PHYSIOLOGICAL ASPECTS OF THE MODE OF ACTION OF NITROFLUORFEN AND OXYFLUORFEN

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David Edward Vanstone

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INSPIRATION

It is a good thing to give thanks unto the Lord, and to sing praises unto thy name, O most High to shew forth thy lovingkindness in the morning, and thy faithfulness every night, upon an instrument of ten strings, and upon the psaltery; upon the harp and with solemn sound. For thou, Lord, hast made me glad through thy work: I will triumph in the works of thy hands. O Lord, how great are thy works! and thy thoughts are very deep.

Psalm 92: 1-5

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ABSTRACT

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Root uptake and translocation of ^{14}C-nitrofluorfen [2-chloro-1-(4-nitrophenoxy)-4-(trifluoromethyl)benzene] and ^{14}C-oxyfluorfen [2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl)benzene] was measured in fababean (*Vicia faba* L) and green foxtail (*Setaria viridis* (L.) Beauv.) plants; metabolism by fababean leaf discs and green foxtail leaf segments was also studied. The herbicides were taken up readily from nutrient solution by both species. Nitrofluorfen was translocated more extensively than oxyfluorfen, although translocation of both herbicides was small. Less than 10% of nitrofluorfen or oxyfluorfen was metabolized by either species after 24 hours. Metabolism did not influence the herbicidal action of either herbicide.

The use of electrolytic conductivity as a measure of cell membrane disruption was tested on buckwheat (*Fagopyrum esculentum* Moench. cv. 'Tokyo') leaf discs from plants which had been sprayed with oxyfluorfen. All treatments which resulted in visual tissue damage also increased conductivity in solutions where the discs were floated. The highest conductivity measurement (most membrane disruption) was

obtained from the samples which showed severe visual injury. Similar membrane disruption was caused by oxyfluorfen treatments ranging from 1 g/ha to 1000 g/ha. Once membrane disruption began it proceeded exponentially with time. A comparison of the pattern of conductivity change induced by oxyflurofen and paraquat (1,1'-dimethyl-4,4'-bipyridinium ion) implies that oxyfluorfen has a different mode of action from paraquat.

Light was found necessary for the herbicidal activity of oxyfluorfen following foliar treatment to buckwheat. Plants were not injured when placed in the dark for as long as four days after treatment. When they were then brought to the light, injury occurred, albeit more slowly than when plants were placed in the light immediately after treatment. The rate of injury increased as after-treatment light intensity was increased to 6000 $\mu W/cm^2$. The most effective wave length of light was 565-615 nm. This wavelength of effectiveness coincided with the region of absorption by a xanthophyll-protein complex. Chlorophyll content was not reduced by oxyfluorfen. Preliminary evidence suggests that photosynthesis was affected only after the leaf tissue began to wilt.

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FORMAT

This thesis has been written in manuscript style. The original work of the author appears in three manuscripts which have been submitted for publication in Weed Science:

Manuscript 1. Root uptake, translocation and metabolism of nitrofluorfen and oxyfluorfen by fababean and green foxtail.

Manuscript 2. Electrolytic conductivity - a rapid measure of herbicide energy.

Manuscript 3. Light activation of the diphenylether herbicide - oxyfluorfen.

INTRODUCTION

Nitrofluorfen and oxyfluorfen control many grassy and broadleaf weed species when applied either pre or post emergence. An understanding of the mode of herbicidal action would help weed scientists to prescribe a more effective weed control from these herbicides than is obtained at present.

Three important physiological factors relating to herbicidal action are uptake, translocation and metabolism. Uptake determines the amount of herbicide present within the plant, translocation regulates the amount of herbicide which reaches the anatomical site of action, and metabolism usually inactivates the herbicide.

The herbicidal action of certain diphenyl ethers depends upon light, although the actual mechanism has not been reported. Since light activation controls herbicidal action, its mechanism must be understood before the mechanism of herbicidal action can be clearly explained.

Experiments were conducted to determine how the mode of action of nitrofluorfen and oxyfluorfen is affected by root uptake, translocation and metabolism by fababean and green foxtail (Manuscript 1). Further experiments dealt with the question of light activation (Manuscript 3) for which a special technique was adopted for evaluating herbicidal activity (Manuscript 2).

LITERATURE REVIEW

1. Diphenylether Herbicides

1.1 Introduction. Many diphenyl ethers have been tested for herbicidal properties. Bifenox (methyl 5-[2,4-dichlorophenoxy] -2-nitrobenzoate) (Kurger *et al.*, 1974), fluorodifen (p-nitrophenyl,α,α,α-trifluoro-2-nitro-p-tolyl ether) (Ebner *et al.*, 1968) and nitrofen (2,4-dichlorophenyl p-nitrophenyl ether) (Matsunaka, 1969) produce contact injury to newly emerged annual broadleaf and grassy weeds. Bifenox applied to the foliage of susceptible seedlings caused leaf necrosis within several hours (Kruger *et al.*, 1974). At sub-lethal dosages chlorotic leaf spots developed but did not have a persistent effect on subsequent plant growth. Pre-emergence treatments resulted in small necrotic lesions on the tissues which came in contact with the herbicide while emerging through the soil.

Chloroxuron [3-(p- p-chlorophenoxy phenyl)-1,1-dimethylurea] contains a diphenylether moiety and a urea moiety. It does not produce prominent contact injury (Feeny *et al.*, 1974) but actively inhibits the Hill reaction of photosynthesis (Moreland, 1969). This type of injury is typical of urea herbicides but not diphenylether herbicides, so chloroxuron will not be discussed in this literature review.

1.2 Uptake and Translocation. Fluorodifen has been reported to be absorbed by leaves (Walter *et al.*, 1970), cotyledons (Ebner *et al.*,

1968) and roots (Rogers, 1971; Eastin, 1969b; 1971b). Little absorption of fluorodifen by soybean (*Glycine max* L.) leaves was found unless the herbicide was applied in 1% surfactant or pure acetone (Walter *et al.*, 1970). Pereira (1970) studied the penetration of nitrofen into seedling cabbage (*Brassica oleracea* L.) leaves of a resistant cultivar, Hybelle, and a susceptible cultivar, Rio Verde. Hybelle plants had more wax per unit leaf surface than Rio Verde plants. The Rio Verde leaves absorbed ^{14}C-nitrofen twice as fast as the leaves of Hybelle plants. Plants of both cultivars grown in the shade had poor cuticle development and were more susceptible than plants grown in full sunlight. To test for penetration, he rubbed the leaves with glass wool to remove the cuticle; rubbing increased phytotoxicity and decreased selectivity. Leaf absorption of fluorodifen and nitrofen was apparently restricted by the cuticular barrier.

When fluorodifen was applied to soybean leaves, translocation was limited to an acropetal direction (Rogers, 1971; Walter *et al.*, 1970). Only traces of radioactivity were found in the stem after 24 and 48 hours, the rest remained in the treated leaf (Walter *et al.*, 1970).

Fluorodifen micro-droplets applied to soybean cotyledons caused a contact burning effect at the point of application (Ebner *et al.*, 1968). This contact action indicated that the herbicide was absorbed but not translocated.

Various studies report that fluorodifen was readily taken up from root treatments by resistant and susceptible species (Rogers, 1971; Eastin, 1969b; 1971b; Walter *et al.*, 1970). Resistant soybeans

progressively absorbed fluorodifen from 10 ppmw fluorodifen treatment (Rogers, 1971). The amount of herbicide per plant that was absorbed after 1, 2, 4, 8 and 16 days was 74.6, 136.4, 222.0, 500.1, and 874.6 µg, respectively.

Root-applied fluorodifen is translocated to a different extent with different species. In the resistant peanut (*Arachis hypogaea* L.) (Eastin, 1969) only 6.5% of the absorbed radioactivity was translocated to the shoot after 144 hours, with the acropetal movement being confined to the stem and petiole. In susceptible cucumber (*Cucumis sativus* L.) (Eastin, 1971) after only 24 hours 25% of the absorbed radioactivity was translocated from the roots to the stem, leaves and cotyledons. Thus immobility of the herbicide was associated with species resistance.

A small amount of root-absorbed fluorodifen was translocated to the stems in morning glory (*Ipomoea hederacea* (L.) Jacl.), grain sorghum (*Sorghum bicolor* Pers.), peanut and soybean, (Walter *et al.*, 1970). However, after 1 week, significantly greater concentrations of fluorodifen were found in the stems of morning glory (0.15 ppm fresh weight) and grain sorghum (0.22 ppm) than in the upper stems of peanut (0.03 ppm) and soybean (0.01 ppm). Since morning glory and grain sorghum are somewhat more sensitive to fluorodifen than are peanut and soybean seedlings, susceptibility of plant species to fluorodifen was correlated to the extent of translocation.

Differential retention and penetration of foliar-applied nitrofen could not account for selectivity between rapeseed (*Brassica campestris* L.), redroot pigweed (*Amaranthus retroflexus* L.) and green foxtail

(*Setaria viridis* (L.) Beauv.) (Hawton and Stobbe, 1971a). In fact, reduced retention on green foxtail and reduced penetration in redroot pigweed decreased the potential herbicidal effect to these two susceptible species. Differential translocation in these three species could not explain selectivity of nitrofen (Hawton and Stobbe, 1971b). Sixteen days after foliar treatment less than 2% of the ^{14}C of the applied ^{14}C-nitrofen was translocated from the treated leaf of any of the three species.

Soil-applied diphenyl ethers are not restricted to uptake by roots. Surface applications of bifenox gave better weed control than when bifenox was incorporated into the soil (Kruger *et al.*, 1974). Since it was not necessary to move bifenox into the root zone, its performance was satisfactory even when there was insufficient moisture for downward movement of the herbicide.

1.3 Mode of action. Light was necessary for bifenox to be herbicidally active against large crabgrass (*Digitaria sanguinalis* (L.) Scop.) (Kruger *et al.*, 1974). The plants died within four days when treated with 1.68 kg/ha of bifenox under light conditions. Plants kept in the dark were tolerant to bifenox.

The subject of light activation of diphenylether herbicides was pioneered by Matsunaka (1969). Having worked with a number of diphenyl ethers, he postulated that all ortho-substituted diphenyl ethers require light for herbicidal activity. He showed that normal green and mutant yellow rice seedlings were susceptible to nitrofen in light. Albino rice seedlings were tolerant to nitrofen in light. He suggested that xanthophylls, which were contained in the yellow

mutants in large amounts, were important in light activation of ortho-substituted diphenylether herbicides.

In support of Matsunaka's postulation, Fadayomi and Warren (1976) found that light was necessary for the herbicidal action of nitrofen and oxyfluorfen. An albino mutant of corn was much more resistant to the herbicides than a mutant greenish-yellow or a normal green seedling. A yellow mutant of soybean was equally as susceptible as the normal seedling. They suggested that yellow pigments were required for the light activation of nitrofen and oxyfluorfen.

Two general hypotheses of the mechanism for light activation of diphenyl ether herbicides have been made (Matsunaka and Shimabukuro, 1974). There may be a direct photo-transformation of the herbicide within the plant, yielding a toxic compound, or, there may be a specific physiological status present only under light conditions causing the plant to be injured by the parent compound.

In disagreement with other workers cited, Pereira *et al.* (1971) suggested that light was not required for nitrofen activity. They treated cabbage seedlings with nitrofen and then placed the plants in light immediately or in the dark for 3 days followed by light for 4 days. They visually rated plant injury at the end of 7 days and found that the plants which had been placed in the dark for 3 days prior to the light treatment were injured more than the plants which had been in the light for the 7-day period. Plants placed for 3 days in the dark, had been exposed to 4 days of light before herbicide injury was assessed, therefore, from this experiment it is not possible to determine whether nitrofen acts in the dark.

Diphenylethers act quickly by producing contact injury. Nitrofen

increased membrane permeability of red beet (*Beta vulgaris* L.) root sections and cabbage leaf sections (Pereira *et al.*, 1971). Increased membrane permeability could account for the rapid contact injury.

