AN ABSTRACT OF THE THESIS OF

DAVID EUGENE SCHAFER (Name) for the DOCTOR OF PHILOSOPHY (Degree)

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Title: SELECTIVITY AND CHEMODYNAMICS OF 3,5-DIBROMO-4-HYDROXYBENZONITRILE IN WINTER WHEAT (TRITICUM AESTIVUM L.) AND COAST FIDDLENECK (AMSINCKIA INTERMEDIA FISCH. & MEY)

Abstract approved: Redacted for privacy

Controlled environment chamber and laboratory studies were carried out to evaluate selectivity and chemodynamics of bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) in winter wheat (Triticum aestivum L.) 'Nugaines', a tolerant species, and coast fiddleneck (Amsinckia intermedia Fisch. & Mey), a susceptible species.

A comparison of ED_{50} values, based upon reduction of whole plant growth, indicated that wheat was 109 times more tolerant of bromoxynil than was fiddleneck. The shoot/root ratios of both wheat and fiddleneck were observed to increase with increasing dosage of bromoxynil. Measurement of necrotic tissue weight of wheat and fiddleneck, as a function of bromoxynil dosage, suggested a sub-lethal translocation effect.

The selectivity of bromoxynil in wheat and fiddleneck was

concluded to be the result of a complex of interactions. Fiddleneck was found to retain twice as much spray solution as wheat. Mathematical analysis revealed that 6.3 percent of the differential toxicity could be attributed to differential herbicide retention. The penetration of bromoxynil-^{14}C into leaf tissue was found to proceed more rapidly in fiddleneck than in wheat. Penetration of bromoxynil-^{14}C could not be related to stomatal densities in either species. Only 4.9 percent of the selectivity existing between wheat and fiddleneck could be attributed to the penetration differential.

Autoradiography and extraction procedures revealed that the label from bromoxynil-^{14}C was more mobile in fiddleneck than in wheat. Higher levels of radioactivity were found in treated leaf, foliage and root extracts of fiddleneck as compared to wheat. Higher levels of insoluble label were found in treated leaves, foliage and roots of wheat as compared to fiddleneck.

In both species, a high percentage of the extractable radioactivity was attributed to bromoxynil-^{14}C. No specific difference in the percentage of total derivatives could be found. The evolution of $^{14}CO_2$ by wheat treated with bromoxynil-^{14}C significantly exceeded that of fiddleneck. This indicated a greater capacity, on the part of wheat, to degrade this herbicide. Mathematical analysis indicated that 88.8 percent of the selectivity existing between wheat and fiddleneck could be attributed to internal physiological and biochemical mechanisms.

Selectivity and Chemodynamics of 3,5-dibromo-4-hydroxybenzonitrile in Winter Wheat (*Triticum aestivum* L.) and Coast Fiddleneck (*Amsinckia intermedia* Fisch. & Mey)

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David Eugene Schafer

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Redacted for privacy

Head of Department of Farm Crops

Redacted for privacy

Dean of Graduate School

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INTRODUCTION

Subsequent to the elucidation of the herbicidal properties of halogenohydroxybenzonitriles by Wain (1963) and Carpenter and Heywood (1963), these compounds achieved widespread usage in Europe and the United States. Ioxynil (3, 5-diiodo-4-hydroxybenzonitrile) has been well accepted by the Europeans. Bromoxynil (3, 5-dibromo-4-hydroxybenzonitrile), on the other hand, has been shown to be more useful for selective weed control in the United States. Provincially, bromoxynil has been used extensively for selective weed control in areas of winter wheat production in eastern Oregon and Washington. Bromoxynil has proven useful in controlling certain broad-leafed weeds, such as coast fiddleneck, which are marginally controlled by other selective herbicides. Occasionally, control of this species, in winter wheat, has been inadequate. The causation(s) of these failures, while tentatively attributed to stage of growth of the weed or to environmental vagaries, are ill-defined.

Few detailed studies on the influence of environment on the toxic action of bromoxynil are available. A more complete

understanding of the internal physiological and biochemical factors of the plant, which reflect on the toxic action of bromoxynil, would be useful in understanding the effectiveness or failure of this compound in controlling broad-leafed weeds. It is obvious that this knowledge is necessary before the interactions of environment and stage of growth with bromoxynil chemodynamics can be comprehended. Initial published reports on bromoxynil dealt mainly with its practical usage and mode of action. The whole realm of selectivity and chemodynamics of bromoxynil in cereal and dicotyledonous species remains virtually unexplored. In reference to halogenohydroxybenzonitriles, as a herbicide class, additional investigations designed to quantitate translocation, metabolic conjugation, and metabolic conversion to the limit of CO_2 are sorely needed. The present study was undertaken to explore these areas. The objectives of the investigations reported herein were twofold; (1) To elucidate and quantitate the plant-herbicide interactions which could be associated with bromoxynil selectivity, (2) To gain insight into the chemodynamics of bromoxynil in tolerant and susceptible species.

The two species selected for study in this thesis possess great differences in tolerance to bromoxynil. Winter wheat (Triticum aestivum L.) 'Nugaines' is extremely tolerant, while coast fiddleneck (Amsinckia intermedia Fisch. & Mey) is very susceptible under most conditions. These differential tolerance characteristics provided an

ideal tool for understanding causative factors involved in bromoxynil selectivity in cereals and dicotyledonous weeds.

Specifically, the role of herbicide retention, penetration, translocation and degradation, as they relate to the selectivity of bromoxynil, were examined. A mathematical model was established to quantitate the contribution of selected plant-herbicide interactions to the selectivity phenomenon.

LITERATURE REVIEW

I. Introduction

The herbicidal properties of the halogenohydroxybenzonitriles (hereafter referred to as the HBNs) were independently reported by Wain (1963) and by Carpenter and Heywood (1963). A third party, Amchem Products Inc., also independently evaluated the herbicidal effects of these compounds (Hart, Bishop and Cooke, 1964). These compounds were initially described as post-emergence, contact herbicides with limited systemic activity. They were recognized to be effective in controlling many broad-leafed weeds in mono-cotyledonous crops.

In subsequent reports, the herbicidal properties of these compounds were further elucidated. Terry and Wilson (1964) reported that both bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) and ioxynil (3,5-diiodo-4-hydroxybenzonitrile) controlled many important weeds in cereals at dosages of 0.2 to 0.5 kg/ha. Weeds in seedling grass crops were controlled by dosages of 0.4 to 0.5 kg/ha. These herbicides were found to be effective against weeds such as common chickweed [Stellaria media (L.) Cyrillo] which are normally resistent to phenoxy alkanoic acid herbicides. Perennial weeds such as canada thistle [Circium arvense (L.) Scop.] and perenial sowthistle (Sonchus arvensis L.) required higher dosages (greater than one kg/ha)

for adequate control. In field tests, spring wheat (Triticum aestivum L.), barley (Hordeum vulgare L.) and oats (Avena sativa L.) tolerated dosages up to two kg/ha with only superficial leaf scorch occurring. Terry and Wilson suggested that growth stage of the crop and the addition of a wetting agent to the spray solution were factors most likely to influence cereal tolerance.

Ball, Cottrell and Terry (1964) found that bromoxynil at 0.5 kg/ha gave effective weed control in alfalfa (Medicago sativa L.) without affecting the crop stand. Alfalfa was sensitive to ioxynil. Red clover (Trifolium pratense L.) and white clover (Trifolium repens L.) were found to be sensitive to both compounds.

Carpenter et al. (1964c) reported that ioxynil-MCPA and ioxynil-mecoprop mixtures were effective in controlling canada and perennial sowthistle. Ioxynil-mecoprop was found to be effective against a broad spectrum of annual weeds.

Carpenter et al. (1964a) concluded that these herbicides exhibited rapid contact action plus a slower systemic effect. While not being soil active, the compounds were effective inhibitors of seed germination in vitro. They suggested that post-emergence activity was influenced by growth stage of the weed, herbicide distribution on the foliage and by environmental factors such as light intensity and temperature.

In this coverage of the literature, the physical and chemical

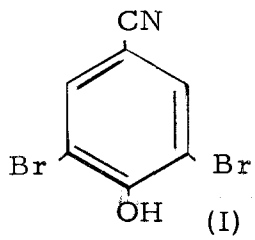
properties, selectivity and chemodynamics, and mode of action of bromoxynil and ioxynil will be reviewed. The paucity of information on bromoxynil, particularly in the areas of selectivity and chemodynamics, will be evident in this review. This has necessitated the inclusion of ioxynil as a representative of the HBNs. While numerous similarities exist between the herbicidal action of ioxynil and bromoxynil, it should be emphasized that extensive extrapolation of informa- from ioxynil to bromoxynil is unwarranted.

II. Properties of Bromoxynil and Ioxynil and Their Relationship to Herbicidal Activity

Initial attempts to use parent HBNs for field weed control were hampered by the solubility characteristics of these compounds. Fortunately, this was a problem that was easily overcome. In addition, the requirements for herbicidal activity were found to be highly dependent upon the identity and location of the substituents on the HBN moiety. It is the purpose of this section to describe these initial investigations on the molecular properties of the HBNs.

A. Chemical and Physical Properties

Bromoxynil (I) and ioxynil (II) are described as high melting crystalline solids. Both compounds are nonvolatile. They are virtually insoluble in water and sparingly soluble in organic solvents. These compounds are relatively stable



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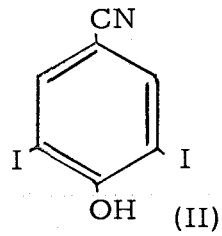

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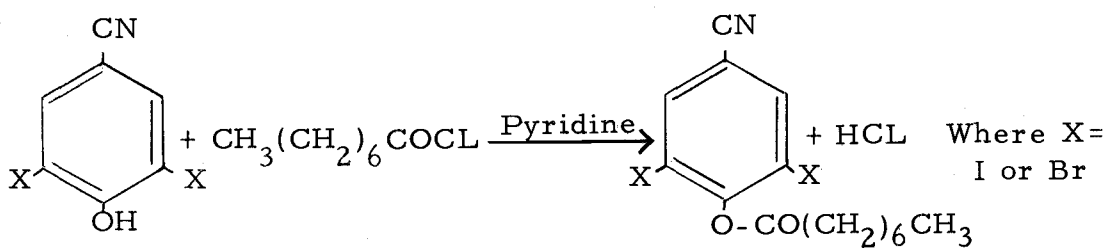
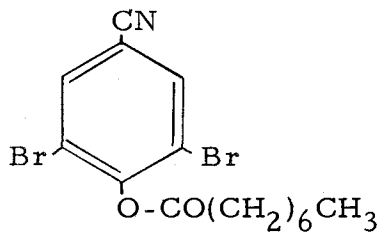
but can be hydrolyzed via strong aqueous alkalis and mineral acids to amide and carboxylic acid derivatives (Heywood, Carpenter and Cottrell, 1964, and Ioxynil and Bromoxynil, 1967).

The limited solubility of the above compounds eliminated the possibility of formulating concentrated solutions for herbicidal use. This obstacle was circumvented in two ways; (1) Both compounds are fairly strong organic acids with a pK of about 4.0. With this characteristic present, both herbicides form salts with alkali metals to give neutral salts in water. Concentrated formulations could then be prepared by incorporating suitable organic solvents. (2) A second means of providing suitable formulations was via conversion of the phenol into ester form.



Bromoxynil (III) and ioxynil octanoate (IV) are low melting crystalline solids



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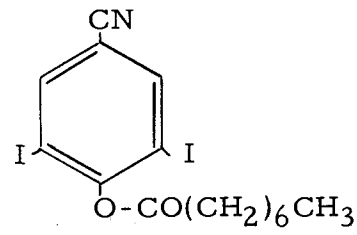


(IV)

which are soluble in organic solvents. Both compounds are non-volatile. They may be stored for up to twelve months at 40 C without decomposition. Slow hydrolytic decomposition in water has been noted. Hydrolysis proceeds rapidly in the presence of aqueous mineral acids and aqueous sodium hydroxide (Heywood, Carpenter and Cottrell, 1964, and Ioxynil and Bromoxynil, 1967).

B. Chemical Structure and Its Relationship to Herbicidal Activity

The relationships existing between chemical structure and herbicidal activity have been described by Carpenter et al. (1964b) and Heywood (1966). In regard to the halogen substituents, among the symmetrical 3, 5-dihalogeno-4-hydroxybenzonitriles, the order of activity is ioxynil > bromoxynil. Mixed halogen compounds assume an intermediate position. The effect of halogen atom position was evaluated via chlorine substitution. The order of activity was found to be 3, 5-dichloro > 2, 5-dichloro > 2, 6-dichloro substitution. These relationships were later found to hold with bromine and iodine substituents. The substitution of hydroxyl, methoxy, amino,

alkylamine and other groups in one or both free (meta) positions of the HBN molecule led to reduction in activity. Replacement of one or both of the halogens with these groups also reduced the herbicidal activity of the compound. All modifications of the cyano group led to less active compounds. Removal of the cyano group destroyed the post-emergence activity of these compounds. The presence and position of a hydroxyl group para to the cyano group was concluded to be necessary for post-emergence activity. In regard to 4-hydroxy derivatives of the HBNs, it was concluded that high activity could be achieved only when the hydroxyl group was readily liberated from the derivative. The herbicidal activity of bromoxynil and ioxynil esters, derived from straight chain fatty acids, increased from the acetate until a chain length of eight carbon atoms was reached, after which activity decreased. Heywood (1967) speculated that esterases, common in living systems, were responsible for the release of the free toxic phenol from the non-toxic ester.

III. Selectivity and Chemodynamics

In order to understand selectivity and chemodynamics of post-emergence compounds in plants, it is necessary to evaluate the various interactions that occur between the herbicide and the target organism. Such interactions include retention and distribution of the herbicide spray, penetration into the foliage, subsequent movement and

changes in herbicide molecular structure.

A. Spray Retention

Differential spray retention between Graminaceous and associated broad-leaf plants has been implicated in the selectivity displayed by HBN herbicides. Carpenter et al. (1964a) evaluated the retention characteristics of barley (Hordeum vulgare L.) and Sinapsis arvensis L. by incorporating 0.1 percent acid red dye in an aqueous solution of ioxynil-sodium. After the plants were sprayed, the dye deposit was washed off and estimated colorimetrically. Using fresh weight as a basis for comparison, barley retained one-fifth as much spray solution as Sinapsis. Since the effective dose was considerably greater than five fold, Carpenter et al. concluded that spray retention did not fully account for the selectivity between these species.

In a recent paper, Davies et al. (1967) reported on spray retention as a factor in differential phytotoxicity of ioxynil to barley (Hordeum distichon L.), field pea (Pisum sativum L.) and white mustard (Sinapsis alba L.). After the plants were sprayed with ioxynil-sodium, they were washed. Estimation of ioxynil was via spectrophotometic analysis, based upon optical absorption of the herbicide at 236.5 mμ. On a dry weight basis, mustard retained 26 times more ioxynil than did barley when no surfactant was included in

the spray. The addition of 0.1 and 1.0 percent Tween-20 surfactant to the spray solution increased retention by barley and changed the retention ratio (mustard/barley) to 11 and eight, respectively. Analysis of growth reduction indicated that Tween-20 influenced spray retention only, and provided no other function in phytotoxicity.

B. Penetration

Foy (1964) has reported on the absorption of ioxynil-^{14}C in barley and fiddleneck (Amsinckia sp.). Microdrops of ioxynil-^{14}C placed on the leaf surface of these species dried rapidly, leaving a powdery residue. From this observation, Foy concluded that penetration of ioxynil was limited.

Davies et al. (1968a) measured the uptake of ioxynil-^{14}C-sodium as an aqueous solution containing 0.1 percent Tween 20. Relatively large amounts (0.1 ml) of the labelled herbicide were placed in nylon rings attached to leaf discs of field pea and white mustard. Uptake by barley was studied by placing the labelled herbicide in a silicone reservoir built up on a leaf section. With all species, rate of uptake was rapid during the first four hours, and slower thereafter. After two and four hours, the uptake ratio of mustard/pea was seven. Over longer durations, this ratio increased. After four hours the uptake ratio of barley/pea was 1.5. When surfactant-free solutions were used on these species, a sharp reduction in uptake of ioxynil-^{14}C was

observed. The entry of ioxynil-^{14}C was found to be unrelated to stomatal density.

C. Translocation

Studies on translocation of ioxynil-^{14}C in barley and fiddleneck have been reported by Foy (1964). By using autoradiographic techniques, he found that translocation from the point of application occurred both acropetally and basipetally, but was extremely slow. After one week of treatment, the label from ioxynil-^{14}C moved approximately one inch in either direction from the microdrop. However, pseudoautoradiograms and contaminants presented numerous problems in his analysis. Foy speculated that initial movement of the herbicide was via diffusion followed by leakage from injured cells.

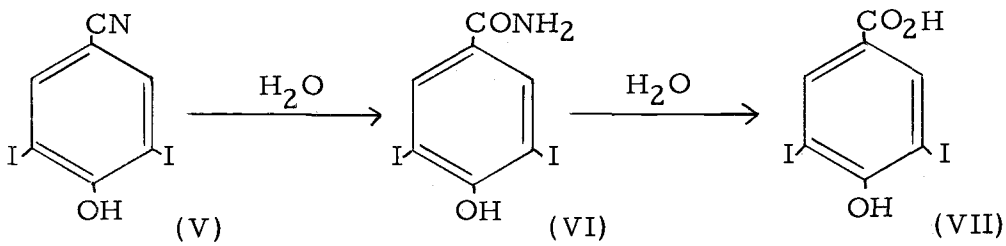
Carpenter et al. (1964a) evaluated translocation of ioxynil by observing phytotoxicity expressed at points removed from the site of application. Pale smartweed (Polygonum lapathifolium L.) plants were enclosed in polythene tubes with one leaf exposed. The plants were then sprayed with ioxynil and the cover removed. After three weeks, chlorosis was noted in both young and older leaves. Carpenter et al. concluded that movement into older leaves was less marked than movement into younger leaves. A 0.2 percent solution of ioxynil applied to wheat leaves produced chlorotic areas around the microdrops, but no chlorosis in new growth.

Limited translocation of ioxynil-sodium, supplied via root treatment, in dwarf bean (Phaseolus vulgaris L.) was observed by Zaki, Taylor and Wain (1967). Analysis of sap from stems revealed the presence of ioxynil in the lower but not in middle stem regions. They concluded that some ioxynil was taken up by bean roots but was poorly translocated.

The translocation of ioxynil-^{14}C in white mustard, cotton (Gossipium sp.), dwarf bean, barley, and field pea was evaluated by Davies et al. (1968a) via autoradiography. While small amounts of the label moved short distances, most of the activity remained close to the site of application. Within a five hour period, activity could be detected down to the base of the petiole of the treated leaf in most species. Acropetal movement in the treated leaves was also noted. Subsequent movement occurred up the stem and into the young leaves. A number of mature leaves were by-passed during translocation. Root treatment resulted in extensive root labelling with only limited translocation. Acropetal movement was observed after stem treatment. Greater mobility of the label was found in barley than among other species. Davies et al. conducted further experiments to compare the effect of leaf removal by cutting with destruction of equivalent leaf areas by ioxynil. These studies indicated a greater translocation effect with mustard than with pea or barley.

