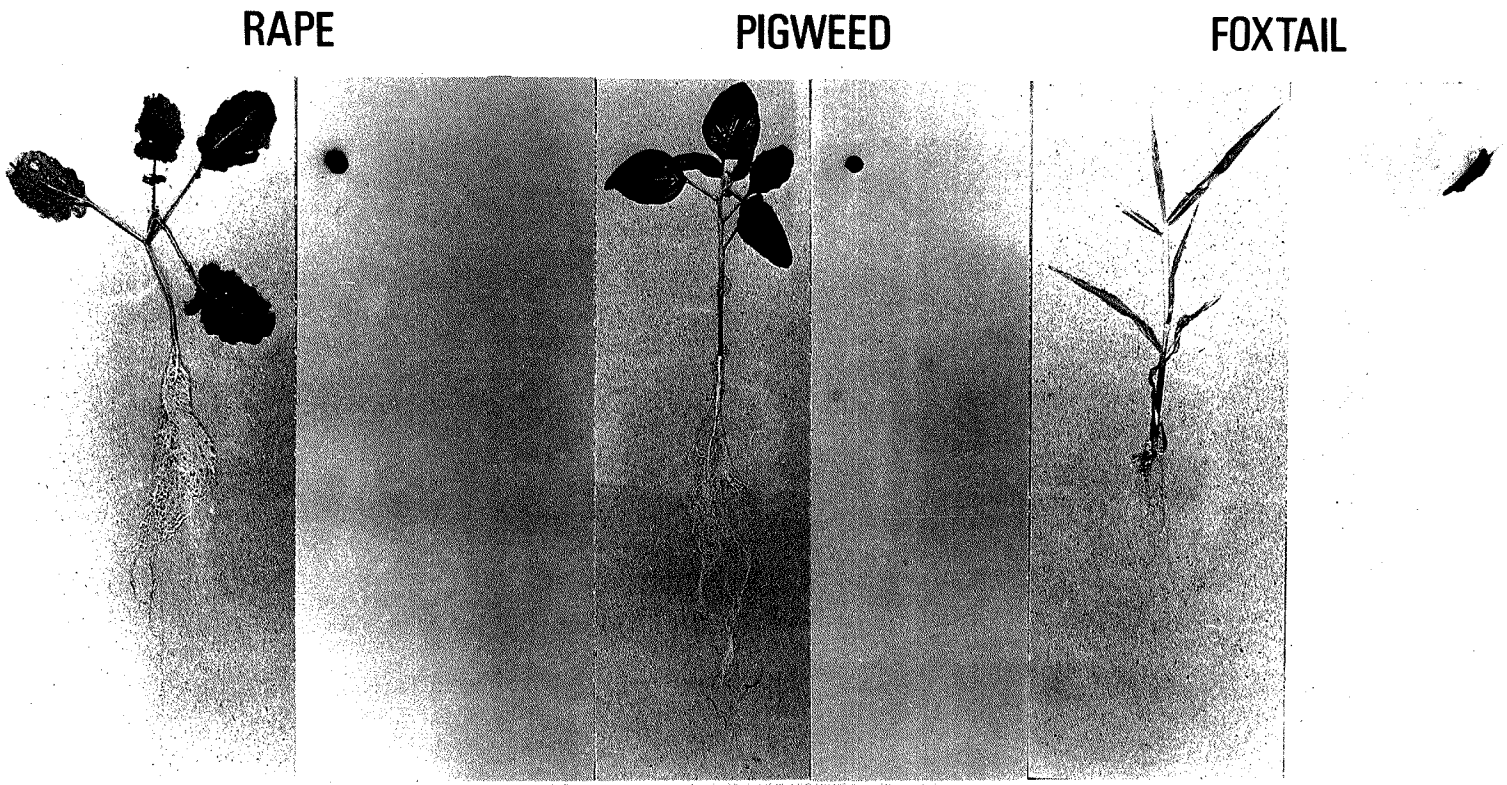
Appendix Table 3. Analyses of variance of % penetration[1] of ^{14}C-nitrofen between species and between dates of harvest.

Source of variation	df	mean square	'F'
	Dates of harvest for rape		
Treatments	2	45.39	1.20^{ns}
Error	24	37.94	
	Dates of harvest for pigweed		
Treatments	2	53.71	5.14*
Error	27	10.46	
	Dates of harvest for foxtail		
Treatments	2	13.95	0.33^{ns}
Error	27	42.16	
	Species for 2 days		
Treatments	2	171.79	9.46*
Error	26	18.15	
	Species for 4 days		
Treatments	2	120.01	2.48^{ns}
Error	26	48.45	
	Species for 8 days		
Treatments	2	90.78	3.97*
Error	26	22.88	

[1] arc sin transformation

*significant at the 5% level

^{ns}not significant at the 5% level.



Appendix Figure 1. Photographs of plants and autoradiographs. Plants were pressed 4 days after treatment with 5 µl of ^{14}C-nitrofen. X0.5.