Moreland *et al.* (1970) determined the effect of three diphenylether herbicides, nitrofen, fluorodifen and MC 1478 (2,4,6-trichlorophenyl-4'-nitrophenyl ether), on phosphorylation and electron transport in spinach (*Spinacia oleracea* L.) chloroplasts and mung bean (*Phaseolus aureus* L.) and white potato (*Solanum tuberosum* L.) tuber mitochondria. The diphenylether herbicides acted primarily as inhibitors of chloroplast non-cyclic electron transport. At high concentrations marginal interference with cyclic electron transport occurred. In mitochondria, MC 1478 and nitrofen acted as electron transport inhibitors when malate, NADH and succinate were added as substrates. The authors suggested that interference with ATP generation could be one mechanism through which the phytotoxicity of diphenylether herbicides is expressed.

1.4 Herbicide Metabolism in Plants. Fluorodifen-1'-^{14}C metabolism occurred via similar pathways in soybean (Rogers, 1971), peanut (Eastin, 1969) and cucumber (Eastin 1971; 1972a), although metabolism was much slower in cucumber than in soybean or peanut (Eastin, 1971). The slow rate of metabolism in cucumber may partly explain why it is susceptible to fluorodifen while peanut and soybean are resistant.

The major metabolic pathway of fluorodifen was hydrolysis of the ether bond and subsequent conjugation with plant constituents. A minor pathway involved reduction of one of the nitro groups before hydrolysis of the ether bond (Rogers, 1971; Eastin, 1972a).

After cleavage of the ether bond was established to be the initial metabolic reaction of fluorodifen, Shimabukuro *et al.* (1973, 1976) demonstrated in peanut leaves the formation of the following conjugates: 5-(2-nitro-4-trifluoromethylphenyl)-glutathione; p-nitrophenyl-6-o-malonyl-β-D-glucoside; p-nitrophenyl-β-D-glucoside (1973) and S-(2-nitro-4-trifluoro-methylphenyl)-N-malonyl-cysteine (1976). These conjugates likely become part of an insoluble fraction which remains in the plant body for the duration of the plant life.

A soluble glutathione transferase enzyme has been isolated and characterized from pea (*Pisum sativum* L.) epicotyls (Frear and Swanson, 1973). This enzyme catalyzes the cleavage of the fluorodifen ether bond by a concerted nucleophilic displacement of p-nitrophenol and glutathione conjunction of the 2-nitro-4-trifluoromethyl-phenyl groups. Cleavage of fluorodifen by glutathione transferase was strongly inhibited by several structural analogs including nitrofen and 2-amino-4-nitro-4-trifluoromethylphenyl ether. Even though these compounds were not effective substrates for the enzyme, their structural similarities apparently resulted in a strong affinity for the enzyme active site.

Glutathione conjugates of fluorodifen were formed in excised roots, hypocotyls, epicotyls, leaves and callous tissues from several plant species (Frear and Swanson, 1973). Tissues from fluorodifen-tolerant plants [cotton (*Gossypium hirsutum* L.), corn (*Zea mays* L.), peanut, pea, soybean and okra (*Hibiscus esculentus* L.)] generally contained higher levels of glutathione transferase than similar tissues from susceptible species [tomato (*Lycopersicon esculentum* Mill.),

cucumber, and squash (*Cucurbita maxima* Ducheane)].

2. Experimental Techniques

2.1 Hydroponic Culture. Plants have been grown in nutrient solution without soil for many years (Hoagland and Arnon, 1938). This culture can accurately supply moisture and nutrients to plant roots and thus remove soil variables so that highly uniform plants may be grown for experimental purposes (Blackendaal *et al.*, 1972). Hydroponics also results in highly efficient growth so that intensive-care greenhouse crops are commercial adaptations (Schwarz, 1968).

Nutrient solution is prepared according to the formula of Hoagland and Arnon (1938) with a slight modification to a more soluble form of iron (Blackendaal *et al.*, 1972). The minerals included in the recipe are potassium, phosphorous, nitrogen, calcium, magnesium, sulfur, iron, boron, zinc, chlorine, molybdenum and manganese. One standard recipe is used for all plants. The solutions may be diluted to one-third, one-half or three-quarter strength according to the plant size and species to be grown.

When plants are grown in nutrient solution, their roots often deoxygenate the solution often below the plant's tolerance level. Air may be bubbled back into the solution by a small electric pump or compressed air. Alternatively, the solution may be changed two or three times per week (Blackendaal *et al.*, 1972).

2.2 Electrolytic Conductivity. The electrolytic conductivity method of assessing tissue injury makes use of the principle that plasmalemma of damaged tissues lose their selective permeability,

thus allowing movement of cellular components from the cell (Wright, 1974). If this tissue is placed in distilled water, the cellular electrolytes will increase the conductivity of the water. The conductivity change then measures the amount of electrolyte leakage and indexes the extent of tissue injury.

The electrolytic conductivity technique has been used widely for evaluating tissue damage caused by cold temperatures (Blazich *et al.*, 1974; Dexter *et al.*, 1932; Sukumaran and Weiser, 1972; Wright and Simon, 1973). The technique is relatively simple, quantitative and reproducible; its results are not confounded by a lengthy measuring procedure (Sukumaran and Weiser, 1972). A close association has been observed between conductivity and visual assessment of cold injury (Blazich *et al.*, 1974). Wright (1974) attributed increased conductivity to an increase in membrane permeability allowing electrolytes to leak from the cell. However, the technique has been limited to a direct comparison of similar tissues (Dexter *et al.*, 1932; Hudson, 1961) because differences in tissue structure, dry matter and electrolyte content will alter the extent of exosmosis.

In one report where conductivity measurements were used to measure herbicidal injury, leaf samples were floated and the conductivity was measured directly in the herbicide solution (Somabhi, 1975). After 14 hr, glyphosate [N-(phosphonomethyl)glycine] treatments, 50 μM and 100 μM, gave higher values than the water check. Under the conditions of that experiment glyphosate apparently caused membrane disruption.

Other weed workers (Price and Putnam, 1969; Sutton and Foy,

1971; Reid and Hurt, 1970; Pereira *et al.*, 1971a), although not using electrolytic conductivity, recognized that herbicides could cause an increase in membrane permeability. They measured the efflux of betacyanin from red beet root sections spectrophotometrically and utilized it as an index of membrane permeability.

2.3 Light - Its Measurement and Plant Responses. Light by definition is that part of the electromagnetic spectrum visible to the human eye (Webster, 1963). The physical characteristics of light have been thoroughly discussed at other times (Calvert and Pitts, 1966; Bickford and Dunn, 1972). Light is energy and the quantum energy of light is inversely related to its wavelength.

Light is most often measured in terms of illuminance, defined as the luminous flux per unit area (Downs and Hellmers, 1975). Illuminance is expressed as

lux (lx) = lumens/m^2 or

foot candles (ft-c) = lumens/sq ft. 1 ft-c = 10.76 lx.

The lumen therefore is a unit expressing photopic sensitivity to visual spectrum. Photopic sensitivity to wavelength change follows a nearly normal distribution with a maximum at 555 nm. Therefore, a relative luminous efficiency has been assigned for each wavelength with a maximum value of one at 555 nm. Light meters for measuring illuminance are equipped with filters to provide a spectral response as nearly like the standard curve as possible.

Illuminance measurements for any biological response other than photopic vision are not correct, because other responses do not have the same spectral sensitivity (Downs and Hellmers, 1975). They are

useful only to indicate relative energy at various distances or different times using the same kind of light source. Comparisons of light sources with different spectral quality would not be valid. Consequently, many investigators recommend total energy measurements as the only valid system. Several systems measuring irradiance, spectral irradiance or incident quanta have been used.

Light energy in the 400-700 nm range may be measured directly in $\mu W/cm^2$ or in langleys (g-cal/cm^2/min) with a thermopile. One langley per min equals 69,800 $\mu W/cm^2$ (Downs and Hellmers, 1975). Although instrument reads out in absolute energy units, it does not relate radiant flux density to plant growth because it receives infrared energy as far as 66 m. This long wavelength energy contributes little to plant growth, so plants may grow and respond differently under two spectrally different light sources of similar energy. In this respect fluorescent lighting is 4 times more efficient than incandescent lamps.

Another type of measurement used is to determine the incident quanta between 400 and 700 nm with uniform spectral sensitivity (Downs and Hellmers, 1975). This energy is referred to as photosynthetically active radiation, and the units used are microeinsteins/m^2/sec. This could be improved by including the radiation of 700-800 nm, which is implicated in the phytochrome system.

Spectroradiometers provide the most descriptive measurement. They measure the spectral irradiance of 400-800 nm and describe the energy level in the various effective regions. Their widespread use has been hindered by high cost.

Whether illuminance, irradiance, incident quanta or spectral irradiance are selected for measuring radiant flux density, instrumentation and measurement errors and inconsistencies do exist. For this reason, a full description of the light source should be included with any reported measurement.

Downs and Hellmers (1975) suggested four possible ways in which light energy absorbed by the plant may be dissipated. It may be (1) converted into heat; (2) reradiated as fluorescence; (3) used as the primary energy source to run an initial reaction upon which many subsequent reactions depend; (4) utilized to sensitize a reaction which then becomes susceptible to other kinds of light or to accelerate chemical reactions that would otherwise take place very slowly.

Bickford and Dunn (1972) summarize the specific plant responses to light. First, of fundamental importance, light causes photosynthesis. Second, of structural importance, light is needed during the synthesis of chlorophyll, anthocyanins, and carotenoids. Third, of regulatory importance, light is often implicated in connection with seed germination, seedling growth and development, flowering, phototropism, photoplasmic viscosity and modifications of "biological clocks".

A number of pigments occur in plants. Initially the green pigment of plants was recognized as the substance responsible for light absorption in photosynthesis, absorbing red and blue light but not green (Bidwell, 1974). However, there are a number of different plant pigments of various colors. Notably there are the blue and red anthocyanins, the yellow to brown xanthophylls and the orange to red carotenes (Chichester, 1972).

MANUSCRIPT 1

Root Uptake, Translocation and Metabolism of Nitrofluorfen and Oxyfluorfen by Fababean (*Vicia faba* L.) and Green Foxtail (*Setaria viridis* (L.) Beav.)

Abstract. Root uptake and translocation of nitrofluorfen [2-chloro-1-(4-nitrophenoxy)-4-(trifluormethyl)benzene] and oxyfluorfen [2-chloro-1-1(3-ethoxy-4-nitrophenoxy)-4-(trifluormethyl)benzene] was measured in fababean and green foxtail plants, and metabolism was studied using fababean leaf discs and green foxtail leaf segments. Both herbicides were taken up readily from nutrient solution but translocation was limited. In fababean, 4.6% of the nitrofluorfen label was translocated to the shoot as compared to 2.2% of the oxyfluorfen labe. Less than 10% of nitrofluorfen or oxyfluorfen taken up in leaf discs on excised leaves was metabolized after 24 hr. Metabolism did not influence the herbicidal action of either herbicide.

INTRODUCTION

Nitrofluorfen and oxyfluorfen control many broadleaf and grassy weed species when applied preemergence or postemergence (Rohm and Haas, 1973a; 1973b).

Fadayomi and Warren (1977) found limited translocation of oxyfluorfen from the roots to the shoots of sorghum (*Sorghum bicolor* (L.) Moench.) or pea (*Pisum sativum* L.). Similar results were found with fluordifen (p-nitrophenyl α,α,α-trifluoro-2-nitro-p-tolyl ether) where herbicide uptake by the roots was rapid, but translocation to the shoots was limited (Eastin, 1969; 1971; Walter *et al.*, 1970).

Degradation of diphenylether herbicides occurs via several pathways. A major pathway shown for fluorodifen is hydrolysis of the ether linkage and subsequent conjugation with plant constituents

(Eastin, 1969; 1971; Rogers, 1971), some of which have been characterized by Shimabukuro *et al.* (1973). Nitrofen (2,4-dichlorophenyl-p-nitrophenyl ether), unlike fluorodifen, was not cleaved at the ether bond, but ring cleavage occurred (Hawton and Stobbe, 1971).