D. Internal Selectivity Factors

Carpenter et al. (1964b) proposed that transformation of HBNs in plant tissue, using hydrolysis of ioxynil as an example, might follow a pathway such as:



They discounted the possibility that the parent phenol (V) had to be 'activated' by the plant via hydrolysis since the biological activity of the amide (VI) and carboxylic acid (VII) derivatives were much less than that of the parent phenol.

Hart, Bishop and Cooke (1964) have reported on the fate of ring labelled ioxynil-^{14}C in wheat. They found that the herbicide was metabolized and reincorporated into starch, gluten and glucose fractions of mature grain.

The metabolism of ioxynil by dwarf bean was evaluated by Zaki, Taylor and Wain (1967). These workers employed a ceric sulphate: sodium arsenite reagent to locate iodo compounds on paper chromatograms. Five days after root treatment with ioxynil-sodium, sap from the lower stem region contained iodide, ioxynil and the benzoic acid derivative. Sap from middle stem regions contained only iodide. No amide derivative was found in either stem location.

Davies et al. (1968b) evaluated chlorophyll destruction and decrease in reduction of 2, 3, 5-triphenyl tetrazolium chloride in ioxynil-treated leaf discs and sections of resistant and susceptible species. Three to five times the concentration of ioxynil was required to bring about a response in barley that was comparable with that noted in white mustard. Residue experiments showed that ioxynil disappeared rapidly from barley plants after 24 hours of treatment. In mustard, however, ioxynil residues remained high throughout 28 days of treatment. Davies et al. also employed ioxynil-^{14}C to study breakdown of the herbicide in barley, pea, and mustard. Within four days, traces of the amide derivative were extracted from pea and mustard leaves. About one percent of the activity recovered from barley was attributed to amide and benzoic acid derivatives. Unknown compounds in barley contributed about five percent to the extracted activity. From the foregoing results, Davies et al. suggested that barley was able to decompose ioxynil. However, no measurements were made on $^{14}CO_2$ release or on unextractable labelled compounds.

The absence of bromoxynil from the foregoing citations is noteworthy. A complete void exists among published literature in regard to the selectivity and chemodynamics of this compound. Further studies in this area are definitely warranted.

IV. Mode of Action

Studies on the biochemical mode of action of HBNs have implicated inhibition of photosynthesis and respiration, impairment of biosynthetic and biodegradation reactions, free radical formation and halide ion toxicity. These factors will be considered sequentially in this section.

A. Photosynthetic Inhibition

Kerr and Wain (1964a) isolated chloroplasts from dwarf bean leaves and measured the reduction of ferricyanide potentiometrically. The molar concentrations required to produce 50 percent inhibition of the Hill reaction were found to be: 10^{-6} ioxynil, 1.8×10^{-5} bromoxynil and 10^{-3} 3,5-diiodo-4-hydroxybenzoic acid. They concluded that a close correlation existed between the herbicidal activity of these compounds and their ability to inhibit photoreduction.

Using chloroplasts isolated from broad bean (Vicia faba L.), Paton and Smith (1965a) have shown that ioxynil produced 50 percent inhibition of CO_2 fixation at ca 10^{-5} M, ATP formation at ca 5×10^{-4} M, and NADP reduction at ca 5×10^{-3} M. These results indicated that ioxynil inhibited both electron transport and non-cyclic photophosphorylation. The inability of phenazine methosulfate to relieve inhibition of ATP formation led these workers to speculate that a sensitive photophosphorylation site may exist between the plant

cytochromes and photosystem I.

In a subsequent paper, Paton and Smith (1965b) reported on the action of ioxynil on the photoreduction of endogenous plastoquinone in isolated chloroplasts of broad bean. O-phenanthroline, a known inhibitor of the Hill reaction, was used as a reference in comparing activity of ioxynil in inhibiting the photoreduction of plastoquinone. The addition of O-phenanthroline and ioxynil to the chloroplast extracts resulted in high percentages of plastoquinone remaining in oxidized form. Plastoquinone is considered to be the acceptor of electrons from the photosystem II mediated reaction involving splitting of water. In an attempt to by-pass this site of inhibition, ascorbate/indolephenol was added to cultures containing the inhibitors. This addition was effective in relieving the inhibition via O-phenanthroline but not ioxynil. From this evidence, Paton and Smith speculated that plastoquinone may be involved in more than one site in photosynthetic electron transport. In addition to inhibiting the system II reaction, ioxynil also had a blocking action at some other part of the photosynthetic electron transport chain.

Friend and Olsson (1967) isolated chloroplasts from broad bean and sugar beet (Beta vulgaris L.) and found that ioxynil at 5×10^{-4} M inhibited the photoreduction of endogenous plastoquinone. However, contrary to the findings of Paton and Smith (1965b) the addition of ascorbate/indolephenol did relieve the inhibition of plastoquinone

reduction. Friend and Olsson concluded that ioxynil inhibited plastoquinone reduction at the same site as O-phenanthroline.

B. Respiratory Inhibition

The involvement of HBNs in respiratory inhibition has been adequately demonstrated. Kerr and Wain (1964b) using mitochondria isolated from pea found that ioxynil and bromoxynil depressed the uptake of inorganic phosphate and, to a lesser extent, oxygen, by the particles. The calculated P/O ratio was reduced to zero at 4×10^{-5} M ioxynil and 9×10^{-5} M bromoxynil, indicating that both compounds were effective in uncoupling oxidative phosphorylation.

Foy and Penner (1965) found that ioxynil was effective in inhibiting the rate of oxidation of succinate and a-ketoglutarate at 0.75×10^{-6} to 0.75×10^{-3} M concentrations in mitochondrial fractions of cucumber (Cucumis sativus L.).

Working with mitochondria preparations from bean root tips, Paton and Smith (1967) found that ioxynil reduced oxygen uptake at high concentrations. They also found that ioxynil blocked the reduction of ubiquinone.

C. Inhibition of Biosynthetic and Biodegradation Reactions

The foregoing evidence suggests that HBNs can function as electron transport inhibitors and as uncoupling agents. The

inhibition of these functions would likely influence ATP dependent biosynthetic reactions. Mann, Jordon and Day (1965) studied the effect of ioxynil upon incorporation of leucine-1-^{14}C into protein of barley coleoptiles and hemp sesbania [Sesbania exaltata (Raf.) Cory] hypocotyls. The incorporation of leucine in both species was inhibited by ioxynil at 2 and 5 ppm. This inhibition was greater in sesbania than in barley. Mann, Jordon and Day, however, attributed the limited incorporation of leucine into protein to inhibition of amino acid uptake from the culture medium. They discounted the possibility that protein synthesis was directly affected by ioxynil. They concluded that process(es) required for both uptake and peptide bond formation were blocked.

Paton and Zalik (1968) reported on the effect of ioxynil on the free amino acid content of leaves of wheat and tartary buckwheat [Fagopyrum tataricum (L.) Gaertn.], a susceptible species. Free amino acids were increased 2.6 percent in wheat and 15.0 percent in tartary buckwheat two days after spraying. Protein amino acids, on the other hand, decreased 4.8 percent in wheat and 31.2 percent in tartary buckwheat.

Mann and Pu (1968) found that ioxynil at 20 mg/liter inhibited the incorporation of malonic acid-2-^{14}C into lipids of hemp sesbania hypocotyls by more than 25 percent.

Evidence has been accumulated to show that HBNs inhibit

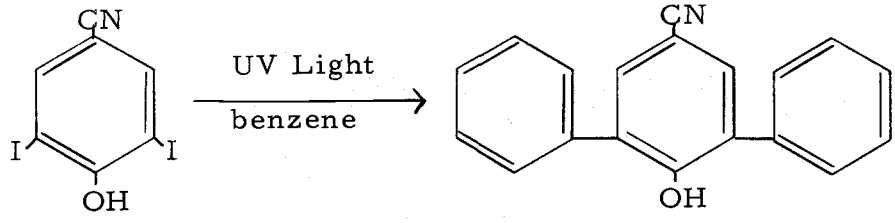
degradation of protein and starch. Ashton, Penner and Hoffman (1968) found that 10^{-3} M ioxynil inhibited proteolytic activity in cotyledons of three day old squash (Cucurbita maxima Dusch.) seedlings by 83 percent. Bromoxynil at 10^{-4} M inhibited proteolytic activity by 68 percent.

In a subsequent paper, Penner and Ashton (1968) reported that bromoxynil did not influence activity per se of isolated proteolytic enzymes of squash. They found that bromoxynil inhibited the proteolytic enzyme(s) development in the cotyledons of intact embryos but not in excised cotyledons. The inhibition was partly overcome by benzyladenine. Penner and Ashton suggested that bromoxynil not only interfered with the hormonal control mechanism, but also proteinase synthesis.

In a more recent paper, Penner (1968) found that the development of amylase activity, controlled by the embryo in distal halves of intact barley seeds during the first two days of germination, was prevented by 10^{-4} M bromoxynil. This inhibition was not overcome by simultaneous addition of gibberellic acid. Bromoxynil did not inhibit gibberellic acid induced amylase synthesis in de-embryonated halved barley seeds. Bromoxynil also inhibited the development of low levels of amylase activity found in two day old squash cotyledons, which could be overcome by benzyladenine.

D. Free Radical Formation and Halide Ion Toxicity

Other pertinent clues to the mode of action of HBNs have been provided by Wain (1964) and Ugochuckwu and Wain (1965). Ioxynil in benzene undergoes photochemical reactions in which both iodine atoms are replaced by phenyl groups.



It was suggested that a similar reaction might take place in plants and the radical reaction may contribute to herbicide activity. Heywood (1966) contests this possibility on the grounds that (1) the activity of bromoxynil is almost as high as that of ioxynil when the halogens are known to be very much less active in photochemical reactions, and (2) that other iodine compounds such as 4-hydroxy-3, 5-diiodobenzoic acid or esters are nearly devoid of herbicide activity but would be expected to react with similar ease in the UV photochemical reaction.

Wain (1964) and Zaki, Taylor and Wain (1967) noted that ioxynil and its benzoic acid derivative were degraded in tissue of bean, with liberation of iodide, suggested that the iodide ion could be a factor involved with ioxynil toxicity. Investigations by Wain et al. (1966) have shown that sensitivity to iodide is widespread among legumes and several important weed species. On the other hand, many

monocotyledons and cruciferous plants show considerable tolerance to iodide.

V. Concluding Remarks

This review of the literature has indicated several areas in the physiology and biochemistry of bromoxynil which warrant investigation. The factors governing selectivity of this compound in cereals and broad-leafed weeds are yet to be elucidated. Even with ioxynil, the relative contribution of spray retention, penetration, and internal factors to total selectivity have been estimated but not quantitated (Davies et al., 1967, 1968a, 1968b). Associated with these considerations, the whole realm of chemodynamics of bromoxynil remains unexplored.

METHODS AND MATERIALS

Winter wheat (Triticum aestivum L.) 'Nugaines', a highly tolerant species, and coast fiddleneck (Amsinckia intermedia Fisch. & Mey), a susceptible species to bromoxynil were selected for study. Caryopses of wheat and nutlets of fiddleneck were planted one to 1.5 cm deep in quartz sand contained in seven-cm-square plastic pots. The pots were watered daily until emergence of the seedlings, after which time one-half strength complete nutrient solution (Machlis and Torrey, 1956) was supplied on alternate days. Sufficient water or nutrient solution was supplied to permit the excess to pass through each pot. During the first 10 days, the pots were maintained in a greenhouse room. The temperature of this room ranged from 15 to 25 C. Light intensity varied from 1,500 to 2,500 ft-c as measured by a Weston Model 756 illuminometer.

After 10 days, the plants were thinned to one seedling per pot and transferred to controlled environment chambers. The temperature was maintained at 20 C during a 10-hour photoperiod and at 15 C during a 14-hour dark period. Fluorescent plus incandescent bulbs were used as a light source. Light intensity within the chambers ranged from 2,000 to 3,500 ft-c depending upon the age of the fluorescent bulbs. Relative humidity of the chambers ranged from 60 to 75 percent. The aforementioned growth chamber regime was used, as a standard environment, for all studies unless otherwise

specified.

A supply of technical bromoxynil octanoate (octanoic acid ester of 3, 5-dibromo-4-hydroxybenzonitrile) was obtained from Chipman Chemical Company, Portland, Oregon. Bromoxynil octanoate labelled in the cyano group with ^{14}C was obtained from May and Baker Ltd., Dagenham, Essex, England. This sample contained 4.03 mg with a specific activity of 10 mc/mM. Radiochemical purity of the bromoxynil-^{14}C sample, as determined by isotopic dilution, was 99 percent.

The labelled herbicide was dissolved in 16 percent acetone (v/v) to yield an activity of 0.021 μc per 10 μliters of solution. An aliquot of the original formulation was diluted to a five percent acetone (v/v) solution to yield an activity of 0.004 μc per 10 μliters of solution. When not in use, these formulations were stored in a refrigerator at 5 C.

Where applicable, experimental data were subjected to analysis of variance. Statistical analyses for these experiments are given in the Appendix of this thesis.

I. Dosage-Response of Wheat and Fiddleneck to Bromoxynil

An experiment was conducted to determine the relative toxicity of wheat and fiddleneck to bromoxynil. Wheat and fiddleneck, grown under the standard environment, were sprayed at the two-tiller and

12 to 14 leaf stage, respectively. Each treatment included 10 plants in a randomized block design.

Preliminary tests showed no adverse effect of spraying either species with 84 percent acetone (v/v) solution. In order to accommodate the high concentrations of bromoxynil octanoate required for a dosage-response in wheat, the herbicide was formulated in 90 percent acetone (v/v). The herbicide concentrations applied ranged from 13 to 416 g/ha for fiddleneck and 1,584 to 95,335 g/ha for wheat. A control treatment (90 percent acetone) was included for each species. Spraying was conducted with a precision greenhouse sprayer delivering 281 liters/ha at 58 cm height. A Teejet 9501-E nozzle was used.

Subsequent to treatment, the plants were maintained for three weeks under the standard environment. At the termination of the experiment, dry weight measurements were made for necrotic and live foliar tissue, and for root systems.

II. Spray Retention by Wheat and Fiddleneck

This experiment was initiated to quantitate spray retention by wheat and fiddleneck. Plant stage of growth and spray parameters were the same as those outlined in the Dosage-Response Experiment. Three replicates with two plants per replicate were included for each species. The spray solution consisted of 90 percent acetone (v/v) containing 0.25 percent (w/v) Brilliant Vital Red dye. After the

plants were sprayed, they were excised at the crown base and washed with distilled water. The quantity of spray solution retained per plant was measured by determining absorbance of the recovered dye solution at 498 mμ with a Hitachi Perkin-Elmer Model 139 spectrophotometer.

Leaf area of the test plants was estimated by mounting leaves and flattened crown bases on bond paper with clear tape, xeroxing the plant mounts, and cutting out the xerox impressions. A linear relationship, existing between area and weight of the bond paper, permitted the construction of a standard curve. Foliage areas in cm^2 of the leaf impressions were obtained by comparing their weight with the standard curve. The dry weight of foliar parts was also determined.

Spray retention by the two species was established on the bases of μliters spray solution retained per plant, μliters retained per g dry weight of foliage, and μliters retained per cm^2 of foliage area.

Surface tension in dynes/cm of the various formulations pertinent to these investigations was measured by a stalagmometer.

III. Penetration of Bromoxynil-^{14}C Into Foliage of Wheat and Fiddleneck

Two experiments were conducted to ascertain penetration of bromoxynil into foliage of wheat and fiddleneck. The first experiment

was designed to evaluate kinetics and specific differences of bromoxynil penetration. A second experiment was designed to quantitate herbicide penetration into adaxial and abaxial leaf surfaces of the two species.

A. Kinetics of Penetration

Wheat at the three to four tiller stage and fiddleneck at the 14 to 16 leaf rosette stage were tested for penetration of the herbicide. A volumetric micropipette was used to deliver 10 μliters of bromoxynil-^{14}C solution, containing 0.004 μc of activity to the second oldest leaf on a tiller of wheat and to the fourth to sixth oldest leaf of fiddleneck. Care was taken to avoid placement of the microdrop on the midrib of the fiddleneck leaves. Treated leaves of fiddleneck were marked with white tags. To prevent runoff of the microdrops, treated leaves of wheat were held in a horizontal position by inserting them through notched wooden stakes.

After durations of 6, 24, 48 and 96 hours, the treated leaves were harvested. Each treatment included five replications in a randomized block design. A four-cm-segment of each treated leaf was immersed and shaken in four ml of 25 percent ethanol in toluene (v/v), contained in a scintillation vial, for 30 seconds to remove the unabsorbed herbicide. The leaf segment was flushed with one ml of the washing solution after being removed from the vial. Ten ml of scintillation solution containing 0.5 percent (w/v) PPO (2,5-

diphenyloxazole) and 0.005 percent (w/v) POPOP [1,4-bis-2-(5-phenyloxazolyl)-benzene] in toluene was added to each vial. Activities of the leaf washings were determined with a Packard Tricarb Model 314 EX liquid scintillation counter. Counting efficiency was determined by the internal spike method (Wang and Willis, 1965) using toluene-^{14}C. All samples were corrected for background radiation. Percent penetration was calculated by subtracting the activity of the washings from that applied to each leaf. The accuracy of this method of determining penetration of bromoxynil-^{14}C was checked by two tests: (1) To determine loss via volatilization, filter paper discs were spotted with bromoxynil-^{14}C, placed in open dishes, and exposed to the growth chamber environment for eight days. No loss of activity from the paper discs was observed during this period. (2) The effect of duration of washing was evaluated. Microdrops of bromoxynil-^{14}C solution were placed on wheat and fiddleneck leaves as previously described. Twenty-four hours after treatment, leaf sections of the two species were washed for 10, 20, 30 and 60 second durations with the washing solution. No significant differences in recovered activity were found in either species. This indicated that the wash was effective in removing unabsorbed herbicide without leaching the compound from the leaf section.

B. Penetration Into Adaxial and Abaxial Leaf Surfaces of Wheat and Fiddleneck

The procedures used in this experiment were the same as those outlined in Experiment III-A, with the exception that microdrop applications were made on adaxial and abaxial surfaces of wheat and fiddleneck leaves. Treated leaves were harvested after a duration of 48 hours. A randomized block design was used. Each treatment was replicated four times. Stomatal numbers on adaxial and abaxial leaf surfaces of wheat and fiddleneck were obtained via microscopic observation. Ten replicated counts were made for each leaf surface of the two species.

IV. Translocation of Bromoxynil-^{14}C in Wheat and Fiddleneck

Two experiments were initiated to trace the movement of the label from bromoxynil-^{14}C in wheat and fiddleneck. In the first experiment, autoradiograms were prepared to determine gross distribution of the label, if any, in the two species. A second experiment was conducted to quantitate the extractable and non-extractable forms of activity in treated leaves, foliage and roots of wheat and fiddleneck.

