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.