The present study was undertaken to compare the rate, extent, and significance of root uptake, translocation, and metabolism of nitrofluorfen and oxyfluorfen by fababean, a broadleaf species, and green foxtail, a grass species.

MATERIALS AND METHODS

Root Uptake and Translocation. Fababean and green foxtail plants were grown from seed in silica sand. On alternate days the sand was soaked with half-strength Hoagland's nutrient solution and allowed to drain. The plants were grown throughout the experiment in a growth chamber maintained at 25C day and 15C night temperatures and 60% relative humidity. Light was supplied using Sylvania Gro-lux WS fluorescent tubes at an intensity of 4500 $\mu W/cm^2$ during a 16-hr photoperiod.

Eighteen days after planting, when green foxtail was in the 4-5 leaf stage and fababeans were in the 3 leaf stage, individual seedlings were transferred to aluminum foil-covered bottles and grown in half-strength Hoagland's nutrient solution until treatment 3 days later. Plants were transferred to 16 x 150 mm test tubes containing ^{14}C-herbicides and treated for 8 hr. They were then transferred to unlabelled herbicide solutions for 16 hr. All treatments were prepared in 0.1% v/v formulation blank and half-strength nutrient

solution (TABLE 1).

At the end of the 24-hr treatment the roots of all plants were washed in distilled water. Plants were then either pressed and frozen in preparation for autoradiography or returned to fresh nutrient solution for another 4 days before they were prepared for autoradiography.

The amount of herbicide taken up by the roots was determined by measuring the amount of radioactivity remaining in the treatment solutions through liquid scintillation spectrophotometry and then subtracting this value from the amount originally applied.

Whole plants were autoradioagraphed using Kodak no screen X-ray film (Yamaguchi and Crafts, 1958). The plants were maintained under frozen conditions throughout the autoradiography period to overcome the possibility of spurious results caused by uneven drying.

A quantitative assessment of translocation was obtained by combusting plant shoots and roots using a liquid combustion method (Claus and Behrens, 1976). Each dried sample (30 mg) was digested in a closed container by 20 ml of concentrated H_2SO_4 and 10 ml of 0.05 M $Na_2Cr_2O_7 \cdot 2\ H_2O$ swirled together. The resultant CO_2 was trapped by 1 ml of methyl cellosolve-ethanolamine (1:1 v/v) and assayed using liquid scintillation spectrophotometry.

Metabolism. Fababean and green foxtail plants were grown in a soil mixture (clay loam : sand : peat/2:1:1) to the 2 leaf stage and 4-5 leaf stage, respectively. Growing conditions were maintained at 25C day and 15C night temperatures. Light was supplied using Sylvania cool white fluorescent tubes at an intensity of 2550 $\mu W/cm^2$

TABLE 1. Herbicide treatments for the root uptake and translocation study

Treatment		
nitro ring-^{14}C-nitrofluorfen	1 ppm	(sp. act. 3.18 μCi/mg)
	5 ppm	(sp. act. 1.18 μCi/mg)
nitro ring-^{14}C-oxyfluorfen	1 ppm	(sp. act. 2.35 μCi/mg)
	5 ppm	(sp. act. 1.10 μCi/mg)
$^{14}CF_3$-oxyfluorfen	1 ppm	(sp. act. 3.30 μCi/mg)
	5 ppm	(sp. act. 1.13 μCi/mg)

during a 16-hr photoperiod.

Leaf discs, 8 mm in diameter, from the fababean first leaf and the complete third leaf blade of green foxtail were used in the metabolism study. Triplicate samples, each containing 20 leaf discs or leaf blades, were taken to test the rate of herbicide metabolism in both species (treatment list, TABLE 2).

The ^{14}C-herbicides were dissolved in 0.35 M mannitol and vacuum infiltrated into freshly cut leaf samples. The treated samples were removed from the treatment emulsions after 15 min and incubated in petri dishes at 25C in constant light (Sylvania cool white fluorescent and incandescent at an intensity of 3800 $\mu W/cm^2$) for 4 or 24 hr. The samples were then frozen and extracted with acetone. The herbicides were separated from their metabolites by thin-layer-chromatography (TLC) with silica gel plastic-backed plates[4] using a solvent system of benzene : isopropyl ether (2:1). The ^{14}C-compounds were located on the chromatogram (nitrofluorfen, R_f = 0.60; oxyfluorfen, R_f = 0.55; unidentified metabolites, R_f = 0) and quantified by liquid scintillation spectrophotometry.

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4. Baker-Flex silica gel 1B2-F.

TABLE 2. Herbicide treatments for the metabolism study

Treatment		
nitro ring-^{14}C-nitrofluorfen	10^{-6} M	(sp. act. 3.43 μCi/mg)
nitro ring-^{14}C-nifrofluorfen	10^{-5} M	(sp. act. 3.43 μCi/mg)
nitro ring-^{14}C-oxyfluorfen	10^{-6} M	(sp. act. 2.61 μCi/mg)
nitro ring-^{14}C-oxyfluorfen	10^{-5} M	(sp. act. 2.61 μCi/mg)
$^{14}CF_3$-oxyfluorfen	10^{-6} M	(sp. act. 3.84 μCi/mg)
$^{14}CF_3$-oxyfluorfen	10^{-5} M	(sp. act. 3.84 μCi/mg)

RESULTS AND DISCUSSION

Root Uptake and Translocation. The results of the root uptake study indicate that all treatments of nitrofluorfen and oxyfluorfen were readily taken up by fababean and green foxtail (TABLE 3). Rogers (1971), Eastin (1969; 1971) and Walter *et al.* (1970) found similar results using fluorodifen.

Uptake was affected by concentration of herbicide. At 1 ppm, uptake of nitrofluorfen by fababean was 9.2 µg/plant compared to 35.5 µg/plant at 5 ppm. The uptake of nitrofluorfen was similar to the uptake of oxyfluorfen by both species and at both concentrations. The maximum amount of applied herbicide taken up from any treatment was 50%, so lack of herbicide availability was not a factor in uptake. The approximate 4-fold increase in uptake resulting from a 5-fold increase in concentration suggests that the uptake of nitrofluorfen and oxyfluorfen may be passive.

Autoradiographs of fababean and green foxtail showed no label movement from the roots to the shoots, as illustrated by the nitrofluorfen 5 ppm treatment (FIGURE 1). Five days after treatment the plants showed necrotic leaf spots. The herbicide effect may have been caused because the herbicide was translocated in micro quantities or some unlabelled herbicidally-active identity was translocated to the leaves.

After being autoradiographed, the plants were assessed quantitatively for herbicide translocation. Acropetal movement of ^{14}C

TABLE 3. Root uptake of nitrofluorfen and oxyfluorgen by fababean and green foxtail

Treatment		µg/plant	
		Fababean	Green foxtail
^{14}C-nitrofluorfen	1 ppm	9.2 w	2.1 y
^{14}C-nitrofluorfen	5 ppm	35.5 x	11.1 z
^{14}C-oxyfluorfen	1 ppm	9.4 w	1.6 y
^{14}C-oxyflourfen	5 ppm	34.4 x	6.5 yz

Uptake values followed by the same letter do not differ significantly according to Duncan's Multiple Range (0.05).
Nitro ring-^{14}C and $^{14}CF_3$ values were averaged for oxyfluorfen.

FIGURE 1. Translocation of ^{14}C from ^{14}C-nitrofluorfen root treatment in fababean after 1 day (A) and 5 days (B) and in green foxtail after 1 day (C) and 5 days (D). Treated plant - left; autoradiograph - right

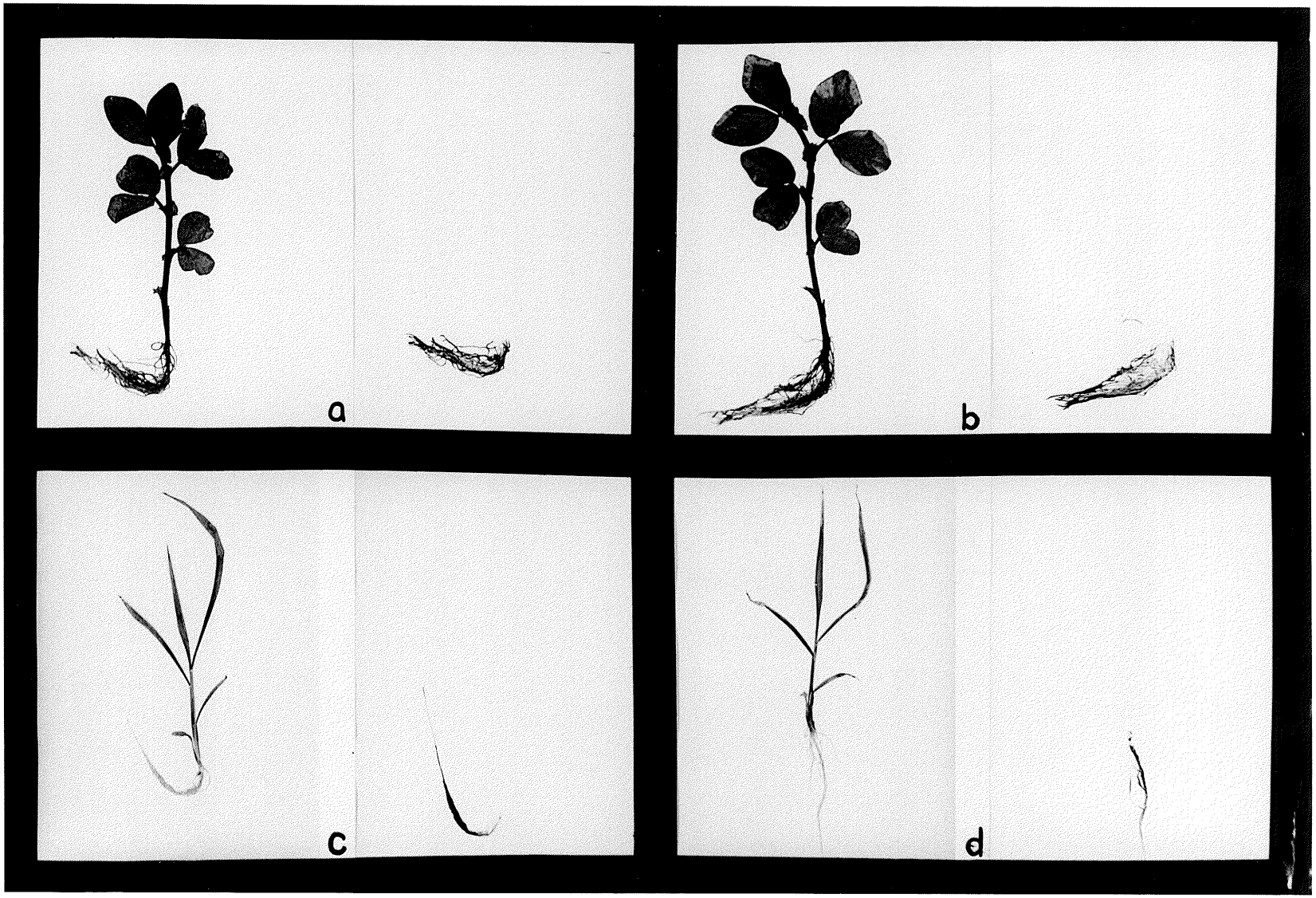

a
b
c
d

TABLE 4. Percent translocation of ^{14}C-nitrofluorfen and oxyfluorfen label from fababean roots

Treatment		Percent translocation 1 day	Percent translocation 5 days	
nitro ring-^{14}C-nitrofluorfen	1 ppm	2.2	5.6	
nitro ring-^{14}C-nitrofluorfen	5 ppm	2.1	8.4	
	mean			4.6
nitro ring-^{14}C-oxyfluorfen	1 ppm	1.2	3.6	
nitro ring-^{14}C-oxyfluorfen	5 ppm	0.7	3.2	
	mean			2.2
$^{14}CF_3$-oxyfluorfen	1 ppm	1.7	3.3	
$^{14}CF_3$-oxyfluorfen	5 ppm	3.4	3.2	
	Overall mean	1.9	4.6	

from ^{14}C-nitrofluorfen or ^{14}C-oxyfluorfen was slight (TABLE 4). Overall, 1.9% was translocated after 1 day and 4.6% after 5 days. During the first day more translocation occurred than on succeeding days, suggesting that the label must become unavailable for translocation.