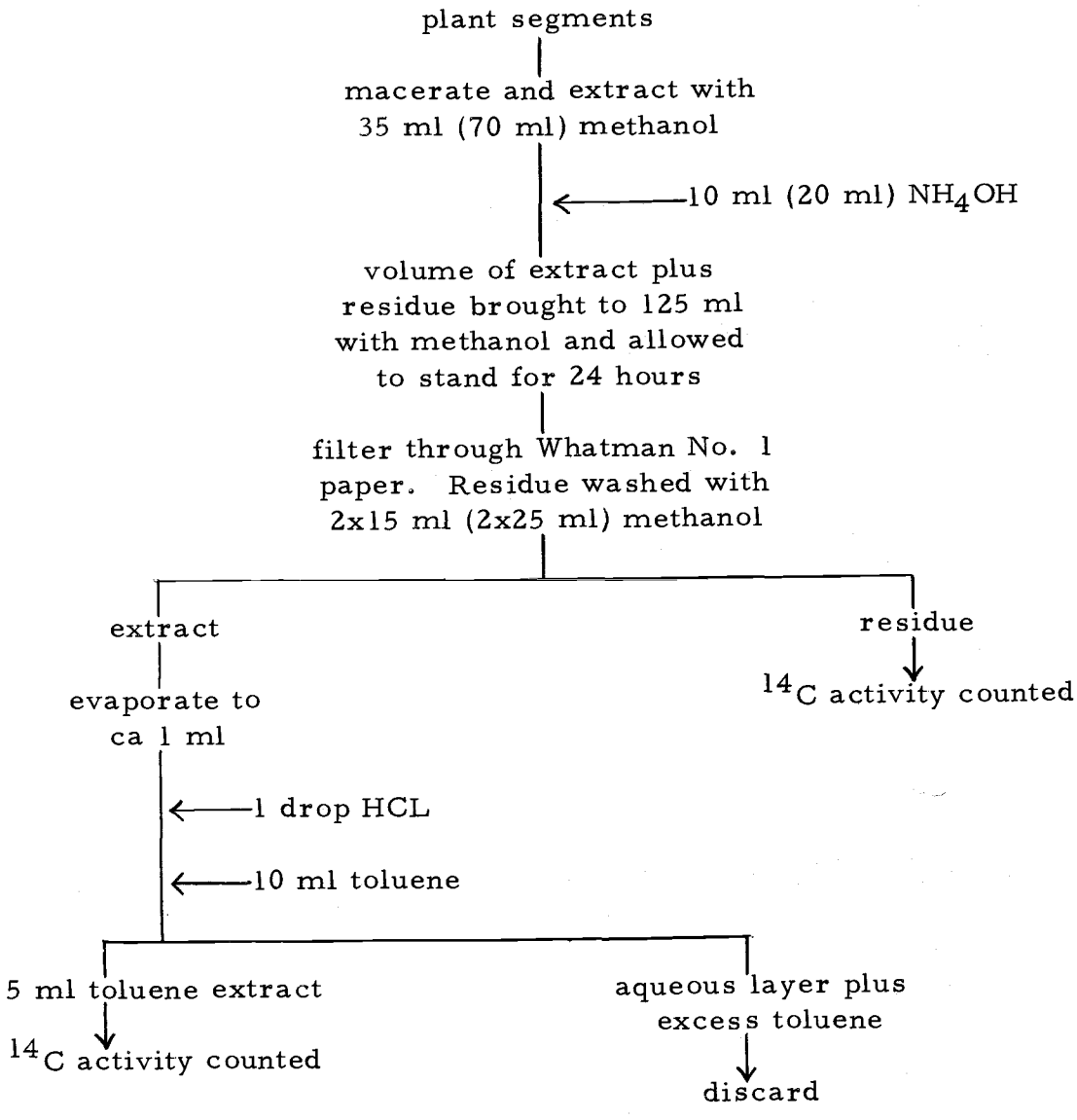
A. Autoradiographic Analysis of Translocation

Bromoxynil-^{14}C was applied as a single microdrop of 20

μliters, containing 0.042 μc activity, to leaves of wheat and fiddleneck. A check treatment (no bromoxynil-^{14}C applied) was included for each species. Wheat plants were in the four to five tiller stage and fiddleneck in the 18 to 20 leaf rosette stage at the time of treatment. After periods of one, two and four days, the plants were sectioned into treated leaves, remainder of the foliage, and roots. These sections were inserted between absorbant paper, placed in a plant press, and dried for 24 hours at 85 C. The plant sections were then mounted on cardboard and placed in contact with Kodak No-Screen X-ray film for a period of five weeks. The X-ray film was then developed according to manufacturer's specifications.

B. Extraction Analysis of Translocation

The extractable and nonextractable activities from treated leaves, foliage, and roots of wheat and fiddleneck were examined after two and four days of treatment with bromoxynil-^{14}C. A 20 μliter microdrop containing 0.042 μc activity was applied to leaves of the two species. Each treatment was replicated three times in a randomized block design. Two plants were included in each replicate. After each treatment duration, penetration of the herbicide into treated leaves was determined by the method outlined in Experiment III-A. Treated leaves, foliage, and roots were extracted by a method modified from that of Collins and Crouch (1965).



In the preceding diagram, solvent and base volumes listed in parentheses were used to accommodate the foliage segments. Activities in toluene extracts from treated leaves and roots were counted by the liquid scintillation method outlined in Experiment III-A. All samples were corrected for efficiency and background. Due to excessive color quenching from interfering materials, the foliage-

toluene extracts were plated out on planchets and counted with a Packard Model 150 Geiger-Muller instrument. Activity in these samples was corrected for background and efficiency. Radioactivity remaining in the residue after extraction was counted in the following manner. Residues were scraped from the filter paper and ground to a powder with a mortar and pestle. Twenty mg of treated leaf residue and 50 mg of foliage and root tissue residue were placed in scintillation vials. Twenty ml of the following gel scintillation formulation was placed in each vial (Ott et al., 1959).

PPO - 5 g

POPOP - 0.1 g

Cab-O-Sil - 40 g

Toluene - 1000 ml

This formulation, upon shaking, permitted the suspension of the residue particles in uniform dispersion. Counting was then done by conventional liquid scintillation methods. These samples were corrected for background and efficiency.

Penetration of bromoxynil-^{14}C was expressed as percent of the applied activity. The activity extracted from the plant segments was expressed as dpm/plant segment and dpm/g fresh weight. The non-extractable activity was expressed as cpm/plant segment and as cpm/g fresh weight.

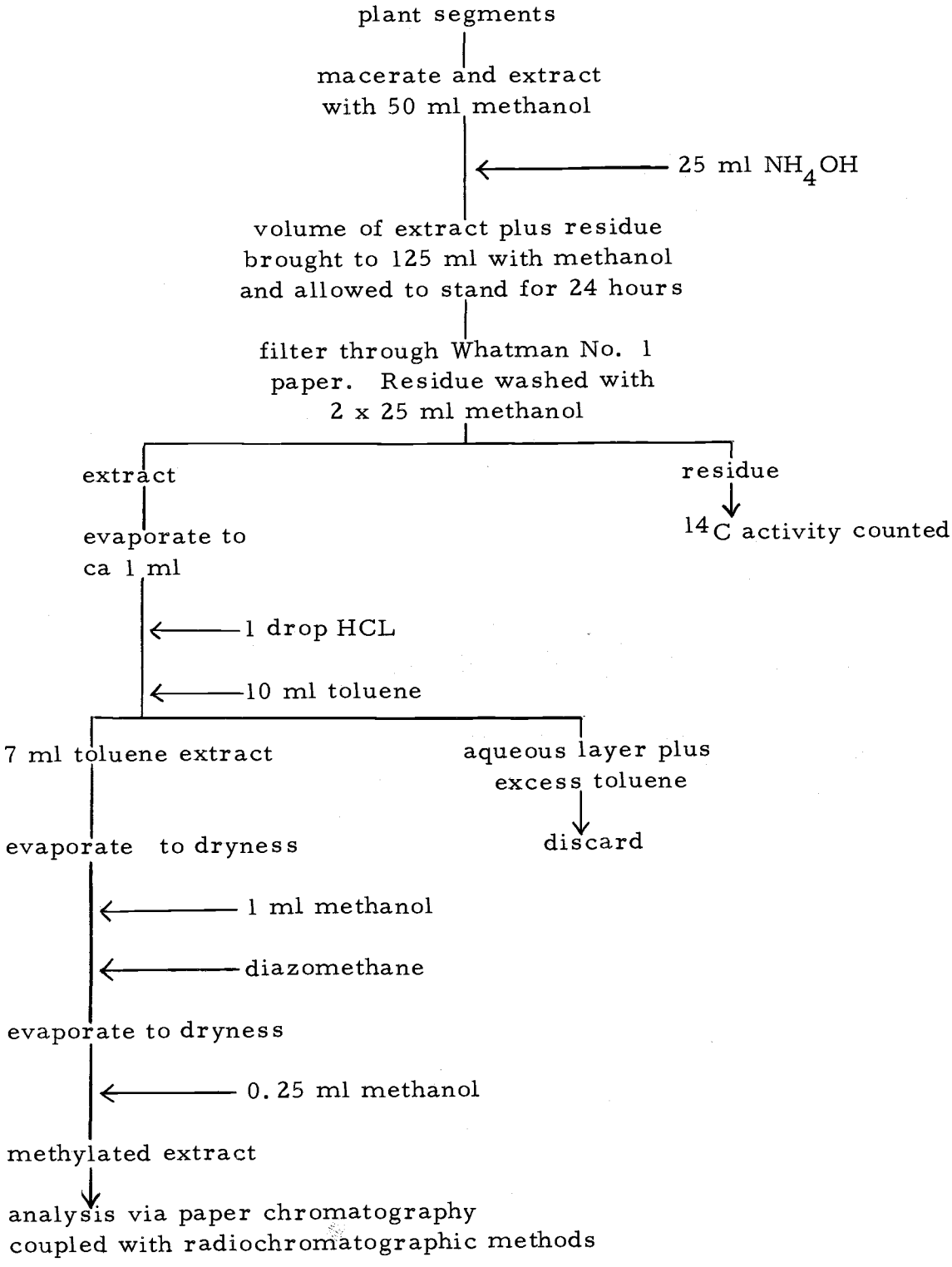
V. Metabolism of Bromoxynil-^{14}C by Wheat and Fiddleneck

The metabolic fate of bromoxynil-^{14}C in wheat and fiddleneck was examined via two investigations. The first experiment was designed to elucidate the identity of extractable activity from leaves and roots of the two species. The possibility of metabolism of bromoxynil-^{14}C to $^{14}CO_2$ in wheat and fiddleneck was examined in a subsequent experiment.

A. Analysis of Breakdown Products

Four 20 μliter microdrops containing a total of 0.168 μc were applied to each plant of wheat and fiddleneck. Treated leaves and root systems were harvested after four and eight days of treatment. Each treatment was replicated three times in a randomized block design. Unabsorbed herbicide present on the leaves was removed by the method given in Experiment III-A, prior to extraction. Treated leaves and roots were extracted by the following method (refer to page 34).

Radioactivity remaining in the residue after extraction was counted by the method outlined in Experiment IV-B.


plant segments
macerate and extract with 50 ml methanol
25 ml NH_4OH
volume of extract plus residue brought to 125 ml with methanol and allowed to stand for 24 hours
filter through Whatman No. 1 paper. Residue washed with 2 x 25 ml methanol
extract
residue
^{14}C activity counted
evaporate to ca 1 ml
1 drop HCL
10 ml toluene
7 ml toluene extract
aqueous layer plus excess toluene
discard
evaporate to dryness
1 ml methanol
diazomethane
evaporate to dryness
0.25 ml methanol
methylated extract
analysis via paper chromatography coupled with radiochromatographic methods

The identity of the radioactive substances present in the methanol extract was established by using paper chromatography coupled with radiochromatographic analysis. Twenty-five μ liters of extract were spotted on sheets of Whatman No. 1 chromatography paper. The parent compound, after being subjected to the extraction procedure, was spotted on the chromatographic paper to determine the Rf of bromoxynil. These chromatograms were developed by descending chromatography. The presence of derivatives was examined by employing two solvent systems. The first consisted of isobutanol:ethanol:ammonia:water = 100:25:7:28 and required a development time of 18 hours. The second, consisting of benzene: acetic acid:water = 2:2:1, was developed for five hours. The presence of activity on five-cm-wide chromatogram strips was detected by using a Packard Model 7201 radiochromatogram scanner system. The proportion of extracted activity present as bromoxynil and other derivatives was calculated by weighing the excised curves. The proportions were then expressed as a percentage of the extracted activity.

B. Metabolism of Bromoxynil-^{14}C to $^{14}CO_2$

Wheat plants at the three to four tiller stage and fiddleneck at the 14 to 16 leaf stage were placed in 250 ml flasks containing one-half strength complete nutrient solution. Each plant was treated with 0.168 μc of bromoxynil-^{14}C as previously described. Twelve hours

after treatment, four plants were placed in 19.5 x 19.5 x 38.0 cm (height) plexiglass chambers. The chambers were rendered air-tight by sealing the base with modeling clay. To safeguard against air leakage, tightened canvas straps were placed around the chambers. A small compressor unit was utilized to pass 180 ml/min of air through an input flask containing 250 ml of 2.0 N NaOH, and the plexiglass chambers. The outgoing air was passed through two flasks of 250 ml 2.0 N NaOH to trap respired CO_2. A trapping period of 11 hours and 50 minutes during a 12 hour photoperiod and 12 hour nycto-period, was conducted over a 10-day period. Ten minutes of each trapping period were utilized to change trapping solutions. The apparatus was disassembeled after five days, to permit replacement of the nutrient solution contained within the plant flasks. A temperature of 20 C was maintained throughout the experiment.

After collection, the CO_2-trapping solutions were heated to 80 C and 50 ml of 1.0 N $BaCL_2$ added. The insoluble $BaCO_3$ was collected by filtration through pre-weighed Eaton-Dikeman No. 613 filter paper. The filter paper plus $BaCO_3$ was dried and weighed. The $BaCO_3$ was scraped from the filter paper, ground to a fine powder, weighed, and placed in scintillation vials. Twenty ml of the Cab-O-Sil scintillation formulation described in Experiment IV-B was placed in each vial and shaken. The counting of the $BaCO_3$ samples was conducted by conventional liquid scintillation methods. All samples were corrected for background and efficiency.

A THEORETICAL TREATMENT OF BROMOXYNIL SELECTIVITY IN WHEAT AND FIDDLENECK

The experimental procedures given in the Methods and Materials section provided estimates of plant-herbicide interactions which could be correlated with the phenomenon of selectivity. The rationale underlying the implementation of these tests was embodied in a mathematical generalization concerned with the causation of selectivity. Hence, the mathematical model proposed in this section can be defined as an a priori generalization directed towards elucidation of bromoxynil selectivity on rationalistic and mechanistic terms. The results of subsequent tests on bromoxynil provided data to test the adequacy of this model.

The correlation of differential plant-herbicide interactions with the selectivity phenomenon is not a new concept by any means. Holly (1964), for example, has reviewed the numerous factors that may account for differential phytotoxicity. For foliar applied compounds, these included differences in spray retention by the shoot and penetration into the shoot; differences in translocation, detoxication, or metabolism to active or inactive products; or by differential effects on metabolic systems at the site of action. However, a shortcoming of most statements concerned with differential phytotoxicity is that no attempt is made to place the contribution of differential plant-herbicide interactions into a meaningful mathematical relationship.

Recently, however, a promising mechanistic approach was offered by Norris (1967). Norris used simultaneous equations to analyze a hypothetical case in which a single woody plant species responded differentially to two herbicides. This analysis was essentially a multiplicative scheme in which the efficiency of control of a given species was controlled by spray retention x penetration x translocation x chemical stability x toxicity of root systems.

The following model is presented to partition the components of selectivity in foliar applied herbicides - specifically bromoxynil in the case of wheat and fiddleneck. This analysis consists of a selectivity equation and a partitioning equation. Since this model employs ratios, the terms are dimensionless. The selectivity equation is given by

$$R_s = R_r \cdot R_p \cdot R_i \qquad [1]$$

where

$$R_s = \frac{(ED_{50})w}{(ED_{50})_f} \qquad [2]$$

represents a ratio of the bromoxynil dosage required to depress the growth of wheat (w) and fiddleneck (f) by 50 percent.

$$R_r = \frac{r_f}{r_w} \qquad [3]$$

indicates the ratio of spray retention (r) by wheat and fiddleneck.

$$R_p = \frac{p_f}{p_w} \qquad [4]$$

represents the ratio of penetration (p) of bromoxynil into foliage of wheat and fiddleneck.

$$R_i = \frac{i_f}{i_w} \qquad [5]$$

Relationship [5] represents the ratio of internal physiological and biochemical factors (i) contributing to differential phytotoxicity. This term may include such differentials as translocation, detoxication, metabolism and selectivity at the site of action. In experimental situations, difficulty in meaningfully quantitating these factors can be resolved by bulking them into one term. When terms [2], [3], and [4] are quantitated, term [5] can be deduced mathematically.

The rationale underlying equation [1] assumes that no more herbicide is available for penetration than has been deposited upon the foliage. In addition, no more herbicide is available for toxic action than has penetrated the foliage. Furthermore, the degree of toxicity expressed by a species is related to the internal mechanisms which may inhibit movement, bind or change the identity of the toxicant. A secondary assumption underlying the use of equation [1] is that ratios > 1.0 indicates selectivity advantage in favor of the more tolerant species, e.g., wheat.

Where all ratios in equation [1] > 1.0 the relative contribution of each term to total selectivity can be partitioned by using the following expressions.

$$\Sigma R = R_r + R_p + R_i \qquad [6]$$

Then the percent contribution of spray retention (r) to total selectivity (S) is given by

$$S_r = \frac{R_r}{\Sigma R} \cdot 100 \qquad [7]$$

penetration (p) by

$$S_p = \frac{R_p}{\Sigma R} \cdot 100 \qquad [8]$$

and internal factors (i) by

$$S_i = \frac{R_i}{\Sigma R} \cdot 100 \qquad [9]$$

If one or more ratios < 1.0 the following selectivity partitioning equation would be appropriate. For example, if $R_c < 1.0$, then

$$\Sigma R = R_a + R_b + \frac{1}{R_c} \qquad [10]$$

In equation [10], R_a, R_b, and R_c refer to hypothetical selectivity factor ratios. Then:

$$S_c = \frac{\frac{1}{R_c}}{\Sigma R} \cdot 100 = \text{negative percent contribution of c to total selectivity} \qquad [11]$$

R_a and R_b's positive percent contribution to total selectivity would be determined according to equations [7] through [9].

The relative nature of the foregoing theoretical treatment must be emphasized. Estimates derived from this analysis are pertinent only to highly specified chemical, physical and biotic environments. Furthermore, where the dosage-response curves of the two species

assume different functions, this analysis pertains only to specified effective dose levels.

Selectivity calculations, based upon this analysis, will be presented for bromoxynil action on wheat and fiddleneck in the Results section of this thesis.

RESULTS

The phenomenon of selectivity and chemodynamics of bromoxynil was approached by considering the pertinent interactions that occur between the herbicide and the target organisms. A comparison of the magnitude of differential phytotoxicity with the relevant contributing factors was the central theme of these investigations. The results of these efforts will be presented forthwith.

I. Dosage-Response of Wheat and Fiddleneck to Bromoxynil

The herbicide dosages supplied in this experiment, with the exception of higher rates applied to fiddleneck, were in the sub-lethal range. In both species, the distal leaf regions were the first areas to express toxic symptoms. Signs of toxicity in wheat were evident within three to five days after treatment. Toxicity was evident in fiddleneck within two to three days after treatment. With fiddleneck, the oldest leaves of the rosette were the first to succumb. In all cases of mortality among fiddleneck, the growing point was the last area affected.