The average translocation of all the nitro ring-^{14}C-nitrofluorfen label was compared to the average translocation of all nitro ring-^{14}C-oxyfluorfen label and was found to be 4.6% and 2.2% with respective herbicides (L.S.D. (0.05) = 2.1%). After 5 days, 8.4% of the nitro ring-^{14}C-nitrofluorfen label was translocated to the shoot. This amount is more than any oxyfluorfen treatments. It may be concluded that nitrofluorfen is translocated to a greater extent than oxyfluorfen.

Limited translocation reported here agrees with Fadayomi and Warren (1977) who found that neither sorghym (*Sorghum bicolor* (L.) Moench.) or pea (*Pisum sativum* L.) translocated oxyfluorfen to any extent.

Metabolism. The metabolism of nitrofluorfen and oxyfluorfen was not extensive (TABLE 5). There were no differences between herbicides or species. Only 0.7% and 0.9% of the herbicide within the fababean and green foxtail leaf samples, respectively, was metabolized after 4 hours. A similar extent of metabolism is reported for time 0 (0.3% and 0.5%, respectively), so perhaps there was a small amount of extrabiological degradation from technical procedures. Tissue injury was beginning to occur at 4 hours (visual assessment). Injury progressed to complete destruction by 24 hours, at which time there was less than 8% metabolism. It may be safely concluded that metabolism

TABLE 5. Percentage metabolism of nitro ring-^{14}C-nitrofluorfen, nitro ring-^{14}C-oxyfluorfen and $^{14}CF_3$-oxyfluorfen by fababean leaf discs and green foxtail leaf segments after 0, 4 and 24 hours[1]

Treatment	Fababean			Green foxtail		
	0 hr	4 hr	24 hr	0 hr	4 hr	24 hr
nitro ring-^{14}C-nitrofluorfen	0.9	0.6	4.9	0.1	0.8	5.4
nitro ring-^{14}C-oxyfluorfen	0.1	1.1	4.1	0.8	0.7	5.8
$^{14}CF_3$-oxyfluorfen	0.0	0.5	7.8	0.5	1.2	4.0
Mean[2]	0.3a	0.7a	5.6b	0.5a	0.9a	5.1b

1. Effect of herbicide rates was not significant. Individual reported values include 10^{-5} M and 10^{-6}M.
2. Values followed by different letters differ significantly according to Duncan's Multiple Range (0.05).

of nitrofluorfen and oxyfluorfen did not inhibit herbicidal action.

There was no difference between the percentage metabolism of nitro ring-^{14}C-labelled and $^{14}CF_3$-labelled oxyfluorfen. Oxyfluorfen labels were retained indiscriminately at the origin of the TLC plates. This would occur if the ether bond was left intact or if the metabolites formed by cleavage of the ether bond were quickly conjugated to plant constituents. Whether or not ether bond cleavage occurred, the fact that the metabolites remained at the origin of the TLC plate suggests that conjugation with plant constituents occurred. This hypothesis is strengthened by reports in the literature of diphenylether conjugation (Eastin, 1969; Kruger *et al.*, 1974; Shimabukuro *et al.*, 1973).

ACKNOWLEDGMENTS

The authors wish to thank Rohm and Haas Canada Ltd. for financial support and for the supply of technical and radioactive herbicides.

Thanks is extended to Mr. Jack Menzies for help with statistical analysis.

MANUSCRIPT 2

Electrolytic Conductivity - A Rapid Measure of Herbicide Injury

Abstract. The use of electrolytic conductivity as a measure of cell membrane disruption was tested on buckwheat (*Fagopyrum esculentum* Moench. cv. 'Tokoyo') plants which had been sprayed with paraquat (1,1'-dimethyl-4,4'-bipyridinium ion) or oxyfluorfen [2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl)benzene]. All treatments which resulted in visible tissue damage significantly increased conductivity of solutions where the discs were floated. The highest conductivity measurement (most membrane disruption) was obtained from paraquat. The highest concentration for each herbicide gave higher conductivity measurements than lower concentrations. A difference in the pattern of conductivity change induced by the two chemicals implies a different mode of action of each.

INTRODUCTION

The electrolytic conductivity technique has been widely used for evaluating tissue damage caused by cold temperature (Blazich *et al.*, 1974; Dexter, 1956; Sukumaran and Weiser, 1972; Wright, 1974). The technique is relatively simple, quantitative, and reproducible, and its results are not confounded by a lengthy measuring procedure (Sukumaran and Weiser, 1972). A close association has been observed between conductivity and visual assessment of cold injury (Blazich *et al.*, 1974). Wright (1974) attributed increased conductivity to an increase in membrane permeability allowing electrolytes to leak from the cell. The technique has been limited to a direct comparison

of similar tissues (Dexter, 1956; Hudson, 1961) because differences in tissue structure, dry matter, and electrolyte content will alter the extent of exosmosis.

Although conductivity measurements have been used extensively to determine tissue damage caused by cold temperatures, there have been no reports on the use of this technique to measure herbicidal injury. The present study was undertaken to determine if conductivity could be used to measure herbicidal injury.

MATERIALS AND METHODS

Plant Material. Buckwheat plants were grown from seed in 10 cm pots containing a mixture of clay : sand : peat moss (2:1:1). All plants were started in the greenhouse and then transferred to a growth cabinet 10 days after seeding. The temperature in the greenhouse was 21C ± 6C. A 16-hr photoperiod was maintained using cool white fluorescent tubes supplying 2700 $\mu W/cm^2$ at the top of the plant canopy. In the growth cabinet, plants were grown at 25C day/15C night temperatures with a 60% relative humidity. Light was supplied by Sylvania Gro-lux WS fluorescent tubes at 5000 $\mu W/cm^2$ for a 16-hr photoperiod. Herbicide treatments were applied 17 days after seeding when the plants had two leaves.

Herbicide Treatments. Plants were sprayed using a pot sprayer. The spray nozzle delivered 130 L/ha at 2.55 kg/cm^2 pressure. Herbicide treatments applied were paraquat at 10, 100, and 1000 g/ha and oxyfluorfen at 1, 10, 100 and 1000 g/ha.

Sampling Technique. At sampling time six leaf discs were cut from the first leaf of 12 sample plants with a #4 cork borer having 8.5 mm diameter. Cutting across major veins was avoided to minimize the conductivity contributed by cut surface. Samples were taken until the tissue was so damaged that it could not be handled.

Conductivity Measurements. After cutting, leaf discs were put into 10.0 ml of double distilled water and placed in a shaking water bath at 25C for 30 min. Electrolyte leakage was then measured with a CDC 114 Radiometer conductivity cell (K = 0.67 cm) connected to a conductivity bridge.

RESULTS AND DISCUSSION

Visual symptoms caused by paraquat toxicity appear to be closely associated with increases in electrolytic conductivity (TABLE 6). Intracellular electrolytes move from the cell because of a loss of membrane integrity. A significant increase in conductivity was observed when there was only slight visual injury (light green mottling of leaf surface) which suggests that membrane integrity is affected early by paraquat action. The conductivity increased with increased visual injury until a conductivity endpoint was obtained when the tissue became completely flaccid (severe injury). At that point the flaccid nature of leaf structure made further conductivity measurements difficult. Conductivity endpoints established for completely flaccid tissue were different from treatment to treatment. This suggests that loss of integrity involves a selective loss of cellular electrolytes possibly by some effect on ion pumps.

TABLE 6. Visual injury and conductivity changes with time in buckwheat plants treated with paraquat

	Paraquat 100 g/ha		Paraquat 1000 g/ha	
Visual injury	Time after spraying (hr)	Conductivity (μmho/cm)	Time after spraying (hr)	Conductivity (μmho/cm)
none	0	37.8	0	35.1
slight	6	46.8	3	40.1
			4	59.6
severe	8	51.9	5	79.2

Electrolytic conductivity comparisons were made between paraquat and oxyfluorfen. Both chemicals act as contact herbicides and require light for activation (Matsunaka and Shimabukuro, 1974; Mees, 1960). The conductivity results presented in Figures 2 and 3 indicate that both herbicides cause a loss of membrane integrity when applied at concentrations which cause tissue injury.

Paraquat has been reported (Black, 1965) to intercept electrons from photosystem I and untimately cause membrane disruption through hydrogen peroxide activity. In our study, paraquat at 100 g/ha and 1000 g/ha caused membrance disruption as measured by conductivity change (FIGURE 2). At both concentrations significant membrane disruption occurred when the tissue showed only slight injury. Paraquat affects cell membranes early in its action. The herbicidal effect on membrane integrity was more pronounced and occurred more quickly at 1000 g/ha than at 100 g/ha. At 1000 g/ha membrane disruption was six times as much during the final 2 hr of tissue injury and ultimately three times more pronounced than at 100 g/ha. The extent of membrane disruption was dependent on the amount of paraquat applied.

When paraquat was applied at a sub-lethal concentration of 10 g/ha, significant conductivity decreases were observed at 6 hr and 8 hr. Diurnal effects could not account for the decrease in conductivity since untreated plants showed no diurnal variability. The decreased conductivity may have been caused by cellular uptake of ions from intercellular spaces, decreased membrane leakage or metabolic shift whereby less electrolytes were present.

FIGURE 2. Electrolytic conductivity change relative to untreated controls of buckwheat at several times after spraying with paraquat