The dosage-response curves for wheat and fiddleneck, expressed as a percent of the control treatment on a dry weight basis, are illustrated on Figure 1. As would be predicted, foliage, root and whole plant dry weight decreased with increasing dosage of bromoxynil. In both species, root growth was suppressed to a greater degree than was foliage growth. The integration of these organ weights, expressed on

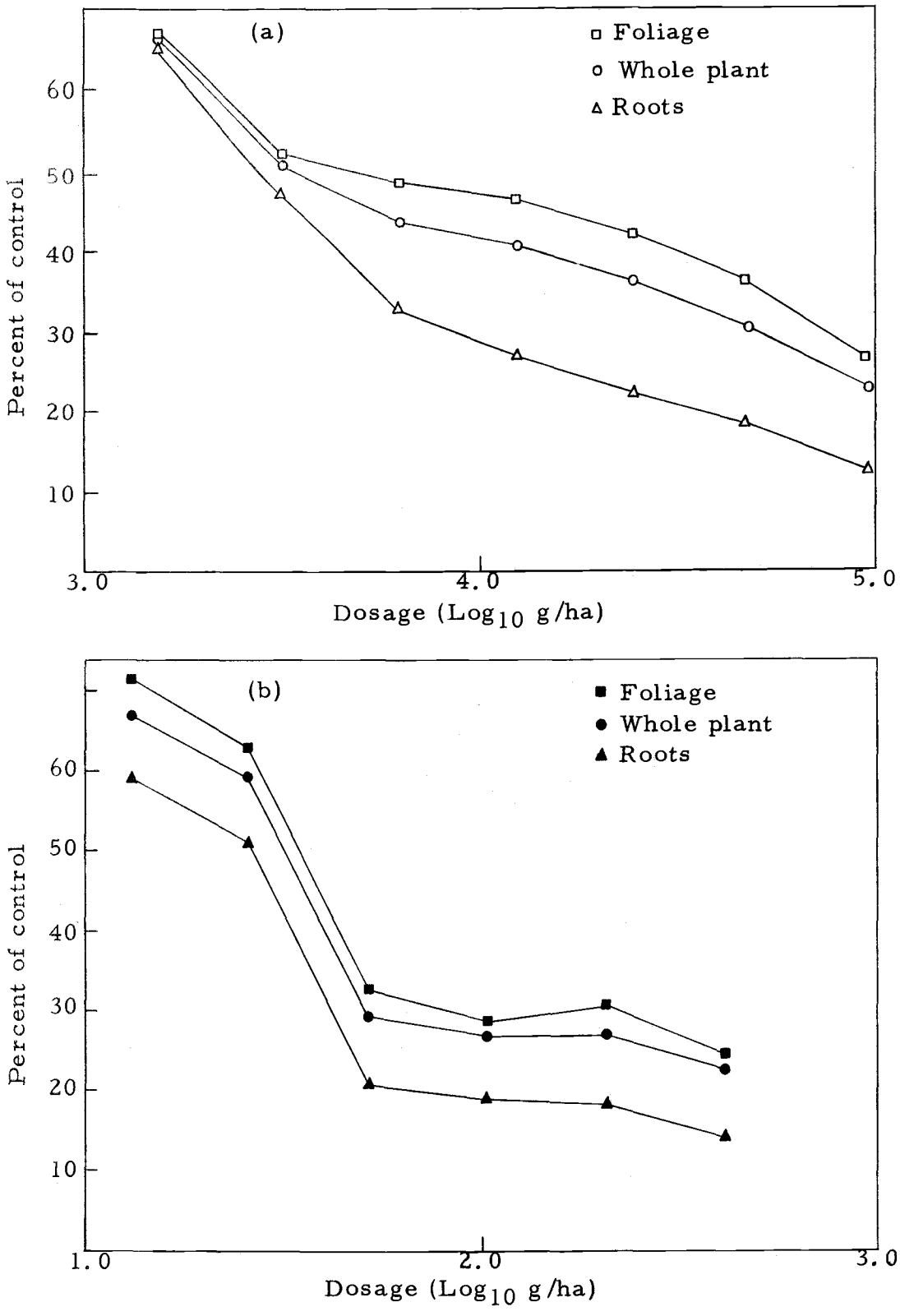


Figure 1. Dosage-response of wheat (a) and fiddleneck (b) treated with bromoxynil.

a whole plant basis, produced a curve of intermediate position. The dosage of bromoxynil required to reduce dry weight of the whole plant by 50 percent (ED_{50}) was ca 3,500 g/ha and ca 32 g/ha for wheat and fiddleneck, respectively. Thus, the selectivity ratio between these two species was ca 109.

The differential response of shoots and roots to increasing dosages of bromoxynil is shown on Figure 2. The shoot/root ratio of wheat increased from 2.5 for the control up to 5.4 for the highest rate tested. Among fiddleneck, this ratio ranged from 2.9 for the control up to 5.3 for the highest rate used.

The foliage dead tissue weights of the two species are illustrated on Figure 3. Significant increases in dead tissue weights were noted among wheat plants in response to increasing dosage. An extensive amount of dead tissue among the fiddleneck controls illustrated a progressive senescence of older leaves of the rosette. The dead tissue weights of fiddleneck treated with increasing bromoxynil dosages were not significantly different (see Appendix Table 2).

II. Spray Retention by Wheat and Fiddleneck

The retention of spray by wheat and fiddleneck foliage was calculated on the bases of whole plants, per g dry weight and per cm^2 of exposed foliage (Table 1). On a whole plant basis, wheat retained 95 µliters as compared to 205 µliters for fiddleneck. A retention ratio

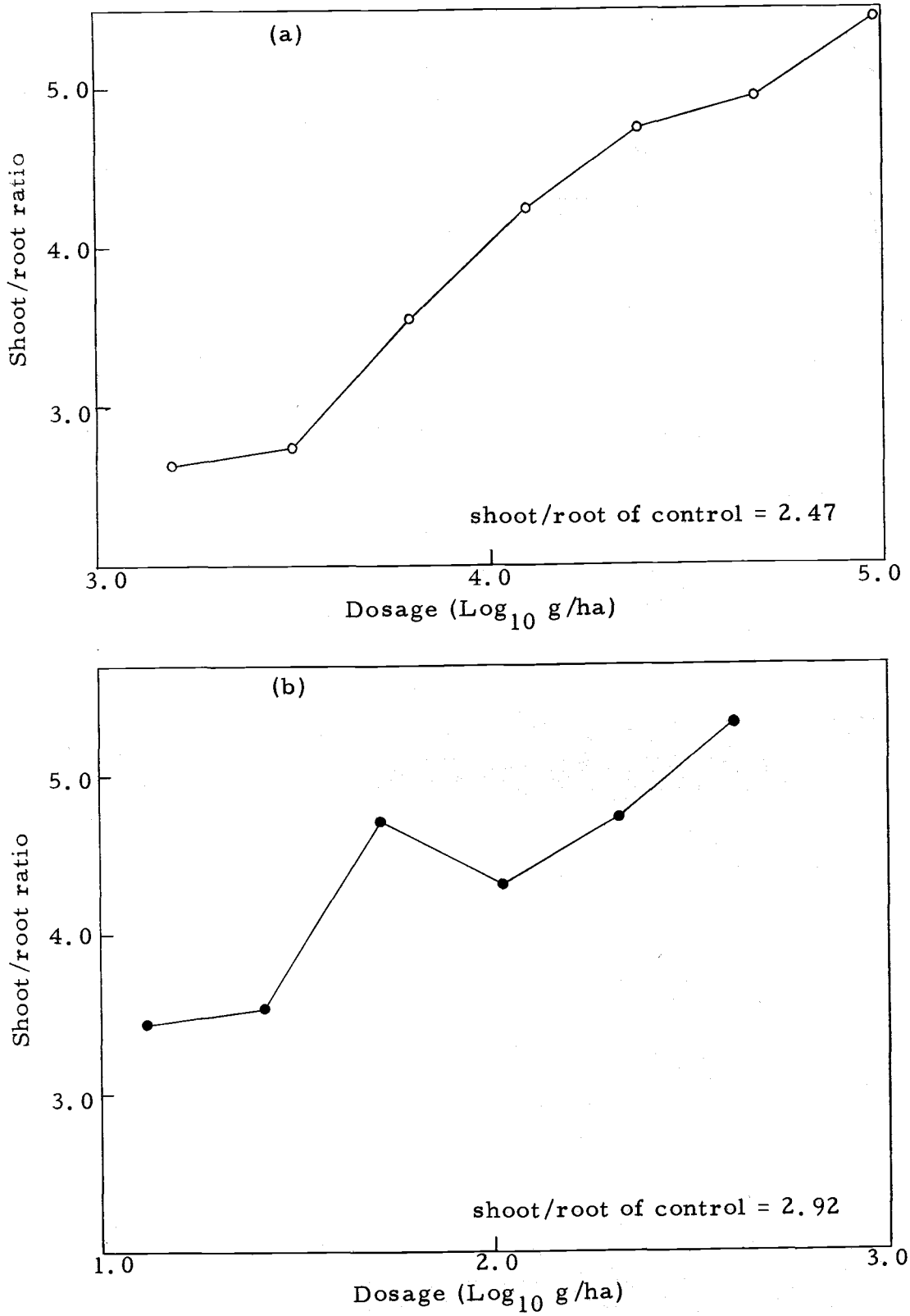


Figure 2. Shoot/root ratios (dry weight basis) of wheat (a) and fiddleneck (b) treated with bromoxynil.

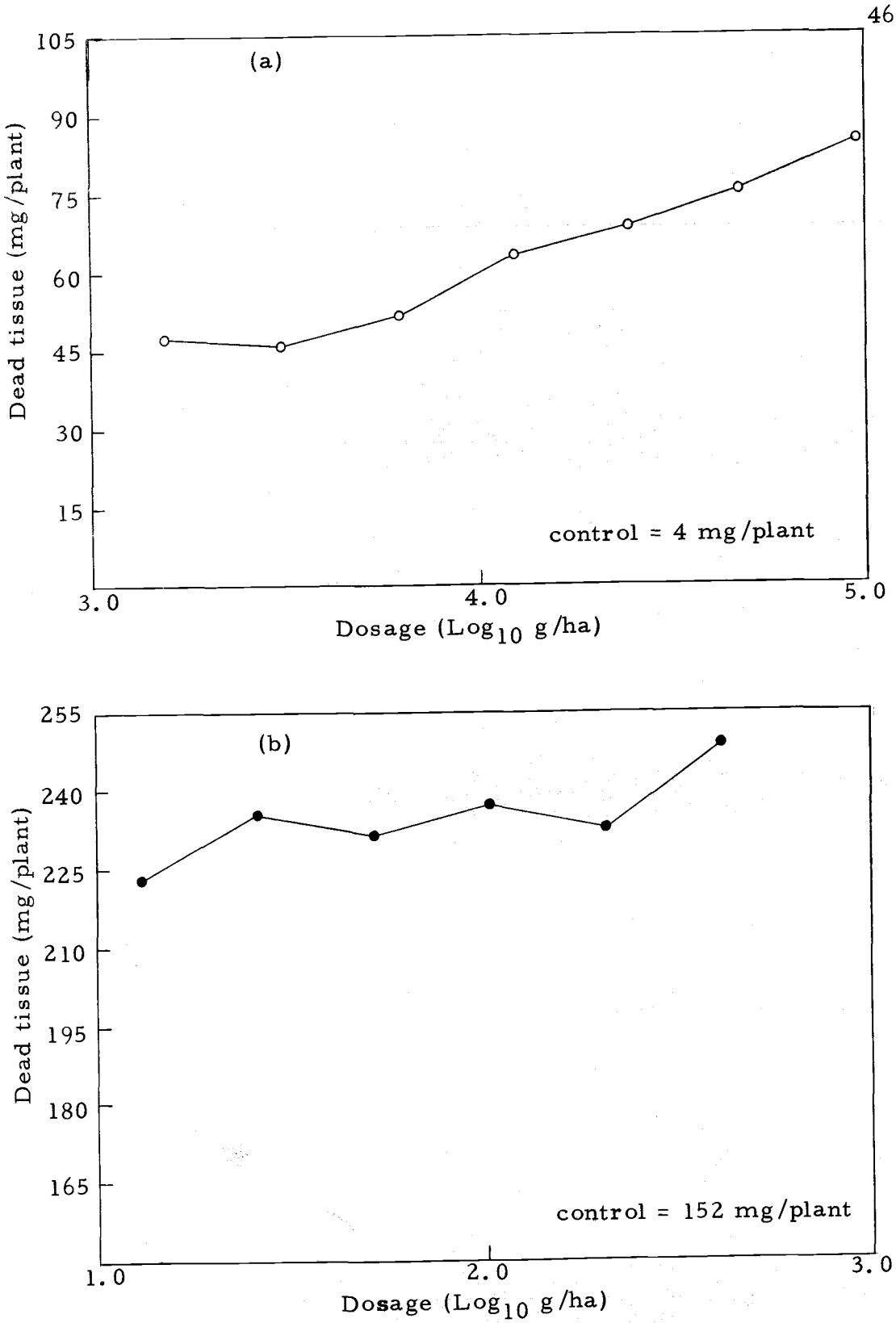


Figure 3. Foliage dead tissue of wheat (a) and fiddleneck (b) treated with bromoxynil.

Table 1. Retention of spray solution by wheat and fiddleneck.

Measurement	Wheat	Fiddleneck	Spray retention ratio (fiddleneck/wheat)
µliters spray solution retained/plant	95	205	2.16
Foliage dry weight mg/plant	267	294	
µliters spray solution retained/g dry weight	356	697	1.96
Exposed foliage area cm^2/plant	72	66	
µliters spray solution retained/cm^2 exposed foliage area	1.33	3.11	2.34

(fiddleneck/wheat) based on this measurement was 2.16. To adjust for any differences in plant mass, spray retention was calculated on a dry weight basis. In this case, wheat retained 356 µliters spray solution per g of dry weight as compared to 697 for fiddleneck. The ratio of retention in this measurement was 1.96. Wheat retained 1.33 µliters of spray per cm^2 of exposed foliage as compared to 3.11 for fiddleneck. The retention ratio in this case was 2.34. In all measurements of spray retention, wheat retained significantly less spray solution than did fiddleneck. However, wheat and fiddleneck were not appreciably different in foliage dry weight and foliage area (see Appendix Table 3 for statistical analyses).

The surface tensions of the various formulations pertinent to these experiments are given in Table 2. A decrease in surface tension

Table 2. Surface tension measurements on formulations pertinent to investigations on bromoxynil.

Formulation	Surface tension (dynes/cm)
distilled water	72
5 percent acetone (v/v)	59
16 percent acetone (v/v)	49
25 percent acetone (v/v)	44
50 percent acetone (v/v)	37
75 percent acetone (v/v)	34
90 percent acetone (v/v)	32
acetone	25
1.1 percent bromoxynil octanoate in 90 percent acetone (w/v)	32
4.5 percent bromoxynil octanoate in 90 percent acetone (w/v)	32
16.8 percent bromoxynil octanoate in 90 percent acetone (w/v)	31
0.25 percent Brilliant Vital Red dye in 90 percent acetone (w/v)	32

was noted as the proportion of acetone in water was increased. The surface tensions of 5 and 16 percent acetone were 59 and 49 dynes/cm, respectively. These formulations represented the acetone mixtures employed in the bromoxynil-^{14}C investigations. These formulations are useful in microdrop applications since the microdrop retains its integrity, without the occurrence of complete wetting. In addition, the surface tensions of 5 and 16 percent acetone are sufficiently low to prevent runoff from wheat foliage. The addition of bromoxynil octanoate and Brilliant Vital Red dye to 90 percent acetone formulations did not alter surface tension.

III. Penetration of Bromoxynil-^{14}C Into Foliage of Wheat and Fiddleneck

A. Kinetics of Penetration

The penetration of bromoxynil-^{14}C into leaves of wheat and fiddleneck, as a function of time, is illustrated in Figure 4. While no significant difference was found between species, a trend towards higher penetration was observed in fiddleneck during longer treatment durations. Penetration of bromoxynil-^{14}C into foliage of these species increased significantly with duration of treatment (see Appendix Table 5). Penetration increased rapidly during the early periods of the experiment, after which the rate of penetration decreased.

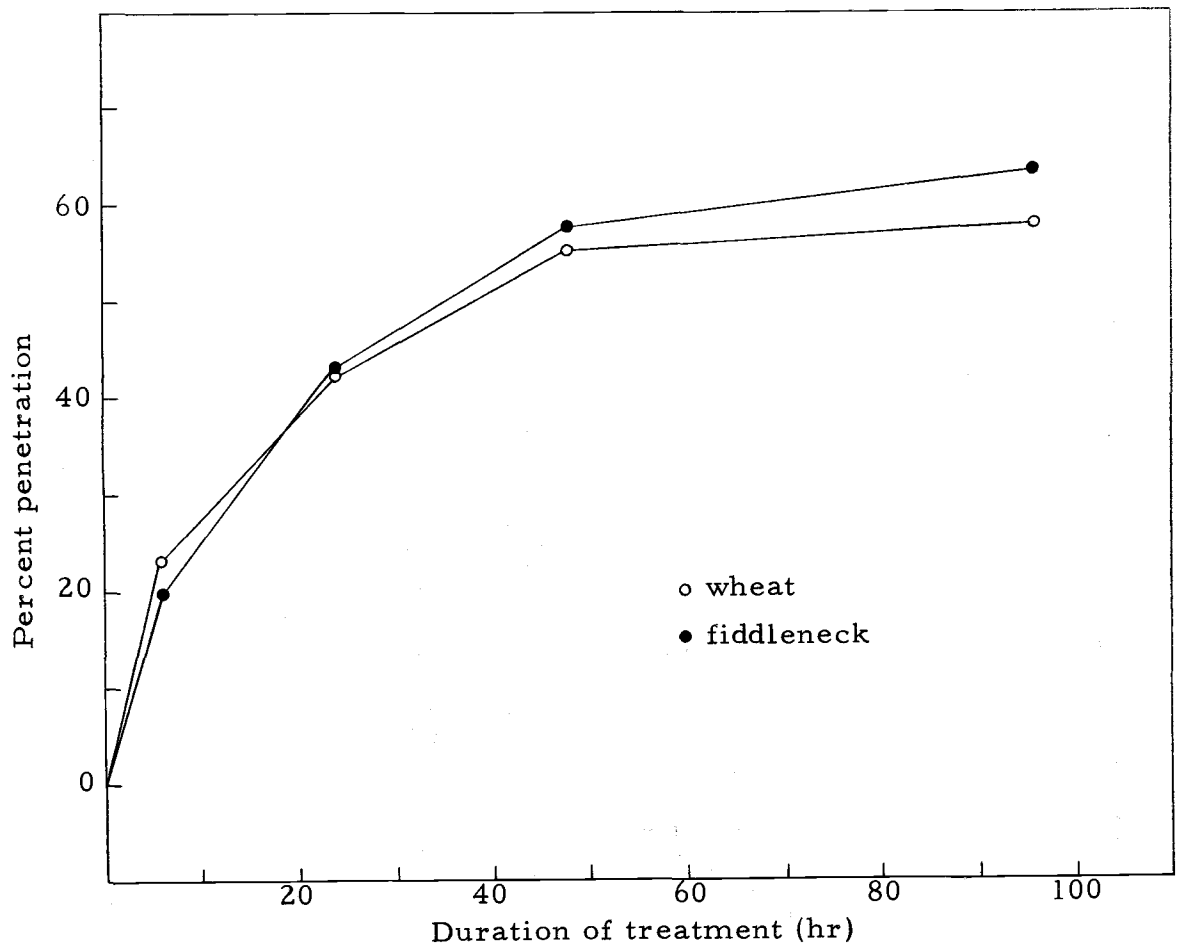


Figure 4. Penetration of bromoxynil-^{14}C into leaves of wheat and fiddleneck.