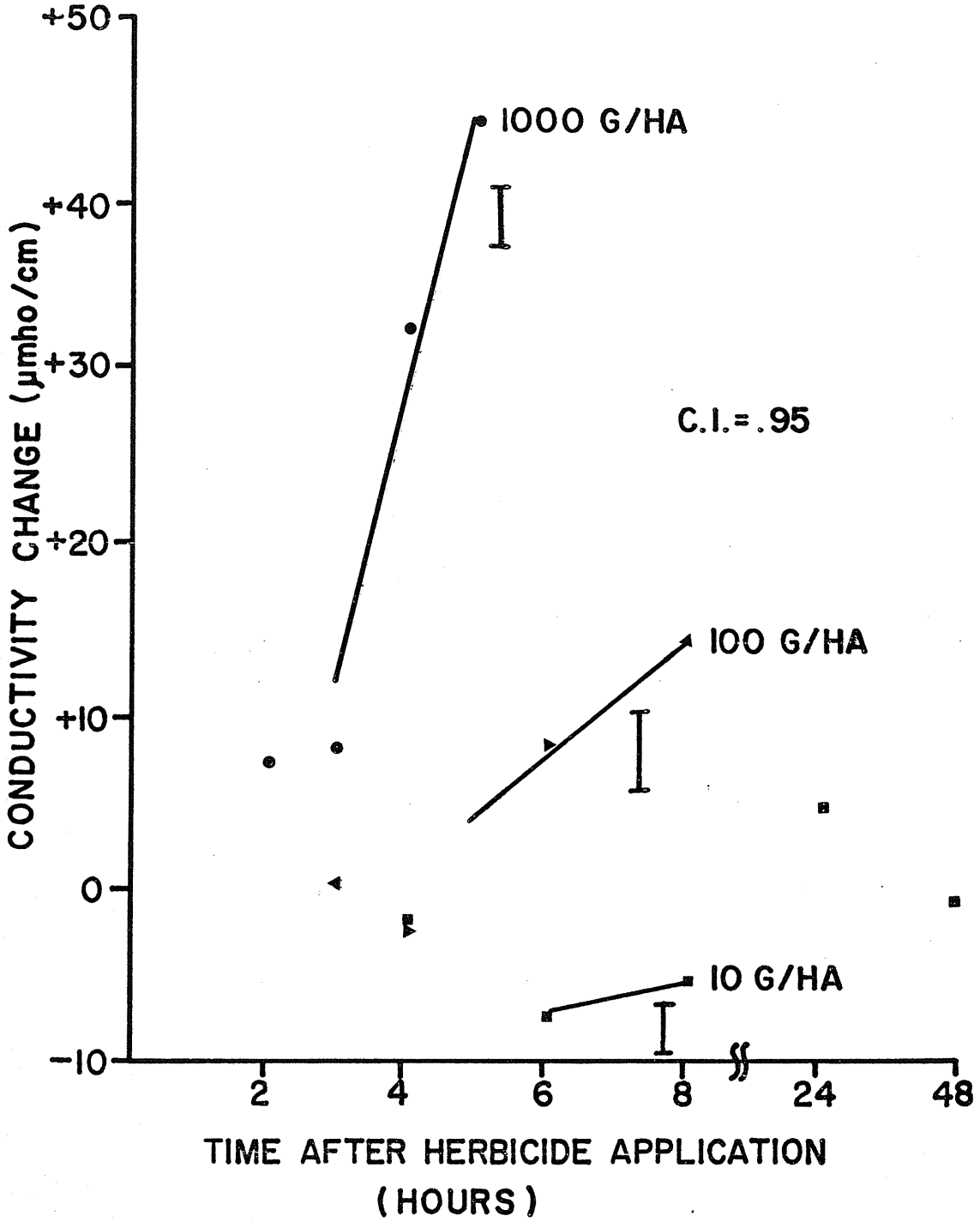
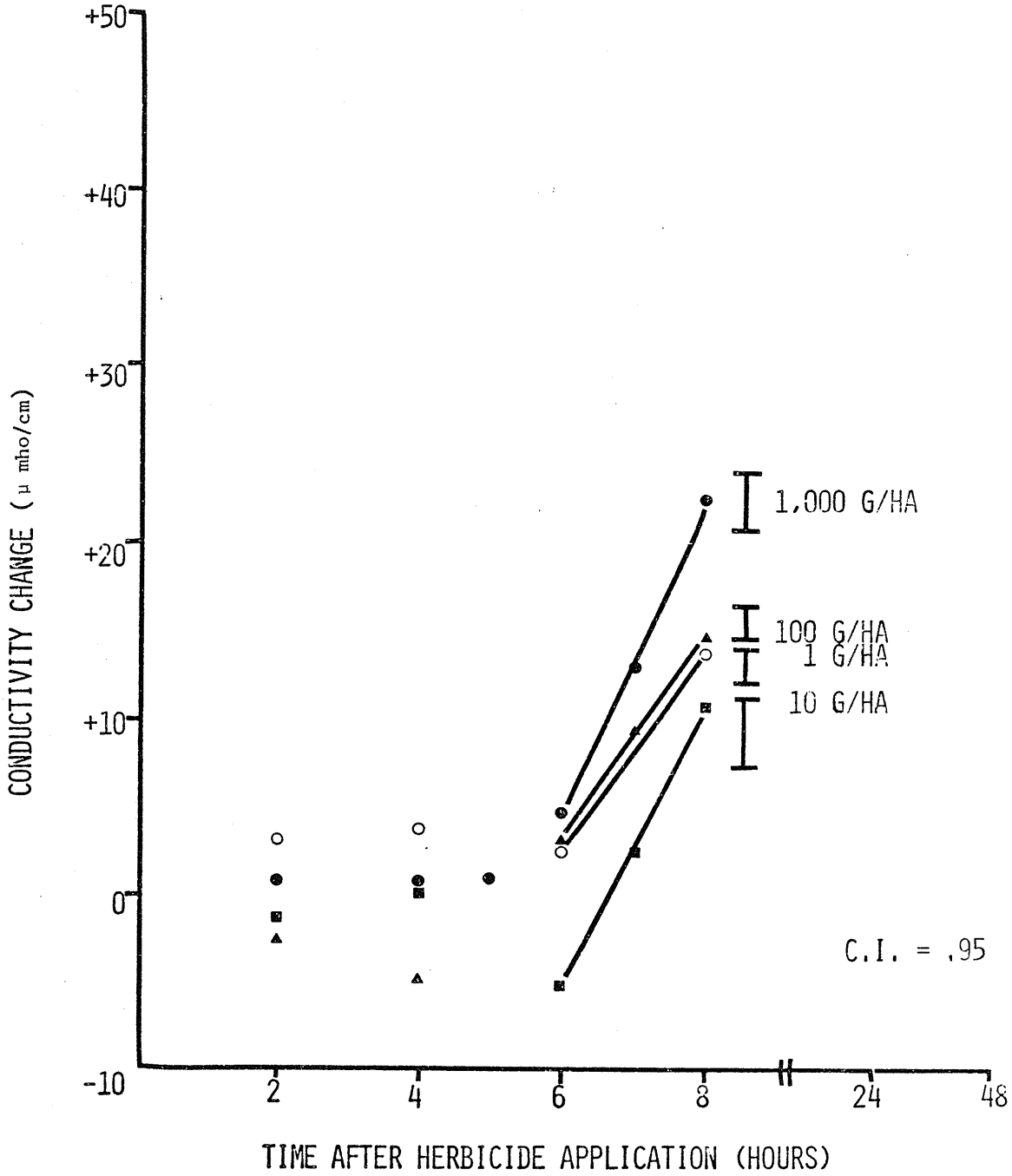

+50
+40
+30
+20
+10
0
-10
CONDUCTIVITY CHANGE (μmho/cm)
1000 G/HA
C.I.=.95
100 G/HA
10 G/HA
2
4
6
8
24
48
TIME AFTER HERBICIDE APPLICATION
(HOURS)

FIGURE 3. Electrolytic conductivity change relative to untreated control of buckwheat at several times after spraying with oxyfluorfen


+50
+40
+30
+20
+10
0
-10
CONDUCTIVITY CHANGE (μ mho/cm)
2
4
6
8
24
48
TIME AFTER HERBICIDE APPLICATION (HOURS)
1,000 G/HA
100 G/HA
1 G/HA
10 G/HA
C.I. = .95

The mode of action of oxyfluorfen has not been reported. Nitrofen (2,4-dichlorophenyl-p-nitrophenyl ether), an analog of oxyfluorfen, has been shown to affect membrane permeability (Hawton and Stobbe, 1971b, Pereira, 1970). Figure 3 shows that oxyfluorfen caused membrane disruption when applied at concentrations from 1 to 1000 g/ha. All treatments required 8 hr to produce severe injury. During the last 2 hr, the treatments of 1, 10, 100, and 1000 g/ha caused conductivity increases of 5.5, 7.9, 5.7, and 8.4 μmho/cm/hr, respectively. The conductivity increases caused by these same treatments was 13.4, 10.4, 14.2, and 21.3 μmho/cm, respectively.

When oxyfluorfen concentration increased 1000-fold, the final conductivity endpoints hardly doubled. With paraquat a 10-fold concentration increase caused the conductivity endpoint to triple. The difference in conductivity response between the two herbicides implies a different mode of action for paraquat and oxyfluorfen.

ACKNOWLEDGMENT

The authors wish to thank Rohm and Haas, Canada for giving financial assistance and supplying the oxyfluorfen for this study.

MANUSCRIPT 3

Light Activation of the Diphenylether Herbicide - Oxyfluorfen

Abstract. Herbicidal activity of foliar-applied oxyfluorfen [2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl)benzene] was light dependent in buckwheat (*Fagopyrum esculentum* Moench. cv. 'Tokoyo'). Plants were not injured when placed in the dark for as long as 4 days after herbicide treatment. When these plants were brought to the light, injury occurred, albeit more slowly than when plants were placed in the light immediately after treatment. The rate of injury increased as light intensity increased. The most effective wavelength light was 565 to 615 nm, suggesting the involvement of a pigment with its absorption spectrum in this region. Chlorophyll content was not reduced by oxyfluorfen. Preliminary evidence suggests that photosynthesis was affected only after membrane integrity was disrupted.

INTRODUCTION

Working with a number of diphenylether herbicides, Matsunaka (1969) postulated that ortho and/or para-substituted diphenyl ethers required light for activation. Normal green and mutant yellow rice (*Oryza sativum* L.) seedlings were susceptible to nitrofen (2,4-dichlorophenyl-p-nitrophenyl ether) in light, while natural albino and artificial albino rice seedlings were tolerant. Xanthophylls, which were contained in the yellow mutants in large amounts, seem to act as the acceptors of light energy for photoactivation.

When nitrofen or oxyfluorfen were applied to albino soybean (*Glycine max* L.) seedlings no injury occurred. Mutant yellow and normal green corn (*Zea mays* L.) and soybean seedlings were injured in the light, but not in the dark (Fadayomi and Warren, 1976).

Two possible mechanisms concerning the light requirement for phytotoxicity are considered (Matsunaka and Shimabukuro, 1974). A direct photo-transformation of the chemical within the plant may yield a toxic compound or a specific physiological status present only under light conditions may cause the plant to be injured by the parent compound.

The objective of this study was to determine the nature of the light requirement for the activity of oxyfluorfen.

MATERIALS AND METHODS

Plant Material. Buckwheat plants cv. 'Tokoyo' were grown in 100 mm-diameter pots containing a soil mixture of clay : sand : peat (2:1:1). The plants were maintained in a growth room at a 16-hr photoperiod with 25C day and 15C night temperatures. Light intensity was 2500 $\mu W/cm^2$ at pot level[6].

Effect of Light on Herbicidal Activity. The plants were sprayed and then either returned to the growth room (2500 $\mu W/cm^2$), placed in a 25C dark chamber or measured immediately for herbicidal injury. After 7 hr, the plants in the light were measured for herbicidal injury. At 24, 48, 72 and 96 hr, plants were removed from the dark

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[6]Sylvania Gro-Lux WS fluorescent tubes.

and herbicidal injury was measured either immediately or after 7 hr in the light.

Detection of Herbicidal Activity. Herbicidal activity was measured using the electrolytic conductivity method (Vanstone and Stobbe, 1977a). Twelve samples were taken at sampling time by cutting six leaf discs from the first leaf of 12 plants with a #4 cork borer having 8.5 mm diameter. After cutting, sets of 6 leaf discs were put into a plastic beaker containing 10.0 ml of double-distilled water and placed in a shaking water bath at 25C for 30 min. Electrolyte leakage was then measured with a conductivity cell[7] (K = 0.67) connected to a conductivity bridge.

Conductivity values were obtained from untreated controls, indicating that ions leaked into the water across uninterrupted membranes or from the cut surfaces of the leaf discs. Similar conductivity values were obtained from treated samples taken immediately after spraying. A conductivity increase from untreated controls ($+\Delta C$) indicates membrane disruption. Herbicidal injury is expressed directly as conductivity change from untreated controls ($+\Delta C$) (FIGURE 5), or as conductivity (FIGURES 4, 6, 7 and 8) from which the conductivity value of the control must be subtracted.

Effect of Light Intensity on Herbicidal Activity. Plants were sprayed, then placed in a growth chamber at 25C, 60% relative humidity. The light intensity at the plant surface was varied by using 1, 2, or

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[7]Radiometer CDC 114

FIGURE 4. Electrolytic conductivity obtained from buckwheat at several times after spraying with oxyfluorfen. Some plants were placed in the dark (solid line); others were placed in the light (dashed line).

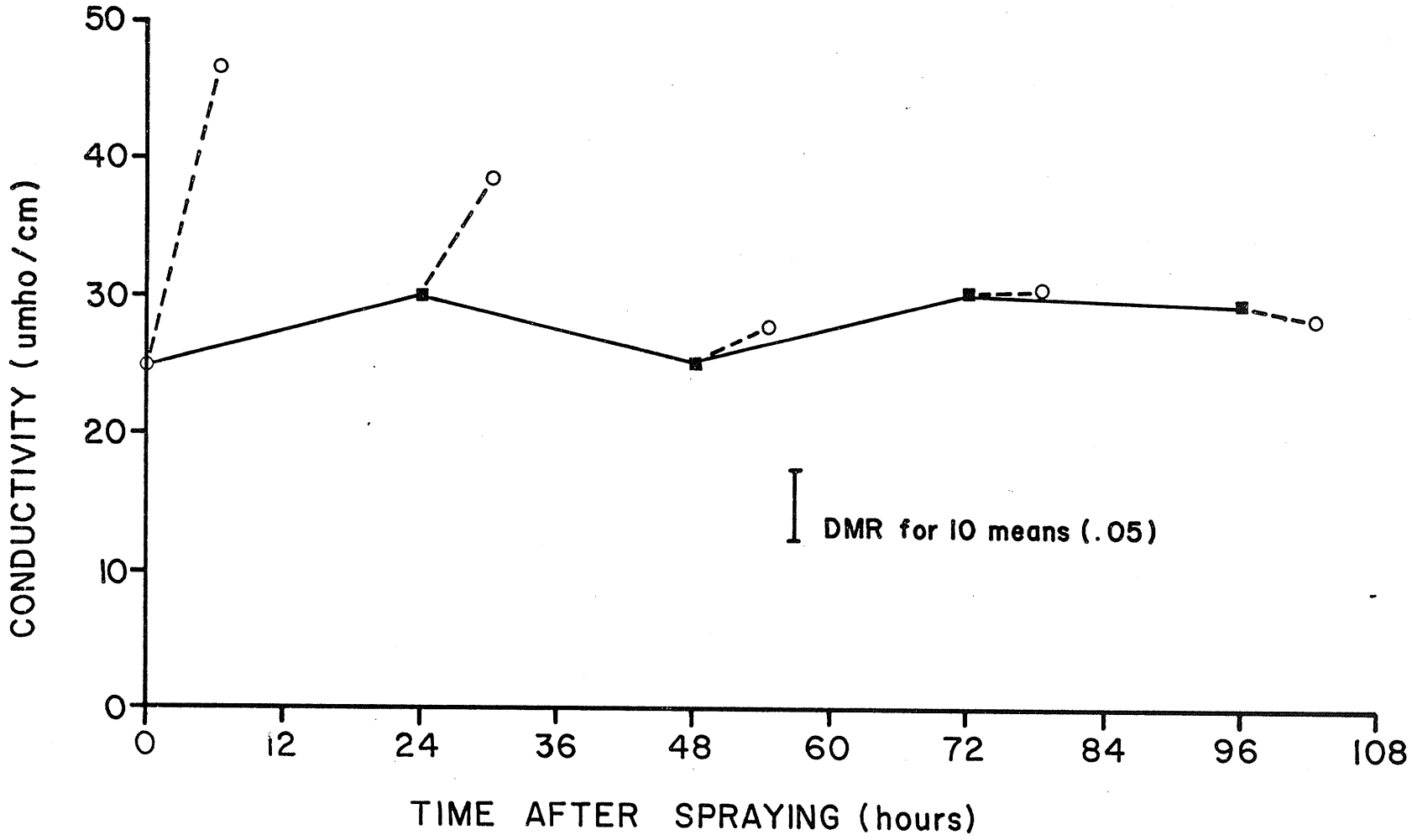

50
40
30
20
10
0
CONDUCTIVITY (umho/cm)
DMR for 10 means (.05)
0
12
24
36
48
60
72
84
96
108
TIME AFTER SPRAYING (hours)

FIGURE 5. Electrolytic conductivity change relative to untreated controls of buckwheat at 4, 6, 8 or 12 hr after foliar treatment with oxyfluorfen. The plants were at the following light intensities after spraying; 500 $\mu W/cm^2$ (○——○), 1000 $\mu W/cm^2$ (□——□), 2000 $\mu W/cm^2$ (▲——▲) and 6000 $\mu W/cm^2$ (△——△).

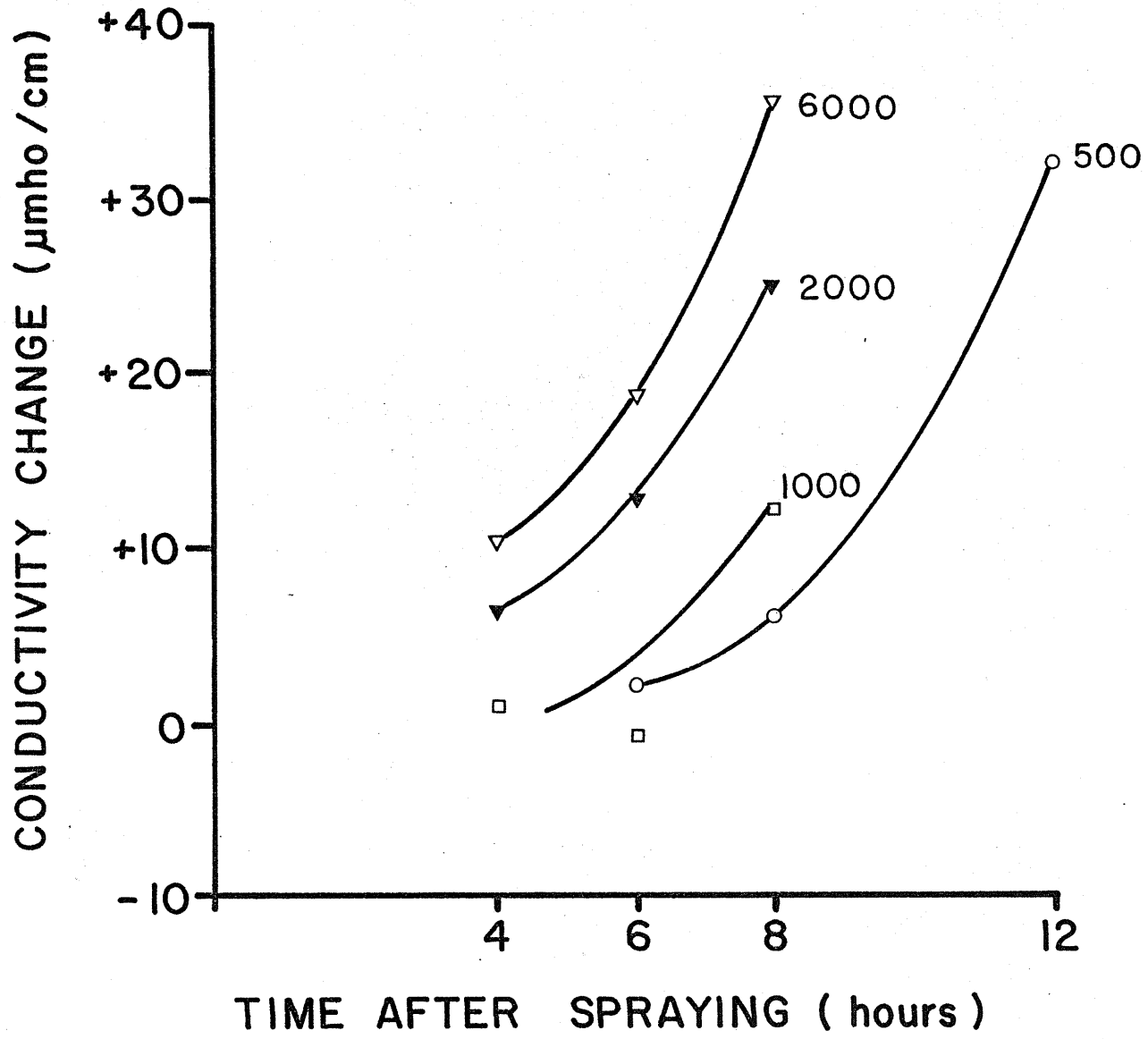

+40
+30
+20
+10
0
-10
CONDUCTIVITY CHANGE (μmho/cm)
6000
2000
1000
500
4
6
8
12
TIME AFTER SPRAYING (hours)

FIGURE 6. Electrolytic conductivity obtained from buckwheat irradiated at several light intensities for 8 hours after spraying with oxyfluorfen

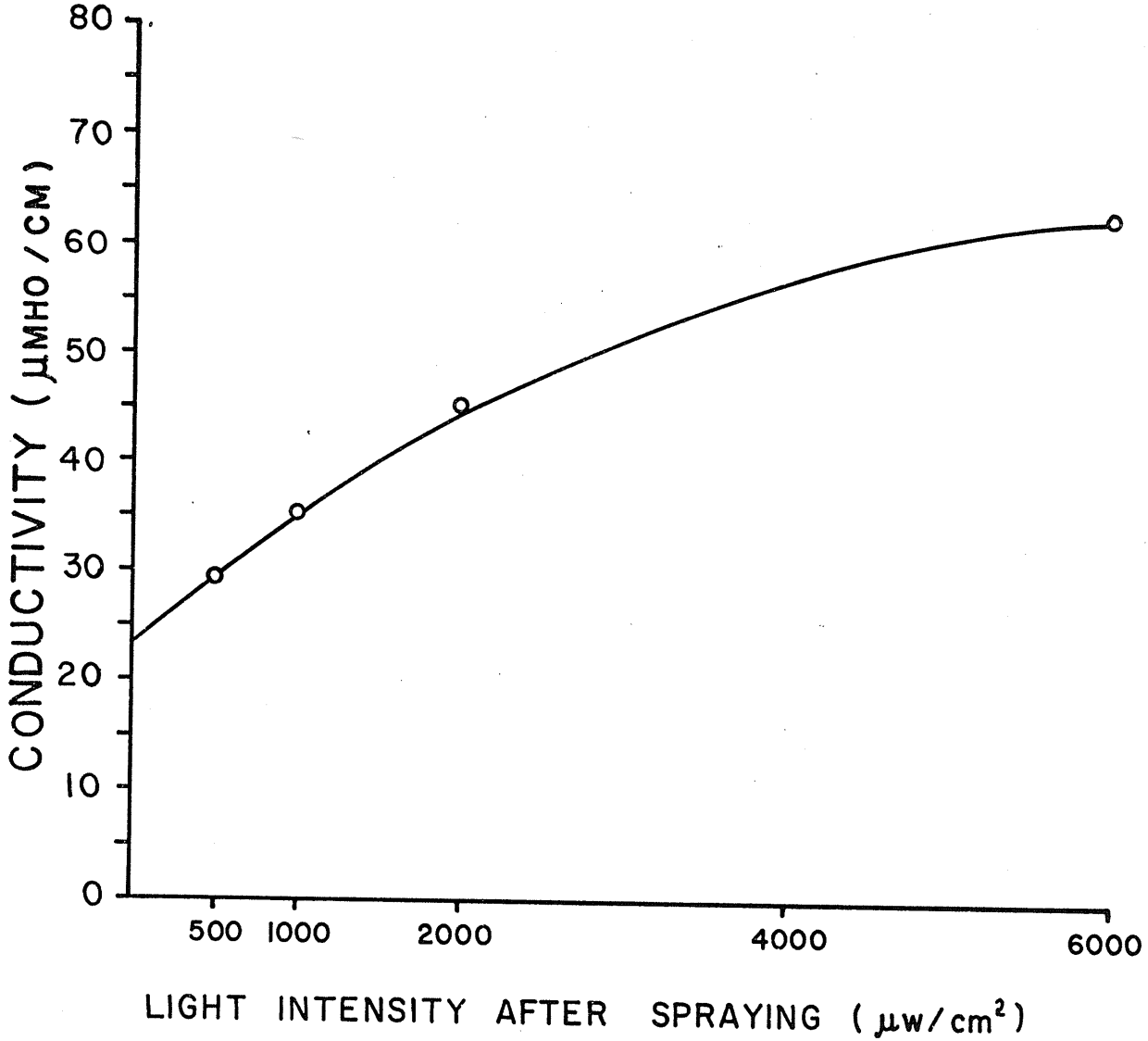

80
70
60
50
40
30
20
10
0
CONDUCTIVITY (μMHO/CM)
500
1000
2000
4000
6000
LIGHT INTENSITY AFTER SPRAYING (μw/cm²)

FIGURE 7. Electrolytic conductivity obtained from buckwheat leaf discs plotted against (irradiation intensity)$^{0.3}$. Conductivity measurements were taken 8 hr after buckwheat plants were sprayed with oxyfluorfen.