B. Penetration Into Adaxial and Abaxial Leaf Surfaces of Wheat and Fiddleneck

The penetration of bromoxynil-^{14}C into adaxial and abaxial leaf surfaces of wheat and fiddleneck after two days of treatment is given on Table 3. No statistical difference in penetration into upper and lower leaf surfaces of either species was found. In this experiment, average penetration of bromoxynil-^{14}C into leaves of wheat and fiddleneck was 53.8 and 65.3 percent, respectively. This represented a statistically significant difference. The penetration of bromoxynil-^{14}C could not be related to stomatal density in either species (see Appendix Table 6 for statistical analyses).

Table 3. Penetration of bromoxynil-^{14}C into adaxial and abaxial leaf surfaces of wheat and fiddleneck, and stomatal numbers on leaf surfaces of the two species.

Leaf surface	Wheat		Fiddleneck	
	Percent penetration	Stomata/ cm^2	Percent penetration	Stomata/ cm^2
Adaxial	53.6	3,250	66.8	7,478
Abaxial	54.0	1,979	63.8	9,519
Average	53.8	2,614	65.3	8,498

IV. Translocation of Bromoxynil-^{14}C in Wheat and Fiddleneck

A. Autoradiographic Analysis of Translocation

Autoradiograms of wheat and fiddleneck treated with

bromoxynil-^{14}C plus controls are presented on Figures 5 and 6, respectively. In both species, a majority of the label remained in the treated leaf. Within treated leaves of wheat, movement of the label was bi-directional, but was predominantly basipetal. Limited movement of the label down the leaf sheath and into other tillers was noted in wheat. Presence of the label in growing points and root systems could not be detected in wheat.

Within treated leaves of fiddleneck, movement occurred both acropetally and basipetally - particularly along the veins. In fiddleneck, the label gradually moved into other leaves and into the root system. Appreciable amounts of the label were found in leaves of intermediate age after four days of treatment.

Control treatments of both species were free of autoradiograms arising from pressure points between the plant mount and the X-ray film. The point-like images appearing on several of the autoradiograms can be attributed to splattering during the microdrop applications.

B. Extraction Analysis of Translocation

The penetration of bromoxynil-^{14}C into leaves of wheat and fiddleneck after two and four days of treatment is given in Table 4. In this experiment, penetration of the herbicide was significantly greater in fiddleneck than in wheat. The ratios of penetration

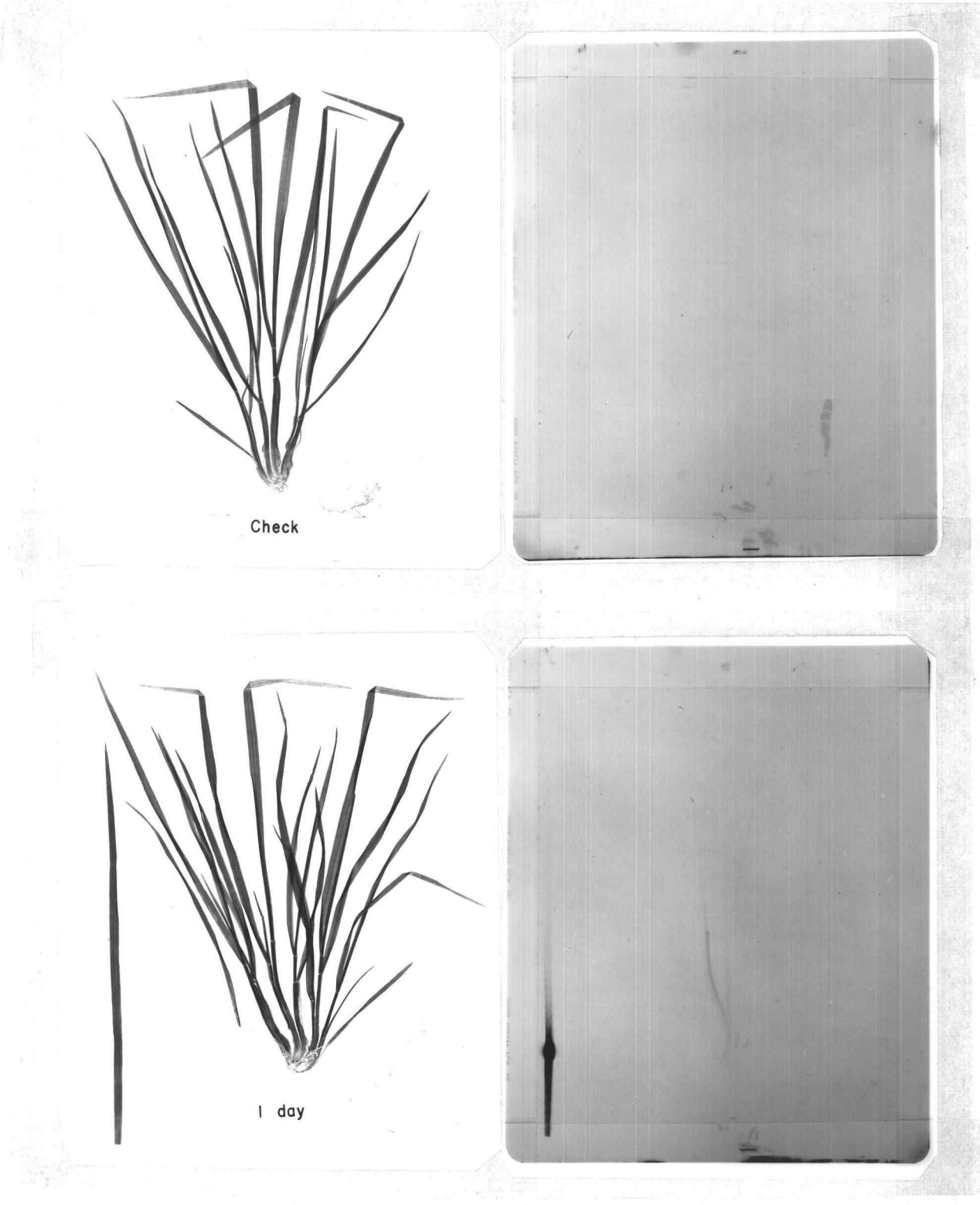
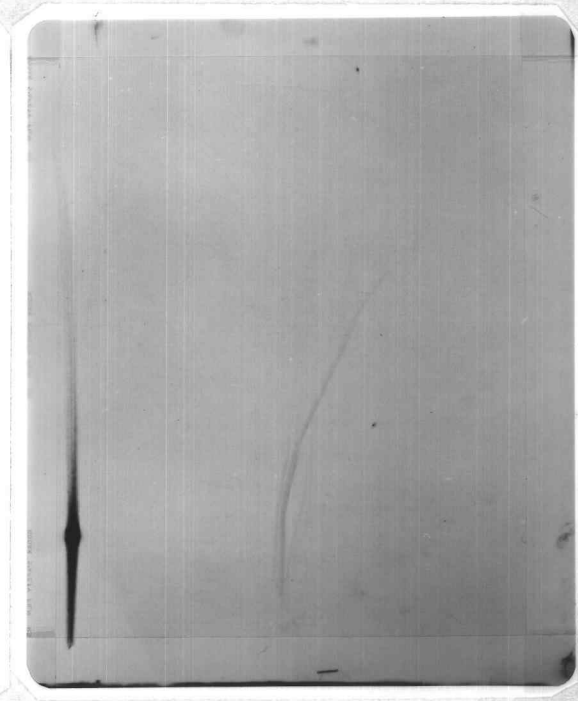


Figure 5. Plant mounts (left) and autoradiograms (right) of the wheat check and wheat treated with bromoxynil-^{14}C over one, two, and four day durations.



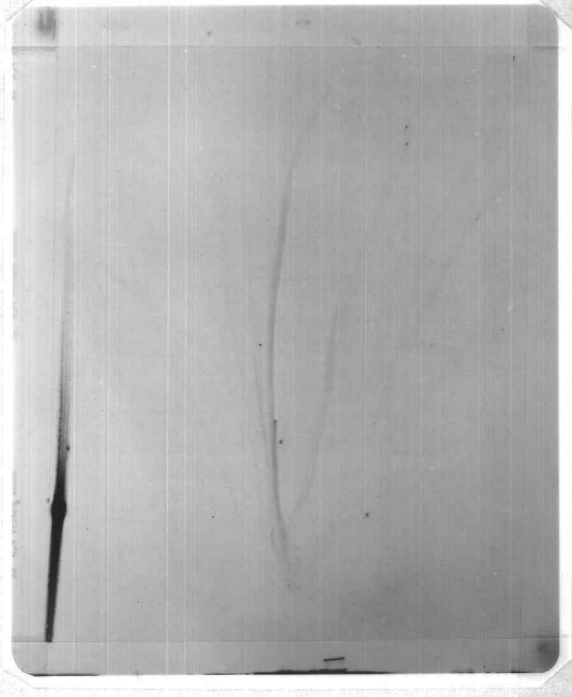


Figure 5. Continued

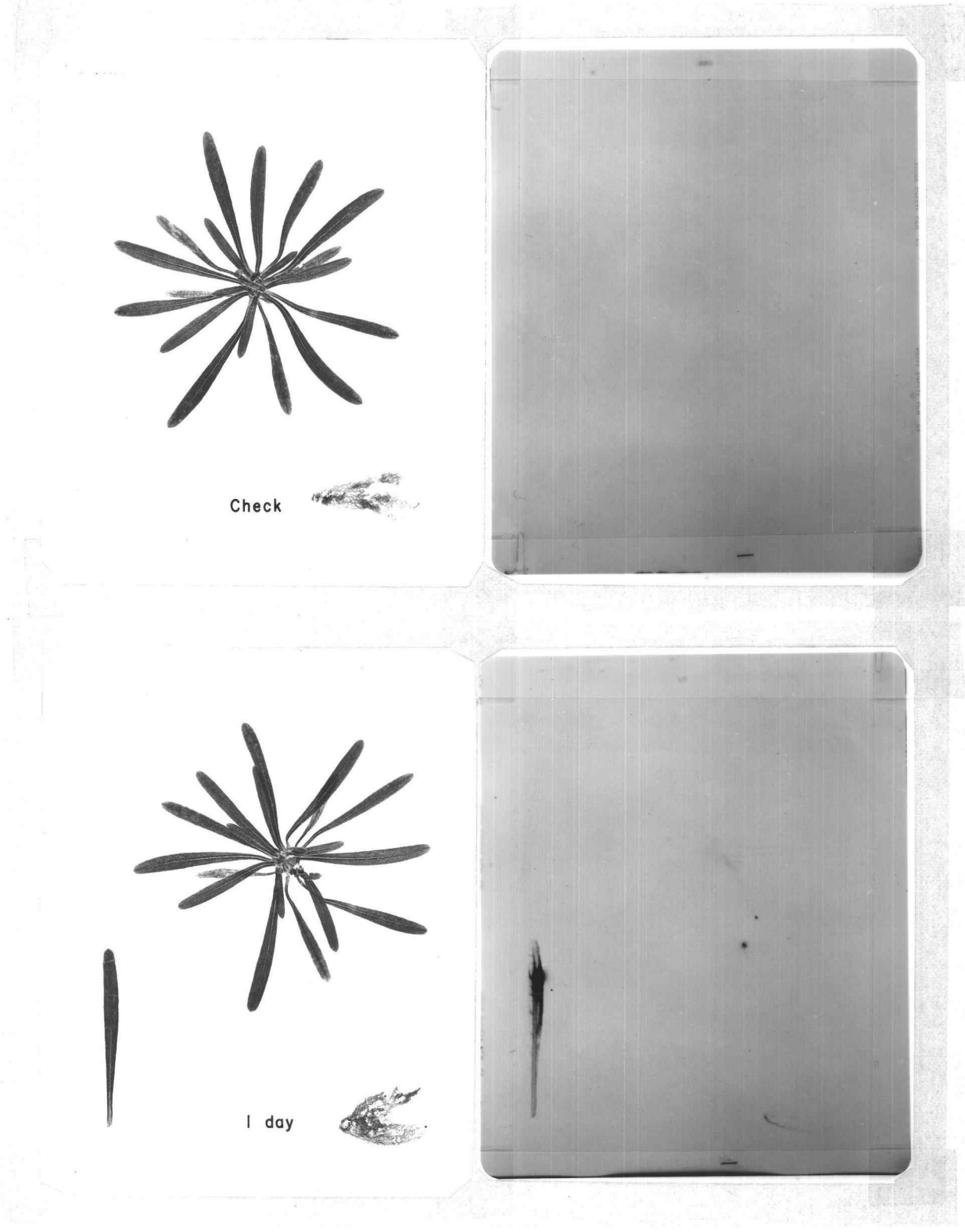


Figure 6. Plant mounts (left) and autoradiograms (right) of the fiddleneck check and fiddleneck treated with bromoxynil-^{14}C over one, two, and four day durations.

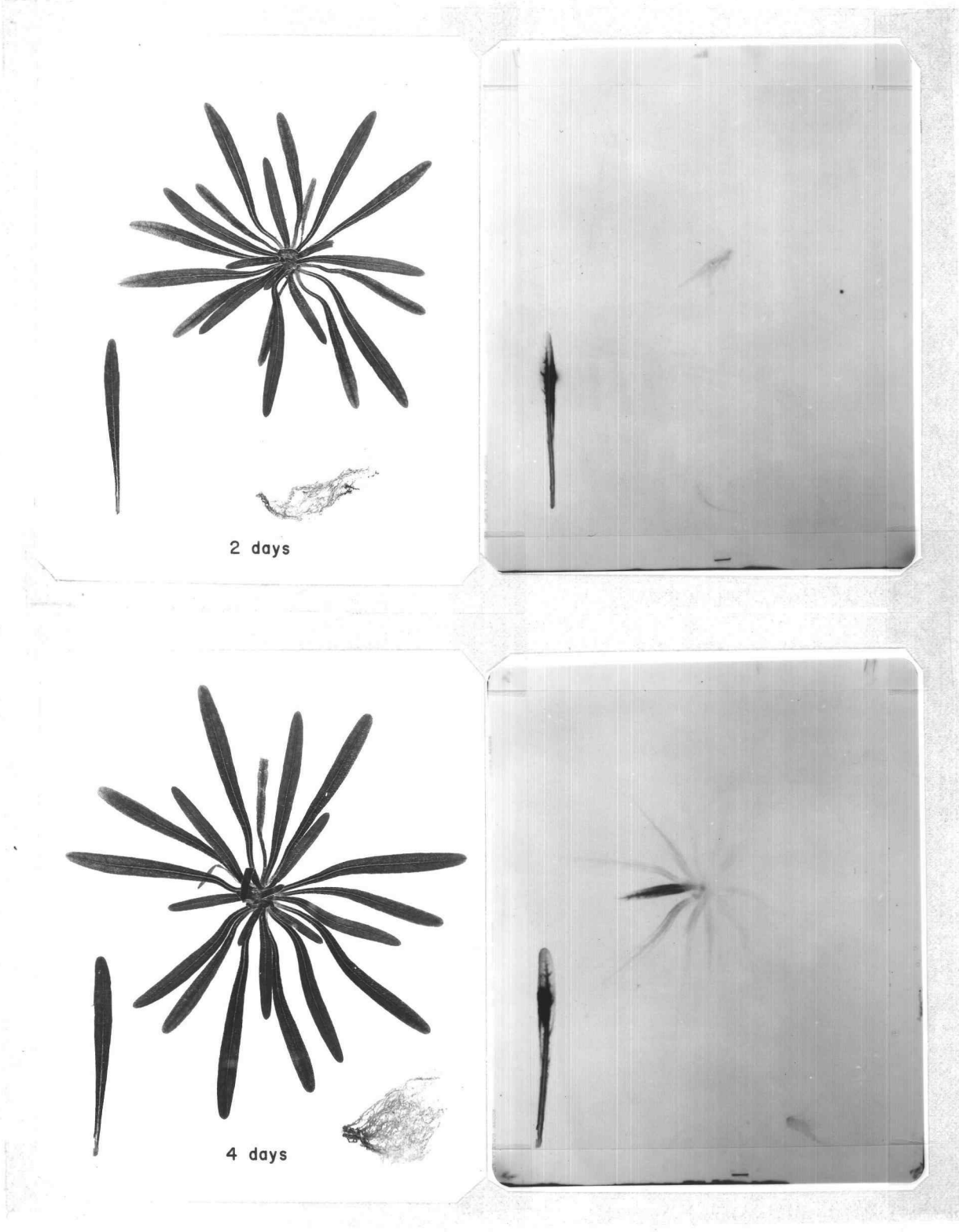


Figure 6. Continued

(fiddleneck/wheat), after two and four days, were 2.14 and 1.67, respectively. In both species, penetration increased over the duration of the experiment (see Appendix Table 7).

Table 4. Percent penetration of bromoxynil-^{14}C into leaves of wheat and fiddleneck after two and four days of treatment.

Species	Duration of treatment	
	2 days	4 days
Wheat	17.5	35.7
Fiddleneck	37.5	59.5

The extractable radioactivity, obtained from plant sections of the two species, is given on Table 5. These data were expressed on two bases. The use of dpm/plant section, an absolute measurement, facilitated the construction of a budget to determine partitioning of the radioactivity within the plant. The use of dpm/g fresh weight, a relative measurement, permitted comparisons of activity per unit of plant mass.

On a dpm/plant section basis, four to five times more activity was obtained from treated leaf extracts of fiddleneck as compared to corresponding extracts from wheat. In both species, a majority of the activity remained within the treated leaf. Extractable activity from treated leaves increased with duration of treatment. More extractable activity was found in fiddleneck foliage as compared to wheat foliage. The quantity of extractable activity from foliage increased with

Table 5. Extractable radioactivity recovered from plant sections of wheat and fiddleneck after two and four days of treatment with bromoxynil-^{14}C.

Measurement	Species	Duration of treatment	
		2 days	4 days
		dpm/plant section	
Treated leaf	Wheat	4,282	4,762
	Fiddleneck	16,608	24,761
Foliage	Wheat	2,067	3,703
	Fiddleneck	3,284	22,176
Root	Wheat	21	127
	Fiddleneck	390	838
		dpm/g fresh weight	
Treated leaf	Wheat	22,131	22,304
	Fiddleneck	56,073	73,851
Foliage	Wheat	451	616
	Fiddleneck	903	4,697
Root	Wheat	5	26
	Fiddleneck	180	254

duration of treatment. The sharp increase in activity found in fiddleneck foliage after four days of treatment was in agreement with the autoradiographic analysis. The amount of extractable activity from roots of fiddleneck greatly exceeded that of wheat. Extractable root activities in both species increased with duration of treatment.

With one notable exception, the foregoing relationships were found in the translocation analysis based on dpm/g fresh weight of tissue. In this analysis, extractable activity did not increase significantly with duration of treatment. This was partly due to growth of the plants during the experiment, and partly due to variation incurred during conversion from the absolute to a relative measurement (see Appendix Table 8 for statistical analyses).