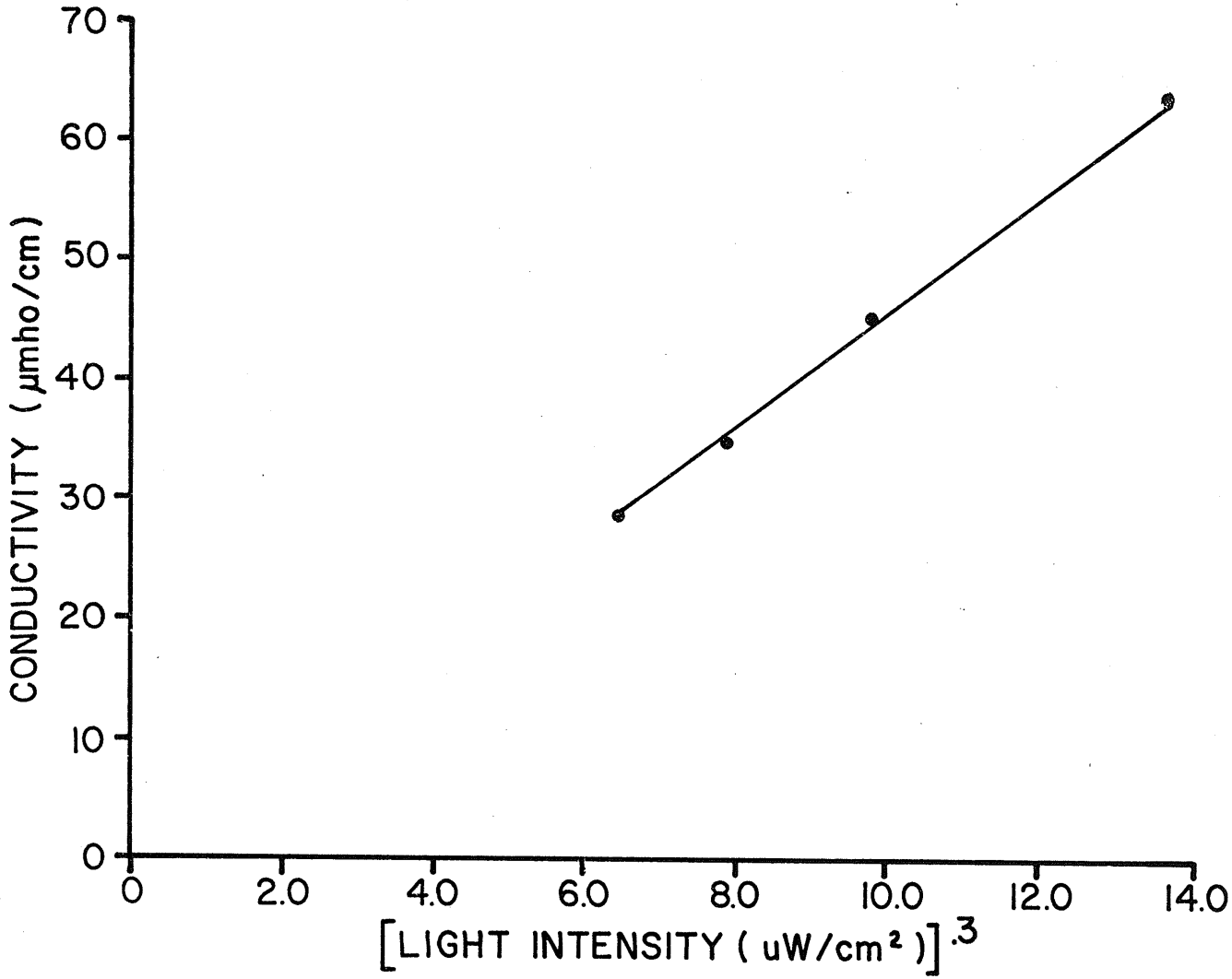
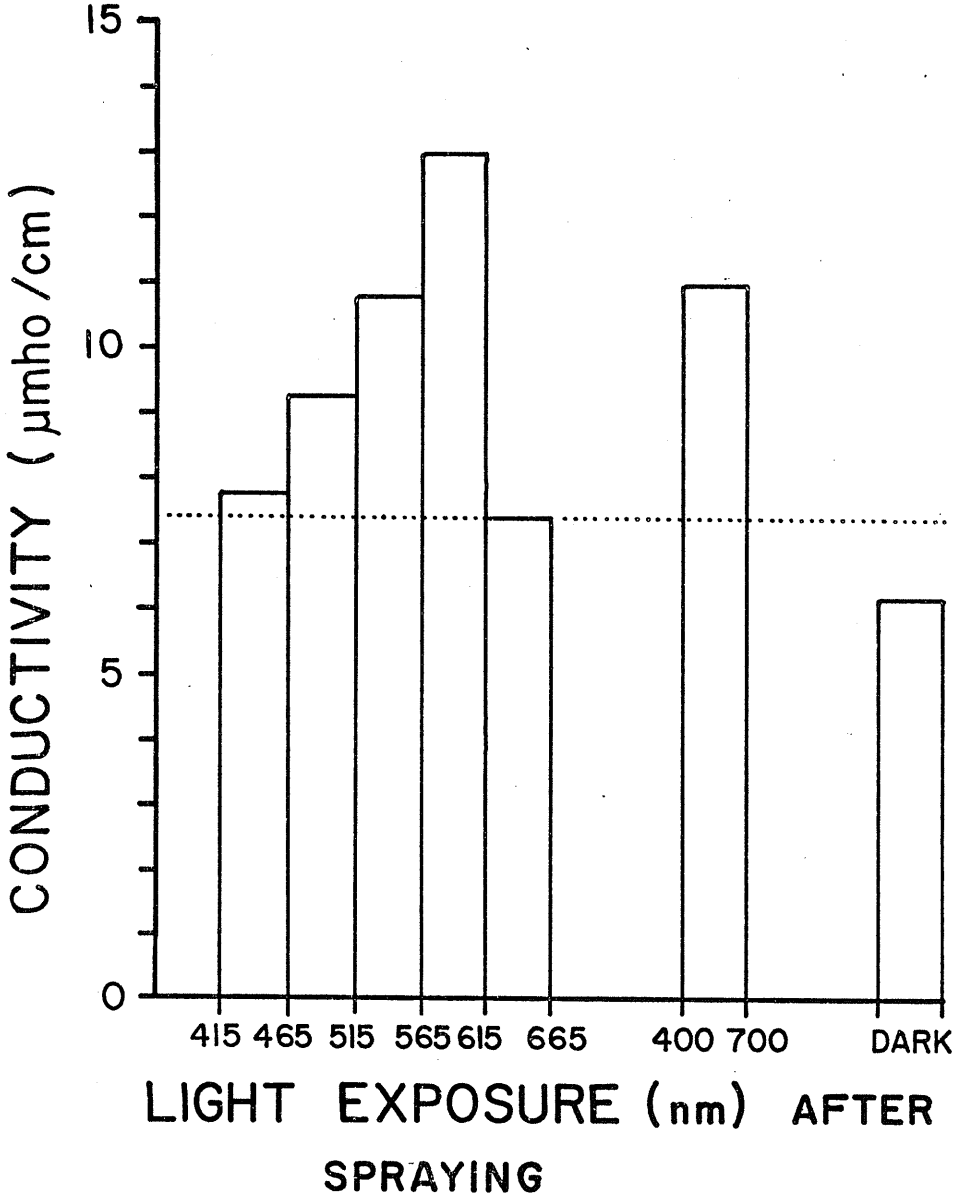

70
60
50
40
30
20
10
0
CONDUCTIVITY (μmho/cm)
0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
[LIGHT INTENSITY (uW/cm²)] .3

FIGURE 8. Electrolytic conductivity obtained from buckwheat irradiated with several wavelengths of light for 19 hours after spraying with oxyfluorfen


15
10
5
0
CONDUCTIVITY (μmho /cm)
415 465 515 565 615 665 400 700 DARK
LIGHT EXPOSURE (nm) AFTER SPRAYING

three light circuits and by varying the distance of the light source[6].

Herbicidal activity was determined at several time intervals after spraying. Measurements were continued until the tissue was completely flaccid (hereinafter referred to as severe injury). It did not mark the point of complete membrane disruption because exposing the leaf discs to boiling water resulted in a further conductivity increase.

Effect of Light Quality on Herbicidal Activity. Plants were sprayed, then placed in a dark room at 25C. A single attached leaf (first leaf) was irradiated for 19 hr with a beam of light from a Xenon arc source[8] filtered by a monochromatic color filter[8]. The filter was adjusted to give the following light conditions: 415 to 465 nm, 465 to 515 nm, 515 to 565 nm, 565 to 615 nm, 615 to 665 nm, or 400 to 700 nm. The light intensity was 240 $\mu W/cm^2$ in all treatments. Herbicidal activity was measured after 19 hr using electrolytic conductivity (four samples of 2 leaf discs). The experiment was repeated and similar results were obtained.

Chlorophyll Content. Chlorophyll was extracted from leaf discs in 80% acetone, separated from water-soluble pigments and quantified colorimetrically (Sestak *et al.*, 1971). A comparison of chlorophyll content was made using five replicates at 0, 2, 4, 6, 8, and 12 hr after spraying.

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[8]Carl Zeiss

RESULTS AND DISCUSSION

Effect of Light on Herbicidal Activity. Plants placed in the dark after spraying with oxyfluorfen were not injured. The electrolytic conductivity measurements showed no significant changes from the control after 96 hr in the dark (FIGURE 4). The etiolated condition of the plants indicated that continuation in the dark would cause irreversible damage unrelated to herbicidal activity. These results are in agreement with Fadayomi and Warren (1976) who demonstrated that light was necessary for herbicidal activity of oxyfluorfen.

Leaf injury was more rapid when plants were irradiated immediately after spraying than when they first received a post-spraying period of darkness. With longer dark periods, the response after being placed in the light was slower. Plants which were maintained in the dark for 48 or more hours plus 7 hours in the light did not show any injury (FIGURE 4). When plants were irradiated longer than 7 hr, stems appeared darkened and withered. The stem injury and slow leaf injury characteristics of plants which had received at least 48 hr of dark plus 7 hr of light indicates that oxyfluorfen was translocated to the stem during the dark period. Irreversible visual injury to the leaves and stems was observed when the plants were irradiated more than 7 hr, regardless of the duration of the previous dark period. Insufficient plants were available for this injury to be measured by conductivity.

Effect of Light Intensity of Herbicidal Activity. Leaves were injured by oxyfluorfen when plants were irradiated at intensities varying from 500 to 6000 $\mu W/cm^2$ (FIGURE 5). At 6000 $\mu W/cm^2$ injury began after

four hr and became severe after 8 hr ($\Delta C = +35$), whereas at 500 $\mu W/cm^2$ injury only began after 8 hr and was not severe until 12 hr ($\Delta C = +32$).

Oxyfluorfen caused cumulative destruction to the buckwheat leaf discs as indicated by the increasing injury with time (FIGURE 5). This type of injury must be caused by more than a constant number of injury-causing molecules. In order to get this accelerating rate of injury oxyfluorfen may be changed in the light to "activated" oxyfluorfen by charge separation. Oxyfluorfen and other light-activated diphenyl ethers have electronegative prosthetic groups such as Cl, CF_3 and NO_2 which are susceptible to charge separation. The critical molecular charge would explain why only ortho and/or para-substituted diphenyl ethers are light-activated. "Activated" oxyfluorfen would exert its herbicidal effect and return to its normal state. It could be recycled many times in the presence of light. This recycling effect would explain accelerating rate of injury of Figure 5 and it would account for the severe injury caused by unusually low applied dosages (Vanstone and Stobbe, 1977).

Conductivity measurements were taken from plants maintained for 8 hr at various light intensities. A plot of conductivity against light intensity showed that oxyfluorfen activity increased hyperbollically as light intensity increased (FIGURE 6). This response indicates that at high light intensity the herbicidal effect approached saturation. Three possibilities could account for the saturation effect at high light intensity: saturation of the response (i.e. total kill), saturation of the herbicide as carrier or saturation of the pigment. There was a higher conductivity measurement from

heat killing the tissue than there was from any herbicide treatment, so it is unlikely that the saturation effect was caused as a result of total kill at high light intensity. The saturation effect apparently was not caused by a lack of herbicide molecules. In another study oxyfluorfen caused similar membrane disruption to buckwheat whether applied at 1 g/ha or 1000 g/ha (Vanstone and Stobbe, 1977). Therefore, it is most plausible to suggest that the saturation effect obtained at high light intensity was caused through saturation of the pigment involved in the light activation of oxyfluorfen.