Nonextractable activity present in plant section residues is presented on Table 6. As with the preceding analysis, measurements on an absolute and relative basis are included. Although determination of absolute counting efficiency is impossible with heterogeneous counting systems due to intraparticulate absorption, the internal spike method was used to correct for efficiency loss in the nonparticulate phase of the scintillation formulation as well as via interparticulate absorption. Thus, these data are represented at the 70 percent counting efficiency level. In both measurements, a majority of nonextractable activity was located in treated leaves. In treated leaf residues, wheat possessed four to five times more

Table 6. Nonextractable activity present in plant section residues of wheat and fiddleneck after two and four days of treatment with bromoxynil-^{14}C.

Measurement	Species	Duration of treatment	
		2 days	4 days
		cpm/plant section	
Treated leaf	Wheat	4,091	4,673
	Fiddleneck	728	1,598
Foliage	Wheat	268	497
	Fiddleneck	0	67
Root	Wheat	102	222
	Fiddleneck	0	0
		cpm/g fresh weight	
Treated leaf	Wheat	18,239	22,669
	Fiddleneck	2,568	4,997
Foliage	Wheat	60	81
	Fiddleneck	0	15
Root	Wheat	31	50
	Fiddleneck	0	0

nonextractable activity than fiddleneck. Small amounts of nonextractable activity could be detected in wheat foliage and roots. Nonextractable activity was found in trace amounts in fiddleneck foliage after four days. This form of activity could not be detected in fiddleneck roots (see Appendix Table 9 for statistical analyses).

A budget of activity distribution in wheat and fiddleneck is outlined on Table 7. The amount of unabsorbed bromoxynil-^{14}C was greater in wheat than in fiddleneck. Extractable and nonextractable activities in wheat plants were of a similar magnitude. However, in fiddleneck, the magnitude of the extractable activity greatly exceeded that of the nonextractable form. The percentage of activity recovered from wheat after two and four days of treatment was 96.1 and 81.7, respectively. The average accountability of activity in wheat was 88.9 percent. After two and four days, 85.2 and 93.8 percent of the applied activity could be accounted for in fiddleneck. The average accountability of activity in fiddleneck was 89.5 percent.

V. Metabolism of Bromoxynil-^{14}C by Wheat and Fiddleneck

A. Analysis of Breakdown Products

The differences in penetration and levels of nonextractable activity between wheat and fiddleneck, noted in Translocation Experiment IV-B, were also found in this experiment. To eliminate

Table 7. Distribution of activity in wheat and fiddleneck after two and four days of treatment with bromoxynil-^{14}C. [1]

Source of activity	Wheat		Fiddleneck	
	2 days after treatment	4 days after treatment	2 days after treatment	4 days after treatment
Unabsorbed	77,463	60,374	58,684	38,027
Extractable				
treated leaf	4,282	4,762	16,608	24,761
foliage	2,067	3,703	3,284	22,176
root	21	127	390	838
Nonextractable[2]				
treated leaf	5,846	6,678	1,040	2,284
foliage	383	710	0	96
root	146	317	0	0
Total activity accounted for	90,208	76,671	80,006	88,086
Activity unaccounted for	3,687	14,224	13,889	5,809

[1] These data are expressed as dpm/plant section. Activity applied to each plant = 93,895 dpm.

[2] Nonextractable activities were converted to 100 percent counting efficiency for the construction of this budget.

redundancy, the detailed results of these measurements will be omitted.

The percentage of extractable activity from treated leaves, present as bromoxynil and its derivatives, using paper chromatograms developed with isobutanol:ethanol:ammonia:water, are presented on Table 8. In both species, a majority of the extracted activity was present as bromoxynil. In addition to bromoxynil-^{14}C, four other labelled compounds were observed in small amounts. No attempt was made to identify these labelled compounds. Although not statistically significant, a lower proportion of activity, present as bromoxynil, was found in wheat as compared to fiddleneck. No differences in the proportions of unknown compounds located at Rf 0.72 and 0.31 could be found between the two species. However, the proportions of unknown compounds at Rf 0.47 and 0.08 were significantly greater in wheat than in fiddleneck. The percentage of breakdown products could not be related to duration of treatment (see Appendix Tables 10 and 11). The activities present in root extracts of wheat and fiddleneck were too low to permit analysis via this method. The high radiochemical purity of the bromoxynil-^{14}C formulation was confirmed in this experiment.

The paper chromatograms developed with the benzene:acetic acid:water solvent possessed only three areas of activity. Bromoxynil displayed a very broad curve on chromatograms developed with this

Table 8. The percentage of extracted activity present as bromoxynil and other compounds in wheat and fiddleneck treated leaves after four and eight days of treatment with bromoxynil-^{14}C.

Compounds	Rf	Wheat		Fiddleneck		Average
		4 days after treatment	8 days after treatment	4 days after treatment	8 days after treatment	
Bromoxynil	0.88	82.8	84.9	84.4	92.4	86.2
Unknown compounds	0.72	7.7	3.3	13.5	4.4	7.2
	0.47	2.6	1.5	0.6	1.3	1.5
	0.31	1.3	4.9	0.6	1.7	2.1
	0.08	5.6	5.4	0.9	0.2	3.0

solvent system. Measurement of the curve areas indicated that the bromoxynil curve was confounded with the two missing components. Hence the results obtained from this solvent system were considered spurious.

B. Metabolism of Bromoxynil-^{14}C to $^{14}CO_2$

The apparent metabolism of bromoxynil-^{14}C to $^{14}CO_2$ was approximately nine fold greater in wheat than in fiddleneck. Figure 7 illustrates the daily $^{14}CO_2$ recoveries from wheat and fiddleneck. In wheat, the recoveries of $^{14}CO_2$ were greater during the nyctoperiod than during the photoperiod. Few differences between photo- and nyctoperiod $^{14}CO_2$ recoveries could be detected in fiddleneck. This accounted for a significant species x collection period interaction (refer to Appendix Tables 13 and 14 for statistical analyses).

The large differences in daily $^{14}CO_2$ recoveries are evident on Figure 8. In order to evaluate total $^{14}CO_2$ evolution over time, accumulative curves are presented. Figure 9 shows the accumulative $^{14}CO_2$ recoveries from wheat and fiddleneck during photo- and nyctoperiods. After 10 days of treatment, wheat had evolved 4.12 and 6.69 percent of the applied activity during photo- and nyctoperiods, respectively, as $^{14}CO_2$. During photo- and nyctoperiods, fiddleneck had evolved 0.51 and 0.76 percent of the applied activity, respectively, as $^{14}CO_2$. Figure 10 illustrates the total accumulated activity of

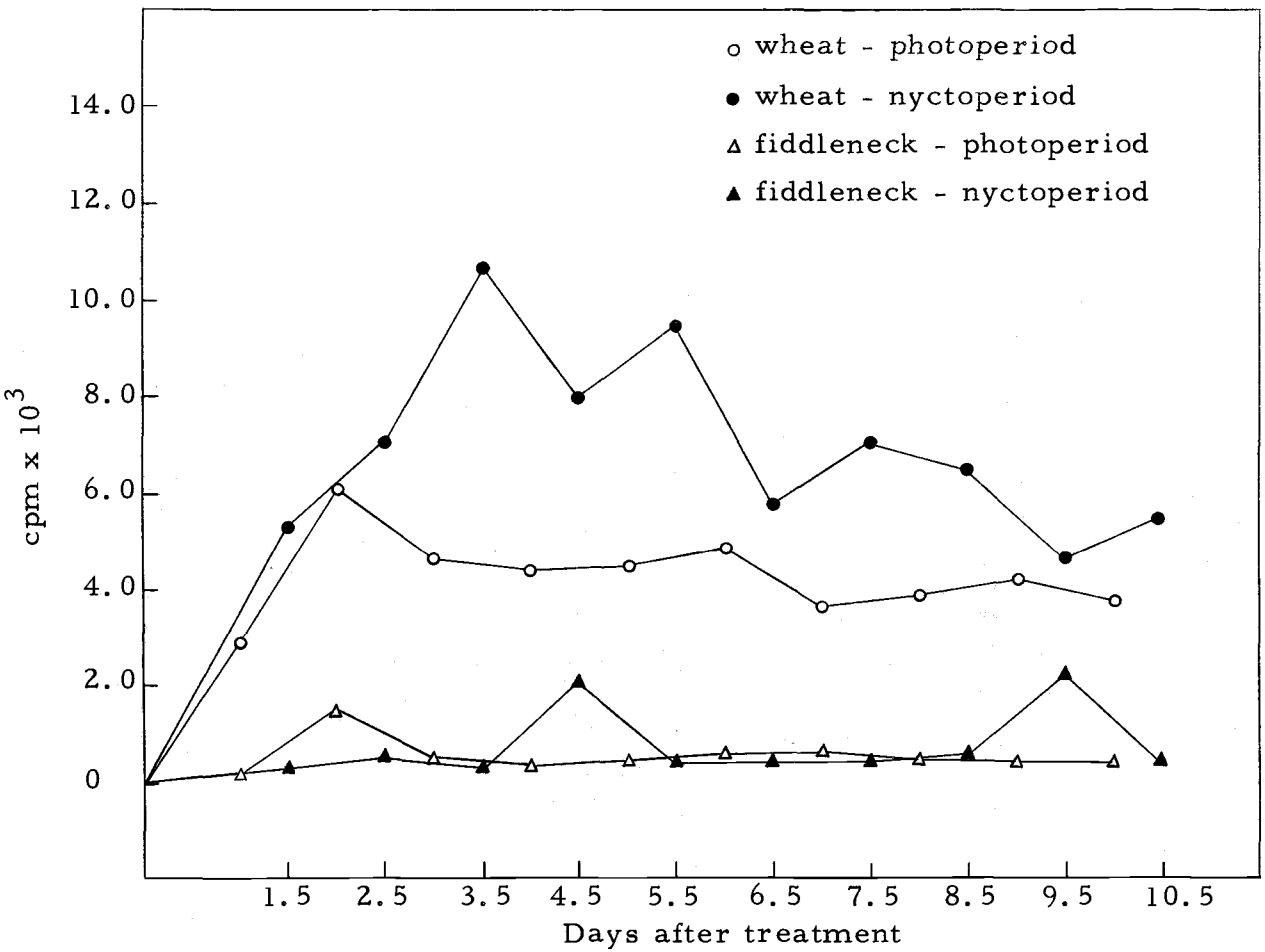


Figure 7. Recovery of $^{14}CO_2$ from wheat and fiddleneck during photo- and nyctoperiods after treatment with bromoxynil-^{14}C.

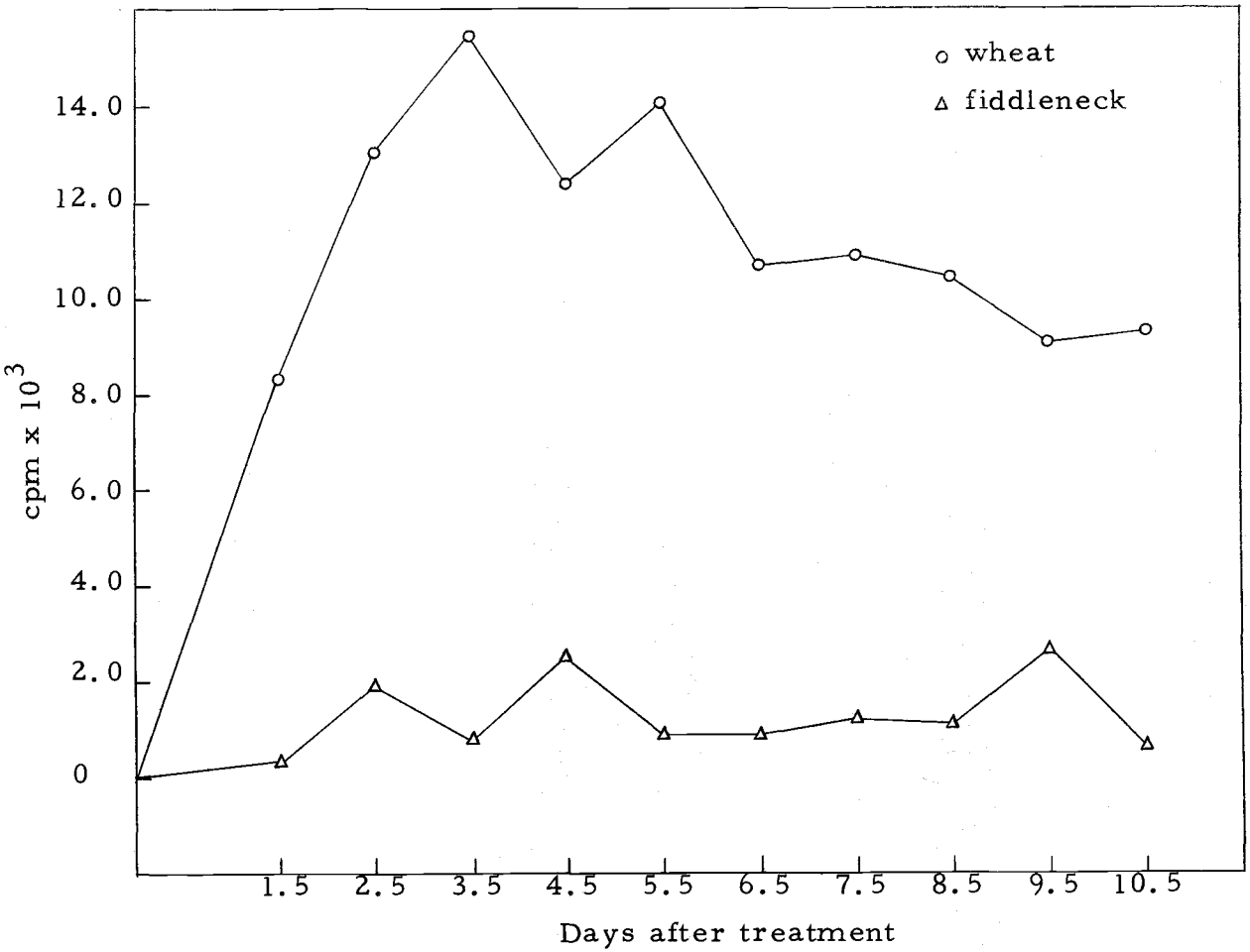


Figure 8. Daily recovery of $^{14}CO_2$ from wheat and fiddleneck after treatment with bromoxynil-^{14}C.

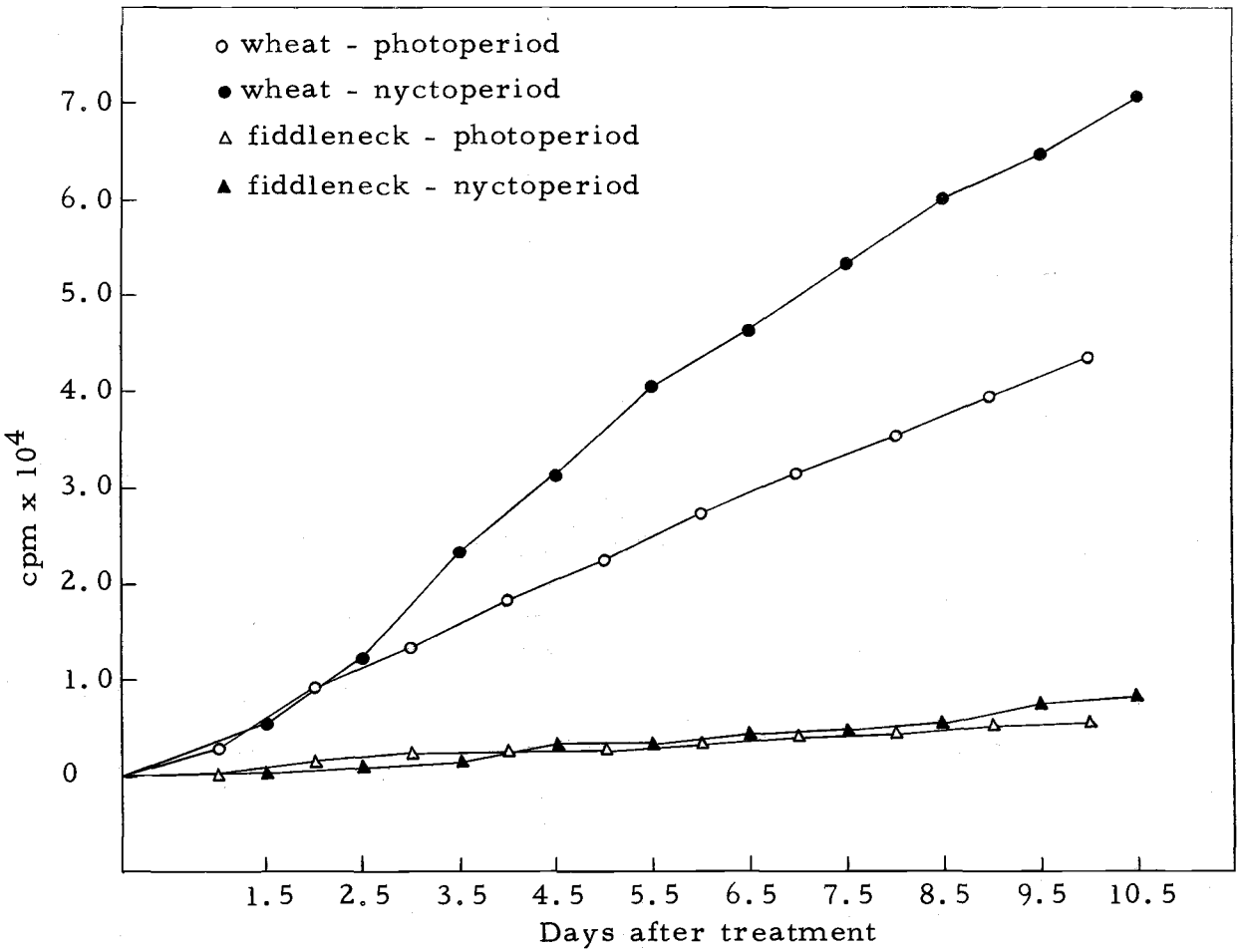


Figure 9. Accumulated recovery of $^{14}CO_2$ from wheat and fiddleneck during photo- and nyctoperiod after treatment with bromoxynil-^{14}C.

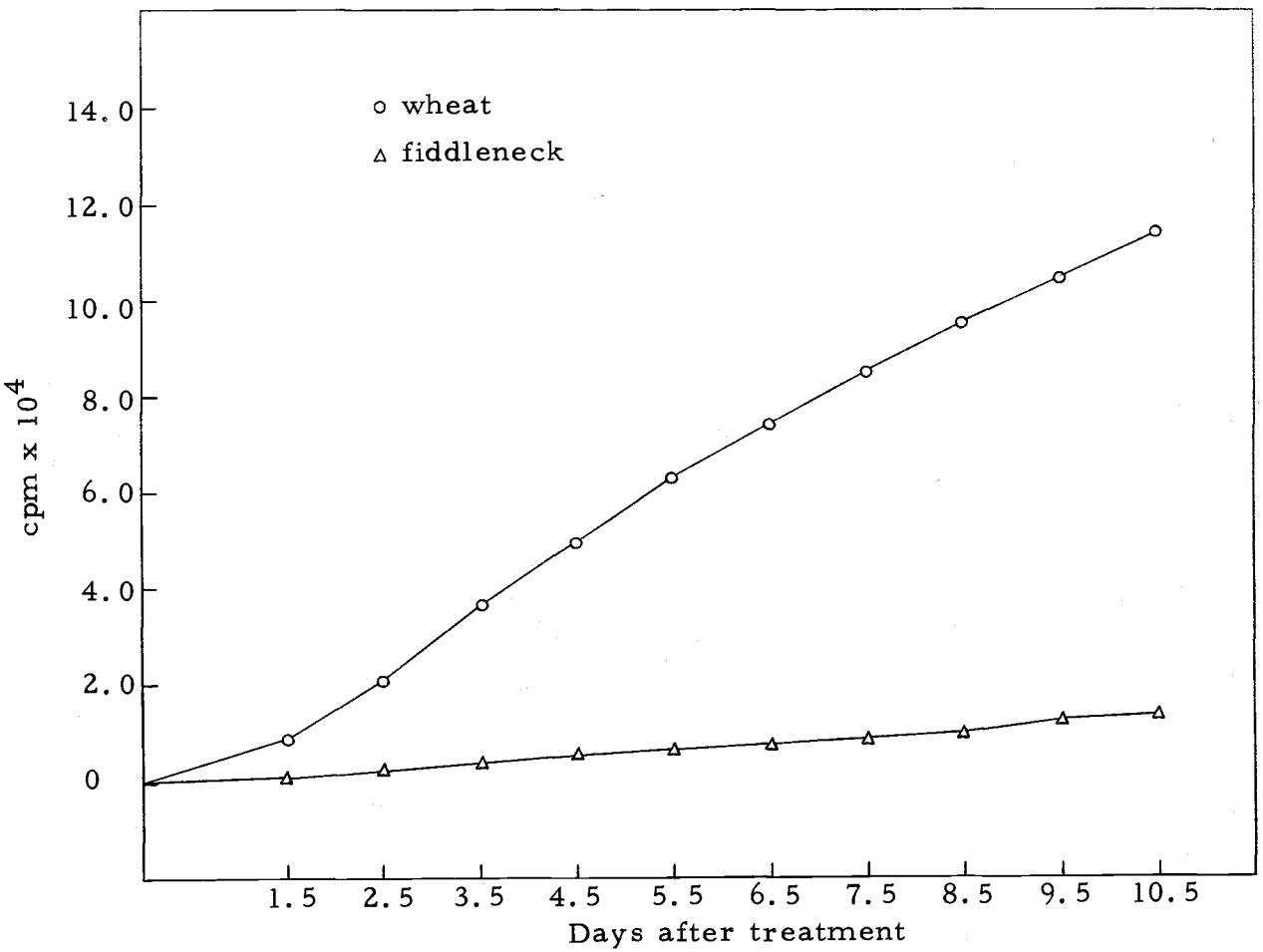


Figure 10. Accumulated recovery of $^{14}CO_2$ from wheat and fiddleneck after treatment with bromoxynil-^{14}C.

$^{14}CO_2$ from wheat and fiddleneck during the 10 days of the experiment. After 10 days of treatment, wheat and fiddleneck had evolved 10.81 and 1.27 percent of the applied activity, respectively, as $^{14}CO_2$.

If the assumption is made that 50 percent of the herbicide penetrated wheat foliage, the $^{14}CO_2$ measurements would represent 8.24 and 13.38 percent of the absorbed activity evolved as $^{14}CO_2$ during photo- and nyctoperiods, respectively. Assuming 70 percent penetration in fiddleneck, 0.73 and 1.08 percent of the absorbed activity was released as $^{14}CO_2$ during photo- and nyctoperiods, respectively. The foregoing values are probably slightly low since 1.39 percent of the treatment time was used to change CO_2 trapping solutions.

VI. The Contribution of Spray Retention, Herbicide Penetration, and Internal Factors to Selectivity of Bromoxynil in Wheat and Fiddleneck

During the course of these investigations, a number of herbicide-plant interactions were considered. It has become increasingly obvious that the phenomenon of selectivity of bromoxynil in wheat and fiddleneck is complex. The following treatment represents an estimation of the contribution of measurable interactions to selectivity. For a complete description of the underlying theory, reference can be made to the preceding section of this thesis.

Table 9 lists the selectivity ratio between wheat and fiddleneck,

Table 9. Measureable factors implicated in selectivity of bromoxynil in wheat and fiddleneck.

Measurement	Basis	Symbolic description	Numerical value	Reference
Dosage-response	ratio of wheat ED_{50}/ fiddleneck ED_{50}	$R_s = \frac{(ED_{50})_w}{(ED_{50})_f}$	109.00	Section I of Results
Spray retention	Average of retention ratios	$R_r = \frac{r_f}{r_w}$	2.15	Section II of Results
Herbicide penetration	Average of significant penetration ratios	$R_p = \frac{p_f}{p_w}$	1.67	Sections III-B and IV-B of Results

and the two other measureable selectivity interactions. By filling in these values for equation [1] (all equations are described in the previous section of this thesis) the following relationship develops.

$$R_s = R_r \cdot R_p \cdot R_i \qquad [1]$$

$$109.00 = 2.15 \cdot 1.67 \cdot R_i$$

R_i (the internal selectivity ratio) is deduced as follows:

$$R_i = \frac{109.00}{3.59} = 30.36$$

The value of R_i indicates that fiddleneck, on an internal basis, is 30.36 times more susceptible to bromoxynil than wheat. In other words, to produce the same toxic effect in both species, 30.36 units of bromoxynil would have to be introduced to the internal tissue of wheat as compared to one unit of bromoxynil in fiddleneck.

Using equations [6] through [9], the contribution of each term to total selectivity can be partitioned.

$$\Sigma R = R_r + R_p + R_i \qquad [6]$$

$$\Sigma R = 2.15 + 1.67 + 30.36 = 34.18$$

The percent contribution of spray retention to total selectivity is given by

$$S_r = \frac{R_r}{\Sigma R} \cdot 100 \qquad [7]$$

$$S_r = \frac{2.15}{34.18} \cdot 100 = 6.29 \text{ percent}$$

penetration by

$$S_p = \frac{R_p}{\Sigma R} \cdot 100 \qquad [8]$$

$$S_p = \frac{1.67}{34.18} \cdot 100 = 4.89 \text{ percent}$$

and internal factors by

$$S_i = \frac{R_i}{\Sigma R} \cdot 100 \qquad [9]$$

$$S_i = \frac{30.36}{34.18} \cdot 100 = 88.82 \text{ percent}$$

These calculations indicate that the majority of selectivity existing between wheat and fiddleneck can be attributed to internal physiological and biochemical mechanisms. While herbicide retention and penetration do contribute to selectivity under the conditions employed in these investigations, their role is a comparatively minor one.

DISCUSSION

I. Dosage-Response of Wheat and Fiddleneck to Bromoxynil

The large difference in selectivity between wheat and fiddleneck, observed after field applications of bromoxynil, was verified in Experiment I. A selectivity ratio of 109, between wheat and fiddleneck, was based at the ED_{50} level. It should be pointed out that this value would change somewhat at other ED levels since the dosage-response curves of wheat and fiddleneck did not assume the same function. The results of Experiment I were in close agreement with those noted by other workers. Davies et al. (1967) reported a 40 percent decrease in growth of barley after spraying with two kg/ha ioxynil, plus 1.0 percent Tween-20 surfactant. The surface tension of the spray solution employed by Davies et al. was 37.9 dynes/cm. In Experiment I, the growth depression of wheat plants sprayed with bromoxynil at two kg/ha was ca 38 percent. The surface tension of the spray solution used in this investigation was 32 dynes/cm. Heywood, Carpenter and Cottrell (1964) reported that 90 percent kill of young plants of fiddleneck was achieved by spraying them with bromoxynil octanoate at 0.25 kg/ha. Extreme depression of growth in fiddleneck was noted in Experiment I subsequent to treatment with bromoxynil at 0.25 kg/ha.

In the dosage-response experiment, extremely high dosages of

bromoxynil (up to 95 kg/ha) were effective in depressing growth of wheat without producing death. New growth was always evident in wheat plants treated with high dosages of bromoxynil. It is notable that Davies et al. (1967) failed to produce death in barley by painting the foliage with high concentrations of ioxynil. They found that treated leaves and leaf sheaths were killed, but new growth was produced. Davies et al. also observed terminal necrosis in leaves of barley sprayed with ioxynil. This phenomenon was also observed among wheat treated with bromoxynil. The occurrence of terminal necrosis in wheat is understandable since terminal portions of the leaves tend to droop and are more exposed to the spray solution. This horizontal position would be accentuated by the force of the spray solution.

The differential response of shoots and roots to bromoxynil has not been alluded to in the literature. An explanation for increasing shoot/root ratios, in response to increasing dosage of bromoxynil, may be approached in two ways. (1) Limited translocation of the label from bromoxynil-^{14}C has been observed. If root tissue was more sensitive to bromoxynil than foliar tissue, an increasing shoot/root ratio would be expected. (2) The increasing shoot/root ratio could be a secondary response. Due to destruction of foliar tissue, the decreased amounts of available photosynthate could be directed to foliar meristematic regions, with the root system being by-passed. In view of the limited translocation of the label from bromoxynil-^{14}C

into roots of these species - particularly wheat, and the fact that the growing point was the last area affected, the latter hypothesis seems more plausible.

The necrotic tissue weights of fiddleneck foliage treated with increasing bromoxynil dosages were not significantly different. However, the growth of fiddleneck plants was greatly depressed by increasing the bromoxynil dosage. This provides evidence that a sub-lethal translocation effect is operative in fiddleneck subsequent to treatment with bromoxynil. While necrotic tissue weight of wheat foliage increased with bromoxynil dosage, the rate of increase did not correspond to the rate of growth depression. These relationships also provided evidence for a sub-lethal translocation effect in wheat, although not as pronounced as that found in fiddleneck. This suggestion is corroborated by the findings of Davies et al. (1967). These workers compared the effect of mechanical removal of leaves of mustard and barley with the effect produced by treating similar leaf areas with ioxynil. Davies et al. concluded that a more extensive translocation effect occurred in mustard as compared to barley.

II. Spray Retention by Wheat and Fiddleneck

Fiddleneck retained about twice as much spray solution as did wheat. Thus, this factor is definitely implicated in selectivity between these two species. Since the addition of Brilliant Vital Red

dye, and bromoxynil to the spray formulation did not alter surface tension, spray retention can be equated with herbicide retention. The differential spray retention by wheat and fiddleneck can be explained by two factors. (1) Wheat possesses a vertically orientated foliage, whereas fiddleneck, in the rosette stage of growth, is characterized by a horizontally orientated foliage. The horizontal projection of leaf area is appreciably less in wheat than in fiddleneck. This characteristic would indicate that spray retained per unit of foliage area could be less in wheat than in fiddleneck. The vertical orientation of wheat foliage would facilitate rebounding of spray droplets upon contact with the leaf surface. (2) The physical nature of the leaf surface differs greatly between wheat and fiddleneck. Wheat leaves are relatively smooth and difficult to wet. Fiddleneck leaves, on the other hand, are easily wetted. Hairs present on fiddleneck leaves are large enough to prevent runoff of the spray, yet are sufficiently dispersed to allow intimate contact of the spray droplet with the leaf cuticle. Both factors considered, it appears that retention of spray on foliage of fiddleneck would not be changed appreciably by alteration of spray surface tension. Surface tension of the spray solution would have an important bearing on the amount of spray retained by wheat.

A morphological factor associated with spray retention and differential toxicity is the fact that the growing point of wheat is located at the base of the foliage and is protected from direct contact

with the spray solution. The growing point of fiddleneck, on the other hand, is exposed and is vulnerable to direct contact with a toxicant in the spray solution.

Differences in spray retention between juvenile plants of cereal species and broad-leafed weeds have been reported to be considerably greater than two-fold. Carpenter et al. (1964a) reported that Sinapsis arvensis L. retained five times more spray solution than did barley. These workers, however, did not present data on the surface tension of the spray solution employed. Davies et al. (1967) found that mustard retained eight to 26 times more herbicide than barley, depending upon the addition of Tween-20 surfactant. The surface tension of the spray solutions employed by Davies et al. ranged from 70.4 to 37.9 dynes/cm. The disparity of the retention differences reported by these workers and retention differences found in this thesis can be explained partially via important differences in spray surface tension of the respective formulations. Spray solutions used in this investigation possessed a surface tension of 32 dynes/cm. A lowered surface tension would increase retention by wheat and probably not influence retention by fiddleneck. This statement is supported by the findings of Blackman, Bruce and Holly (1958). These workers found a large increase in spray retention by barley as the surface tension was reduced from 40 to 30 dynes/cm. The aforementioned disparities may also partially be the result of differences

in spray parameters (e.g., volume of application, sprayer height and spray nozzle design), and specific differences.

III. Penetration of Bromoxynil-^{14}C Into Foliage of Wheat and Fiddleneck

Differential penetration of bromoxynil into leaves of wheat and fiddleneck was shown to be a factor involved in selectivity between the two species. This is in agreement with the findings of Davies et al. (1968a), which implicated differential uptake of ioxynil as a factor in selectivity existing between barley and mustard. The technique employed by Davies et al. was that of Sargent and Blackman (1962). This technique for determining uptake differs appreciably from the method used in these investigations.[1] The Sargent-Blackman method suffers from two shortcomings. (1) Under field conditions, the herbicide droplets dry out, leaving the herbicide deposited on the leaf surface as a thin film. This event is eliminated by the Sargent-Blackman method. (2) Sargent (1965) and Franke (1967) agree that penetration is a diffusion phenomenon. The utilization of leaf discs or sections would decrease the diffusion gradient between the

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[1] Chemical uptake by the Sargent and Blackman method is estimated by placing a relatively large volume of labelled solution within a ring or barrier implanted on a leaf disc or section. These leaf discs or sections are then placed in closed dishes containing moistened filter paper. After a specified time, the labelled solution and barrier are removed. Uptake is determined by counting the radioactivity present in the leaf disc or section.

herbicide and the leaf tissue, since translocation would be interrupted. Hence, measurements via this method would likely underestimate uptake as compared to true uptake under field conditions.

The results of Experiments III-A and IV-B indicated that the rate of penetration of bromoxynil decreased with time of contact. As penetration proceeds, subsequent to treatment, the amount of herbicide remaining unabsorbed is decreased. Thus, the rate of penetration would be attenuated over a time-course. Zukel et al. (1956), for example, found that maleic hydrazide was absorbed at a rate directly proportional to the amount remaining unabsorbed on the leaf. The percent penetration of bromoxynil-^{14}C was found to be less in Experiments IV-B and V-A than in penetration Experiments III-A and III-B. Higher concentrations of labelled herbicide were applied in the former experiments (1.68 μg/microdrop) as compared to the latter experiments (0.16 μg/microdrop). Thus, the unabsorbed herbicide would form a thicker film on the leaf, and could result in proportionately less penetration over time. Hughes and Freed (1961) found that foliar absorption of IAA obeyed the first order rate law. When penetration data of Experiments IV-B and V-A were combined, a plot of $\log_{10}$ unabsorbed bromoxynil versus time on a linear scale resulted in near linear curves for wheat and fiddleneck. Thus, penetration of bromoxynil in these experiments was also conforming to the first order rate law. The time required for 50 percent penetration of bromoxynil into

leaves of fiddleneck and wheat was 73 and 152 hours, respectively.

In Experiment III-B, penetration of bromoxynil into adaxial and abaxial leaf surfaces was found to be unrelated to stomatal densities. From these findings, it may be deduced that cuticular penetration was the primary route of uptake of bromoxynil under the conditions used in this experiment. These findings are in agreement with the report of Davies et al. (1968a). Franke (1967) has speculated that two routes may exist for foliar penetration of solutions. An aqueous route through the cuticle through which water soluble polar compounds may follow, and a lipid pathway through which lipid soluble, apolar substances follow. If, indeed the two pathways exist, it would be expected that bromoxynil octanoate, a lipophilic compound, would follow the lipid pathway through the cuticle.

IV. Translocation of Bromoxynil-^{14}C in Wheat and Fiddleneck

A different translocation pattern was found between wheat and fiddleneck in autoradiographic as well as extraction analyses. Extraction analysis of translocation of HBNs has not previously been reported in the literature. Wheat was characterized by limited amounts of soluble label translocated from treated leaves into adjoining foliage areas and root systems. Fiddleneck, on the other hand, exhibited appreciable amounts of the soluble label in foliage and root sections. The observation that the soluble form of activity was composed mainly

of bromoxynil leads to the speculation that the parent herbicide is more mobile in fiddleneck than in wheat. Reservations regarding the identity of soluble activity in roots and foliage are in order since qualitative analysis could not be made for root activities and was not performed for activities present in foliage segments.

The insoluble fraction of radioactivity, not previously measured by other investigators, was appreciable greater in plant segments of wheat than in comparable segments of fiddleneck. Two hypotheses may be presented to explain this activity. (1) Hydrolysis of bromoxynil octanoate, via esterases would result in the formation of bromoxynil-phenol. Conjugation of the parent phenol with plant proteins and other constituents could occur. These conjugates would be insoluble in the solvent used for extraction. (2) Large amounts of $^{14}CO_2$ were evolved from plants - particularly wheat - after treatment with bromoxynil-^{14}C. Thus, the insoluble activity could represent $^{14}CO_2$ recycled via photosynthesis and subsequent biosynthesis. Both explanations appear equally valid. It is possible that the insoluble fraction may represent both conjugates of bromoxynil and recycled $^{14}CO_2$. Additional investigations, designed to ascertain the identity of the label in the insoluble fraction, would be extremely useful in formulating an explanation for this occurrence.

With the exception of fiddleneck after four days of treatment, a majority of the label remained in the treated leaf. This is in

agreement with the findings of Foy (1964) and Davies et al. (1968a) concerning ioxynil movement. In fiddleneck, the observation that activity moves into younger leaves, while older leaves are by-passed is also in agreement with the reports of Carpenter et al. (1964a) and Davies et al. (1968a). This form of movement is highly suggestive of symplastic translocation. Davies et al., however, found that a greater overall labelling occurred in barley than in dicotyledonous species investigated. The reverse observation was made between wheat and fiddleneck in these investigations. This disparity may be the result of specific differences or differences in the mobility of ioxynil and bromoxynil. Although quantitative data have never been supplied for translocation of ioxynil, and comparisons must be based upon qualitative analysis supplied by Zaki, Taylor and Wain (1967) and autoradiographic analyses by Foy (1964) and Davies et al. (1968a), it is possible that bromoxynil is more mobile than ioxynil in dicotyledonous species.

The role that differential translocation plays in selectivity between wheat and fiddleneck is difficult to quantitate. However, it seems likely that a more extensive translocation in combination with other factors does contribute to the susceptibility of fiddleneck to bromoxynil.

V. Metabolism of Bromoxynil-^{14}C by Wheat and Fiddleneck

The observation that soluble activity extracted from treated leaves of wheat and fiddleneck was mainly attributed to bromoxynil is in agreement with the report of Davies et al. (1968b) on the degradation of ioxynil. No evidence was provided by Davies et al. to show that the unabsorbed ioxynil-^{14}C was removed from treated leaves prior to extraction. This could account for the higher percentage of ioxynil-^{14}C appearing in the parent form than was observed for bromoxynil in these investigations.

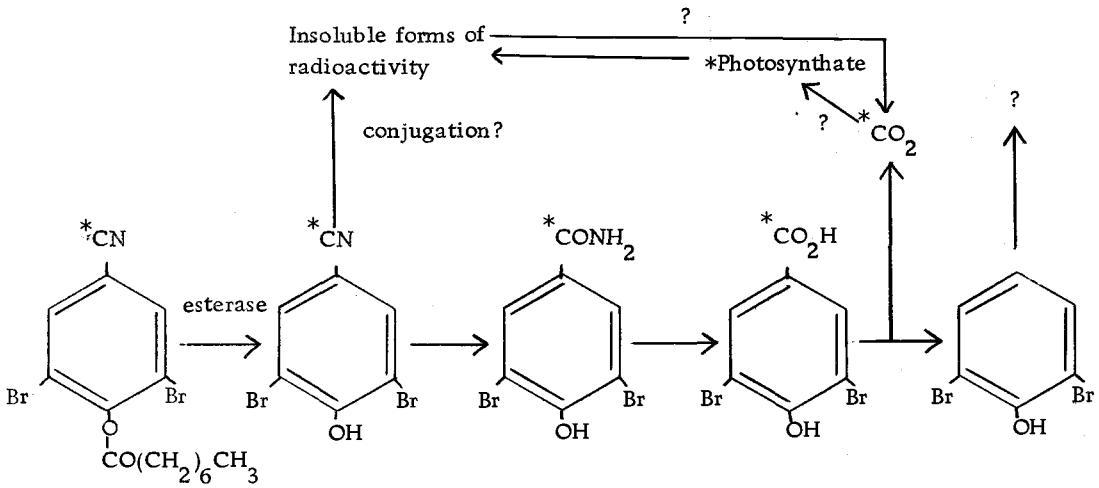
The foregoing evidence on limited accumulation of derivatives of HBNs is surprising, since ample evidence is available to show that rapid degradation of HBNs occurs in plant tissue [Heywood, Carpenter and Cottrell (1964); Davies et al. (1968b); and the $^{14}CO_2$ evolution experiment reported herein]. It is evident that metabolism of the parent phenol to the carboxylic acid derivative must occur prior to decarboxylation. The obvious explanation for these findings is that formation of derivatives does occur, but that they are extremely unstable in the plant and are rapidly degraded. Hence, large accumulations of derivatives could not be detected at any point in time.

Investigations on metabolism of HBNs, using both cyano and ring labelled compounds, would be useful in tracing the fate of the

phenol moity subsequent to decarboxylation. Only limited work has been conducted on this aspect. Hart, Bishop and Cooke (1964) reported that wheat degraded ring labelled ioxynil-^{14}C. Activity in mature grain of wheat was found in starch, gluten, and glucose fractions. However, they did not outline the exact degradation pathway.

Investigations on the evolution of $^{14}CO_2$ from plant tissue treated with labelled HBNs have not previously been reported. After correction for the penetration differential, the ratio (wheat/fiddleneck) of $^{14}CO_2$ evolution was ca 12. A large internal selectivity difference between wheat and fiddleneck was deduced prior to the initiation of Experiment V-B. This deduction was made via the mathematical analysis described earlier. Differential degradation of bromoxynil by wheat and fiddleneck, interacting with species differentials based upon translocation and conjugation, could easily satisfy the internal selectivity difference as deduced by mathematical analysis.

With the foregoing evidence at hand, the following tentative scheme for partial metabolism of bromoxynil is presented.



Other reports [Zaki, Taylor and Wain (1967); and Ioxynil and Bromoxynil, (1967)] show dehalogenation occurring simultaneously with hydrolysis as a mechanism for degradation of ioxynil. Thus, the possibility exists that dehalogenation occurs with bromoxynil. The consequence of these pathways would be the production of less toxic compounds, such as hydroxybenzoic acid, a natural plant constituent. It is becoming evident that these metabolic pathways are functional to a greater degree in cereal species as compared to many broad-leafed weed species.

A definite point, established by the $^{14}CO_2$ evolution experiment, was that wheat degraded bromoxynil to a nontoxic form more rapidly than did fiddleneck. Carpenter et al. (1964b) and Heywood (1966) have emphasized that the presence of the cyano group is essential for the expression of phytotoxicity. This, of course, was the group that was removed more rapidly in wheat than in fiddleneck.

In relating the results of the $^{14}CO_2$ experiment with other investigations on chemodynamics of bromoxynil, two reservations are in order. (1) A constant temperature of 20 C was maintained throughout the experiment whereas other experiments were conducted at 20 C day and 15C night temperatures. (2) Relative humidity within the plexiglass chambers was at 100 percent, as verified by condensation of water on the chamber walls. This may have increased penetration of the herbicide. Both factors may have increased total $^{14}CO_2$

evolution as compared to other experiments.

The observation that $^{14}CO_2$ recoveries were appreciably higher during the dark period in wheat versus the photoperiod is worthy of mention. Tregunna, Krotkov and Nelson (1961 and 1964) and Moss (1966) have provided evidence to show that photorespiration in many species exceeds nyctorespiration. Moss also found that maize (Zea mays L.) did not have this light-stimulated CO_2 evolution. It was speculated that different mechanisms were responsible for light and dark respiration. Zelitch (1958) claimed that respiration in the light was largely due to a glycolate pathway. Light evolution of $^{14}CO_2$, arising from labelled bromoxynil, however, was less than dark evolution. The aforementioned photorespiration mechanism, in this case, would probably only apply to that $^{14}CO_2$ recycled via photosynthesis and Crassulacean acid metabolism. The evolution of $^{14}CO_2$ via decarboxylation of the herbicide would be expected to proceed by an entirely different mechanism. The lesser evolution of $^{14}CO_2$ during photoperiods may be a reflection of $^{14}CO_2$ reincorporation by photosynthesis.

VI. Factors Responsible for Selectivity of Bromoxynil in Wheat and Fiddleneck

The investigations reported in this thesis were concerned with factors responsible for the selective action of bromoxynil in wheat and fiddleneck. The mathematical model employed herein served as a

tool for describing the contribution of certain plant-herbicide interactions to differential phytotoxicity. This form of analysis should prove useful in describing the causation of differential toxicity in any foliar applied, selective herbicide. This analysis, in its present form, can only be used for di-specific comparisons.

Spray retention was found to be a factor in bromoxynil selectivity, under the conditions selected for these experiments. In an overall analysis spray retention only accounted for 6.3 percent of the selectivity difference between wheat and fiddleneck. Carpenter et al. (1964a) and Davies et al. (1967) found that herbicide retention played a larger role in ioxynil selectivity between cereals and broad-leafed weeds. The low surface tension of spray solutions used in experiments reported herein accounts for most of these disparities. Under field applications, spray retention on cereal crops would be expected to be less, since surface tensions of practical spray solutions would probably be greater than 32 dynes/cm. Hence, the selectivity ratio and retention ratio between cereal species and broad-leafed species would increase. Under these conditions, greater import would be placed on retention as a factor in bromoxynil selectivity.

As Davies et al. (1968b) pointed out, gross morphological differences between cereal species and dicot weeds likely contribute to HBN selectivity. The growing point of fiddleneck is exposed while that of wheat is protected from direct contact with a toxicant.

Once deposited upon the foliage, greater amounts of bromoxynil penetrated into fiddleneck as compared to wheat. This constituted another factor involved in bromoxynil selectivity - although a minor one. Penetration differences could only account for 4.9 percent of the differential toxicity of bromoxynil to wheat and fiddleneck.

A key point, uncovered by these investigations, was the large internal differential existing between wheat and fiddleneck in relation to bromoxynil toxicity. These internal differences can be attributed to greater translocation in fiddleneck, and more extensive degradation and possibly conjugation by wheat. It is possible that other internal mechanisms are operative which could contribute to cereal tolerance. In these studies, 88.8 percent of the bromoxynil selectivity difference between wheat and fiddleneck could be attributed to internal factors.

When all of these interactions are considered, it is evident that wheat, sprayed under field conditions, possesses characteristics which offer tremendous protection from bromoxynil damage. Under field applications, spray retention differentials would offer the first line of defense against bromoxynil toxicity. Should this line of defense be breached by the addition of surfactants, spray overlap or overapplication, penetration limitations interacting with internal physiological and biochemical factors could be expected to provide considerable protection for wheat.

At this time, knowledge of the action and fate of bromoxynil in

plants is far from complete. It is this author's opinion that the following aspects of bromoxynil selectivity and chemodynamics deserve further attention. (1) The degradation pathway of bromoxynil remains incomplete. The elucidation of this pathway could be obtained by the use of both cyano and ring labelled bromoxynil and derivatives. (2) Analysis and identification of the label in 'bound' forms of radioactivity, present in plant tissues after treatment with bromoxynil-^{14}C, would be useful. (3) Further analyses of specific differentials of bromoxynil action at the organelle level are warranted. (4) As a corollary to the investigations reported herein, knowledge of bromoxynil penetration, translocation, and degradation, as a function of temperature, light intensity, relative humidity, and plant stage of growth, is within reach.

SUMMARY AND CONCLUSIONS

Growth chamber and laboratory studies were conducted to evaluate selectivity and chemodynamics of bromoxynil in winter wheat and coast fiddleneck.

Dosage-response experiments illustrated high tolerance of wheat and susceptibility of fiddleneck to bromoxynil. Comparison of ED_{50} values, based upon depression of whole plant growth, indicated that wheat was ca 109 fold more tolerant of bromoxynil than was fiddleneck. With increasing dosages of bromoxynil, the shoot/root ratios of both wheat and fiddleneck were found to increase. Measurement of necrotic tissue weight of wheat and fiddleneck over increasing dosages of bromoxynil suggested a sub-lethal translocation effect.

The selectivity found between wheat and fiddleneck was concluded to be the result of a complex of interactions. Fiddleneck was found to retain twice as much spray solution as wheat. Examination of spray surface tension indicated that spray retention could be equated with herbicide retention. Mathematical analysis indicated that 6.3 percent of the differential toxicity between wheat and fiddleneck could be attributed to differences in herbicide retention.

Penetration of the herbicide into leaves of wheat and fiddleneck was estimated by using bromoxynil-^{14}C coupled with leaf washing procedures. Significant specific penetration differences were found in three out of four experiments. It was, therefore, concluded that

differential penetration of bromoxynil was a factor involved in selectivity. Penetration of bromoxynil-^{14}C could not be related to stomatal densities in either wheat or fiddleneck. This suggested that cuticular penetration was the principle route of uptake of bromoxynil. Only 4.9 percent of the selectivity existing between wheat and fiddleneck could be attributed to the penetration differential.

Translocation of bromoxynil-^{14}C in the two species was followed by autoradiography and extraction procedures. Overall movement of the label was more extensive in fiddleneck than in wheat. Higher levels of radioactivity were obtained from treated leaf, foliage and root extracts of fiddleneck as compared to wheat. Appreciable amounts of the label from bromoxynil-^{14}C were found in foliage and root segments of fiddleneck after four days of treatment. Higher levels of insoluble label were found in plant segments of wheat as compared to fiddleneck. This insoluble fraction may represent insoluble conjugates of bromoxynil, recycled $^{14}CO_2$, or both.

Identification of the soluble fraction of the activity from treated leaves of wheat and fiddleneck was made by paper chromatography coupled with radiochromatographic techniques. In both species, a high percentage of extractable activity was attributed to bromoxynil. No specific differences in the level of derivatives could be detected. The metabolism of bromoxynil-^{14}C was further studied via $^{14}CO_2$ evolution. $^{14}CO_2$ evolution from wheat significantly exceeded that of

fiddleneck. This indicated a greater capacity, on the part of wheat, to degrade the herbicide. It was further concluded that derivatives of bromoxynil are formed, but are unstable and quickly degraded.

Mathematical analysis indicated that 88.8 percent of the selective toxicity existing between the two species could be attributed to internal physiological and biochemical mechanisms. Specific differentials in translocation and herbicide degradation capacity contribute substantially to the internal selectivity factor.

The mathematical model for selectivity analysis should be applicable for describing the causation of differential toxicity in foliar applied, selective herbicides.

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APPENDIX

Appendix Table 1. Dosage-response measurements of wheat and fiddleneck treated with bromoxynil.

Bromoxynil dosage (g/ha)	Foliage dry weight (percent of control)	Root dry weight (percent of control)	Whole plant dry weight (percent of control)	Shoot/Root ratio (dry weight basis)	Foliage dead tissue (mg/plant)
			Wheat		
Control				2.47	4
1,584	66.4	64.6	65.9	2.67	47
3,168	52.5	47.6	51.1	2.73	46
6,326	48.3	33.3	44.0	3.58	52
12,637	46.6	27.3	41.0	4.23	63
24,769	42.5	22.1	36.7	4.75	68
47,168	36.0	18.1	30.8	4.91	75
95,335	27.6	12.6	23.2	5.40	85
			Fiddleneck		
Control				2.92	152
13	70.8	59.3	66.3	3.46	223
26	62.4	51.3	59.6	3.55	235
52	32.5	20.2	29.3	4.70	231
104	29.4	19.9	27.0	4.31	237
208	30.1	18.7	27.2	4.70	233
416	25.6	14.1	22.7	5.31	249

Appendix Table 2. Analyses of variance for foliage dead tissue of wheat and fiddleneck treated with bromoxynil. [1]

Source of variation	df	Mean square
	Wheat	
Replications	4	53.75
Dosages	6	1,096.50**
Error	24	94.79
Total	34	
	Fiddleneck	
Replications	4	11,451.75**
Dosages	5	460.60
Error	20	560.20
Total	29	

[1] **Significant at the one percent probability level. Each replicate was composed of two plants.

Appendix Table 3. Analyses of variance for measurements on retention of spray solution by wheat and fiddleneck. [1]

Source of Variation	df	μ liters Spray Solution Retained/Plant Mean Square	Foliage Dry Weight mg/Plant Mean Square	μ liters Spray Solution Retained/g Dry Weight Mean Square	Foliage Area cm^2/Plant Mean Square	μ liters Spray Solution Retained/cm^2 Foliage Area Mean Square
Replications	2	420.50**	3,302.00	1,485.00	236.00*	0.03
Species	1	20,068.33**	1,066.66	196,928.33**	37.66	5.17**
Error	2	0.84	716.67	1,645.33	10.67	0.02
Total	5					

[1] *, **Significant at the five and one percent probability levels, respectively.

Appendix Table 4. Percent penetration of bromoxynil-^{14}C into leaves of wheat and fiddleneck after four durations of treatment.

Duration of treatment (hr)	Wheat	Fiddleneck
6	23.0	19.7
24	42.1	42.7
48	55.4	57.5
96	57.9	63.5

Appendix Table 5. Analysis of variance for penetration of bromoxynil-^{14}C into leaves of wheat and fiddleneck after four durations of treatment. [1]

Source of variation	df	Mean square
Replications	4	49.10
Species	1	15.70
Duration of treatment	3	3,144.60**
SD	3	34.56
Error	28	60.55
Total	39	

[1]**Significant at the one percent probability level.

Appendix Table 6. Analyses of variance for penetration of bromoxynil-^{14}C into adaxial and abaxial leaf surfaces of wheat and fiddleneck, and stomatal numbers on adaxial and abaxial leaf surfaces of the two species. [1]

Source of variation	df	Mean square
	Percent penetration	
Replications	3	13.28
Species	1	511.89*
Leaf surfaces	1	8.26
SL	1	9.40
Error	9	83.25
Total	15	
	Stomata/0.48 mm^2	
	Wheat	
Leaf surfaces	1	186.05**
Error	18	5.83
Total	19	
	Fiddleneck	
Leaf surfaces	1	480.20**
Error	18	17.06
Total	19	

[1] *, **Significant at the five and one percent probability level, respectively.

Appendix Table 7. Analysis of variance for penetration of bromoxynil-^{14}C into leaves of wheat and fiddleneck after two and four days of treatment (From Translocation Experiment IV-B). [1]

Source of variation	df	Mean square
Replications	2	16.46
Species	1	1,432.27**
Duration of treatment	1	1,214.04**
SD	1	10.64
Error	6	30.27
Total	11	

[1]**Significant at the one percent probability level.

Appendix Table 8. Analyses of variance for extractable radioactivity recovered from plant sections of wheat and fiddleneck after two and four days of treatment with bromoxynil-^{14}C. [1]

Source of variation	df	Treated leaf mean square	Foliage mean square	Root mean square
		dpm/plant section		
Replications	2	0.82	26.82	0.15
Species	1	783.92**	290.77*	87.42**
Duration of treatment	1	55.86**	315.70*	22.93**
SD	1	44.20**	223.39	8.76
Error	6	2.60	44.91	1.49
Total	11			
		dpm/g fresh weight		
Replications	2	116.58	1.61	0.11
Species	1	5,481.83**	15.41*	12.20**
Duration of treatment	1	241.74	11.75	0.69
SD	1	232.50	9.88	0.21
Error	6	123.86	2.59	0.47
Total	11			

[1] Activities from treated leaves and foliage were converted to the exponent 10^3 for calculation of analysis of variance. Root activities were converted to the exponent 10^2.

*, ** Significant at the five and one percent probability levels, respectively.

Appendix Table 9. Analyses of variance for nonextractable activity present in plant section residues of wheat and fiddleneck after two and four days of treatment with bromoxynil-^{14}C. [1]

Source of variation	df	Treated leaf mean square	Foliage mean square	Root mean square
		cpm/plant section		
Replications	2	2.46	0.65	0.99
Species	1	31.27**	36.54**	7.89*
Duration of treatment	1	1.63	6.57	1.07
SD	1	0.05	1.97	1.07
Error	6	1.74	1.76	0.84
Total	11			
		cpm/g fresh weight		
Replications	2	46.03	0.02	0.05
Species	1	833.83**	1.20**	0.49*
Duration of treatment	1	35.26	0.10	0.03
SD	1	3.01	0.01	0.03
Error	6	47.18	0.08	0.07
Total	11			

[1] Activities from treated leaves were converted to the exponent 10^3 for calculation of analysis of variance. Foliage and root activities were converted to the exponent 10^2.

*, ** Significant at the five and one percent probability levels, respectively.

Appendix Table 10. Analyses of variance for percent extracted activity present as bromoxynil and other derivatives in wheat and fiddleneck after four and eight days of treatment with bromoxynil-^{14}C. [1]

Source of variation	df	Compounds				
		bromoxynil Rf 0.88 mean square	Rf 0.72 mean square	Rf 0.47 mean square	Rf 0.31 mean square	Rf 0.08 mean square
Replications	2	218.30	99.71	1.77	2.66	44.22
Species	1	62.11	36.40	3.63*	12.00	74.00*
Duration of treatment	1	76.51	135.34	0.13	16.81	0.66
SD	1	26.70	16.10	2.42	4.56	0.27
Error	6	112.88	82.63	0.45	7.57	10.38
Total	11					

[1] *Significant at the five percent probability level.

Appendix Table 11. Analysis of variance for percent extracted activity present as bromoxynil and other compounds. [1]

Source of variation	df	Mean square
Compounds in extract	4	5,485.63**
Error	15	10.37
Total	19	

[1]**Significant at the one percent probability level. Entries used for calculation of analysis of variance were wheat and fiddleneck means at four and eight day treatment periods. LSD (.05) for evaluating differences between arrayed means of compounds = 4.9.