Figure 6 showed a hyperbolic relationship between conductivity and light intensity (I). The same data are shown as a direct correlation between conductivity and $(I)^{.3}$ (FIGURE 7). An exponent below one indicates a complex pathway between light activation and the measurement of injury.

Effect of Light Quality on Herbicidal Activity. Several wavelengths of light were tested for their efficacy in the light activation of oxyfluorfen (FIGURE 8). Leaves were exposed for 19 hr at 240 $\mu W/cm^2$ at all wavelengths. Herbicidal activity was greatest using light with wavelengths of 565 to 615 nm. The work of Matsunaka (1969) and Fadayomi and Warren (1976) showed that plants containing large quantities of yellow pigments were susceptible to diphenylether herbicides and albinos were resistant. They suggested that xanthophylls are involved in light activation of diphenyl ethers. Since xanthophylls absorb in the blue region (400-440 nm), there is an apparent discrepancy between previously reported work and our own work. However, conductivity measurements have shown that oxyfluorfen

increases membrane permeability. It is the protein constituent of membranes which controls semi-permeability (Stoeckenius, 1976). It may be that a xanthophyll-protein complex in the membrane absorbed in the 565-615 nm region, thus causing light activation at those wavelengths. A similar pigment-protein complex causing an absorption shift from the uncomplexed pigment has been reported to occur in the salt-loving bacteris (*Halobacterium halobium*) (Stoeckenius, 1976). A rhodopsin-like pigment abosrbing at 380 nm complexed with protein in the membranes. The absorption maximum of the complex was 570 nm. A carotenoid-protein complex makes living lobsters appear blue. Upon death of the lobster, the complex is broken down and the lobster then appears orange due to the simple carotenoid pigmentation. Pigment-protein complexes do occur normally in nature; a pigment-protein complex induced by oxyfluorfen may explain why light of 565-615 nm effectively activates oxyfluorfen.

Chlorophyll Content. Chlorophyll content of buckwheat leaves was constant during the first 8 hr after spraying with oxyfluorfen (TABLE 7). There was an apparent increase in chlorophyll content 12 hr after spraying. At 12 hr the tissue was shrivelled so that each sample of leaf discs contained more tissue than earlier samples. Oxyfluorfen does not produce bleached leaves. Lack of bleaching and low herbicidal activity in the region of chlorophyll absorption (blue region and red region above 620 nm) indicates that chlorophyll is not involved in the light activation of oxyfluorfen. In a preliminary experiment using infra-red gas analysis phosynthesis was affected only after the leaves began to wilt.

TABLE 7. Chlorophyll content of buckwheat leaves at various times after buckwheat plants were sprayed with oxyfluorfen

Time after spraying (hours)	Chlorophyll content (mg/l)
0	10.2
2	9.6
4	9.5
6	10.5
8	11.1
12	14.3[1]

[1]Differs significantly (0.05) from the other means according to Duncan's Multiple Range Test.

GENERAL DISCUSSION

The mode of action of a herbicide refers to the total array of anatomical, physiological and biochemcial processes affected by the herbicide. Several processes may be affected concurrently or sequentially. The extent to which each process is affected depends primarily upon the herbicide concentration at the site of action. Each process may be affected differently by a change of concentration. This concentration-dependent relationship among processes implies that the mode of action at a given concentration may be different than the mode of action at another concentration. Contrary to normal occurrence, the action of oxyfluorfen was similar when applied from 1 g/ha to 1000 g/ha (FIGURE 3). This response suggests that oxyfluorfen was present in excess at these concentrations. A test of lower concentrations than those tested would have shown a more typical concentration-response relationship.

Mode of action studies provide information leading ultimately to the mechanism of action which is defined as the primary biochemical or biophysical lesion leading to death of the plant (Ashton and Crafts, 1973). It is difficult to localize precisely the mechanism of action because of the complicating influence of overshadowing secondary effects. However, the influence of each process may be separated somewhat by establishing individual time-response relationships.

There are several reasons for studying mode of action. Firstly, it provides a factual background for making recommendations and for "troubleshooting" in the event of herbicide malfunction. Secondly, it often reveals something about the process being affected and thus contributes to fundamental science. Thirdly, such studies aid in the search for new herbicides.

Visual observations form the basis of our understanding of the herbicidal action of oxyfluorfen. Foliar-applied oxyfluorfen caused severe leaf wilting within 8 hours (TABLE 7). Contact injury occurred so quickly that it must have resulted from direct physical disruption of the cell. Other diphenyl ethers apparently cause similar effects (Ebner *et al.*, 1968; Kruger *et al.*, 1974). The wilting symptom would result when cellular water is lost, a phenomenom that would occur when the selective permeability of the plasmalemma is destroyed. Membrane disruption is reported as the mechanism by which nitrofen, a chemical analog of oxyfluorfen, caused injury to red beet root sections (Pereira *et al.*, 1971).

Since oxyfluorfen causes rapid membrane disruption the effects on photosynthesis, respiration, protein synthesis, nucleic acid synthesis or any other physiological process are secondary. It would seem that conclusions by Pereira *et al.* (1971) and Moreland *et al.* (1970) that diphenyl ethers act primarily as inhibitors of electron transport and oxidative phosphorylation are valid only under the *in vitro* conditions of those studies.

Although visual observations are useful in forming the basis of our understanding of herbicide action, these observations are

not sufficiently precise to establish the mode of action of this herbicide. Visual observations cannot be used to accurately describe a time-response relationship, to compare the effect of various herbicide concentrations or to assess the effect of light on herbicidal activity.

Several analytical assessments such as dry weight and plant height were tried unsuccessfully. Herbicidal injury occurred so quickly that differences between control and treated plants could not be detected with these methods.

An electrolytic conductivity measure of injury was developed to overcome the problem of an inadequate assessment of herbicide injury. This technique (Manuscript 2) which is an adaptation from frost hardiness studies (Dexter, 1956) provides a rapid, quantitative and reproducible measure of herbicidal activity. It is ideal for assessing oxyfluorfen activity because it works on the principle that membranes are being disrupted by the herbicide treatment. Since membrane disruption is the likely cause of oxyfluorfen activity, electrolytic conductivity should be sensitive to oxyfluorfen-induced disruption of membranes. Measurements did show, in fact, that membrane disruption began concurrent with the first evidence of visible injury (TABLE 6).

It was found by using electrolytic conductivity that oxyfluorfen activity was light-dependent (FIGURE 4). Plants were injured within 7 hours when irradiated, but they were not injured after 4 days when placed in the dark. Oxyfluorfen causes injury only to plants placed in the light. My conclusion about the light requirement of diphenyl ethers disagrees with Pereira *et al.* (1971) who concluded

that injury from nitrofen was obtained in the dark. Their conclusions were based on visual assessments of plant injury taken 7 days after spraying with nitrofen. Plants receiving the dark treatment were kept in the dark for only 4 days and then brought to light for the remaining 3 days.

The requirement for light at the site of action was shown by the uptake study where there was root uptake of herbicide (TABLE 3) but no apparent damage to roots maintained in the dark (FIGURE 1). The practical significance of light activation is seen in the field where soil incorporation reduces oxyfluorfen activity. The extent of translocation would determine the amount of root-absorbed oxyfluorfen to be light-activated. The present translocation study shows that less than 10% of oxyfluorfen absorbed by fababean roots was translocated to the stems after 24 hr (TABLE 4). If such a small amount were translocated in plants grown under field conditions, this lack of translocation could account for poor herbicidal activity of soil-incorporated oxyfluorfen.

The requirement for light at the site of action is demonstrated further by the type of injury obtained from pre-emergence treatments of oxyfluorfen. Weed seedlings emerge from the soil and grow for several centimeters before they are injured by pre-emergence treatments of oxyfluorfen. A contact burning of the tissue just above the soil is typical of oxyfluorfen activity.

It was observed that foliar-applied oxyfluorfen was dependent on light at the precise site of action. When leaves were partially shaded during experiments, the shaded parts were not injured as

quickly as the unshaded parts.

The work of Matsunaka (1969) and Fadayomi and Warren (1976) showed that plants containing large quantities of yellow pigments and no chlorophyll were susceptible to diphenylether herbicides and albinos were resistant. Based on their work it appears that xanthophylls or carotenoids are involved in light activation of diphenyl ethers. Since yellow pigments absorb blue light (400-440 nm), one would expect blue light to be most effective for activating oxyfluorfen. Our studies show orange light (565-615 nm) to be most effective for activating oxyfluorfen. The apparent discrepancy between previously reported work and our own work may indicate that a pigment-protein complex absorbed in the 565-615 nm region, thus causing light activation at those wavelengths. A similar pigment-protein complex has been reported to cause an absorption shift from the complexed pigment (Stoeckenius, 1976). A rhodopsin-like pigment absorbing at 380 nm complexed with protein in the membranes of the salt-loving bacterium, *Halobacterium halobium*. The absorption maximum of the complex shifted to 570 nm. Since the protein constituent of membranes controls semi-permeability (Stoeckenius, 1976), oxyfluorfen injury to plants may result when the yellow pigment becomes complexed to protein in the membranes.

Since carotenoids may be involved, it could be suggested that oxyfluorfen inhibits the normal protective function of carotenoids and thereby allows photo-oxidation by energized chlorophyll. Photo-oxidation is an unlikely cause of oxyfluorfen-induced injury. It is not necessary for the plant to contain chlorophyll for it to

be injured by oxyfluorfen (Fadayomi and Warren, 1976). Photo-oxidation causes bleaching; oxyfluorfen-induced injury did not cause bleaching (TABLE 7). If photo-oxidation were the cause of injury, then injury would become more severe as light intensity increased, but rather oxyfluorfen injury approached a maximum response at 6000 $\mu W/cm^2$ (FIGURE 6).

Conductivity measurements were taken from plants maintained for 8 hr at various light intensities. A plot of conductivity against light intensity showed that oxyfluorfen activity increased hyperbolically as light intensity increased (FIGURE 6). This response indicates that at high light intensity the herbicidal effect approached saturation. Three possibilities could account for the saturation effect at high light intensity; saturation of the response (i.e. total kill), saturation of the herbicide as carrier or saturation of the pigment. There was a higher conductivity measurement from heat killing the tissue than there was from any herbicide treatment, so it is unlikely that the saturation effect was caused as a result of total kill at high light intensity. The saturation effect apparently was not caused by a lack of herbicide molecules. In another study oxyfluorfen caused similar membrane disruption to buckwheat whether applied at 1 g/ha or 1000 g/ha (FIGURE 3). Therefore, it is most plausible to suggest that the saturation effect obtained at high light intensity was caused through saturation of the pigment involved in the light activation of oxyfluorfen.

Figure 6 showed a hyperbolic relationship between conductivity and light intensity (I). The same data are shown as a direct correlation

between conductivity and $(I)^{.3}$ (FIGURE 7). An exponent below one indicates a complex pathway between light activation and the measurement of injury.

Injury may occur by an interaction of the energized pigment (or energy from the pigment) with the oxyfluorfen molecule. This interaction could lead to an "activated" oxyfluorfen by charge separation within the molecule. Oxyfluorfen and other light-activated diphenyl ethers have highly electronegative prosthetic groups such as Cl, CF_3 and NO_2 which are susceptible to charge separation. The positioning of prosthetic groups on the molecule is critical for activation because only ortho and/or para-substituted diphenyl ethers are light-activated (Matsunaka, 1969). The case for an "activated" oxyfluorfen is strengthened by our inability to detect an oxyfluorfen metabolite responsible for injury (TABLE 5). Failure to detect a suitable metabolite by chromatography implies that either it was present below our detection limits or that "activated" oxyfluorfen is not a metabolite, but a charge separation of oxyfluorfen.

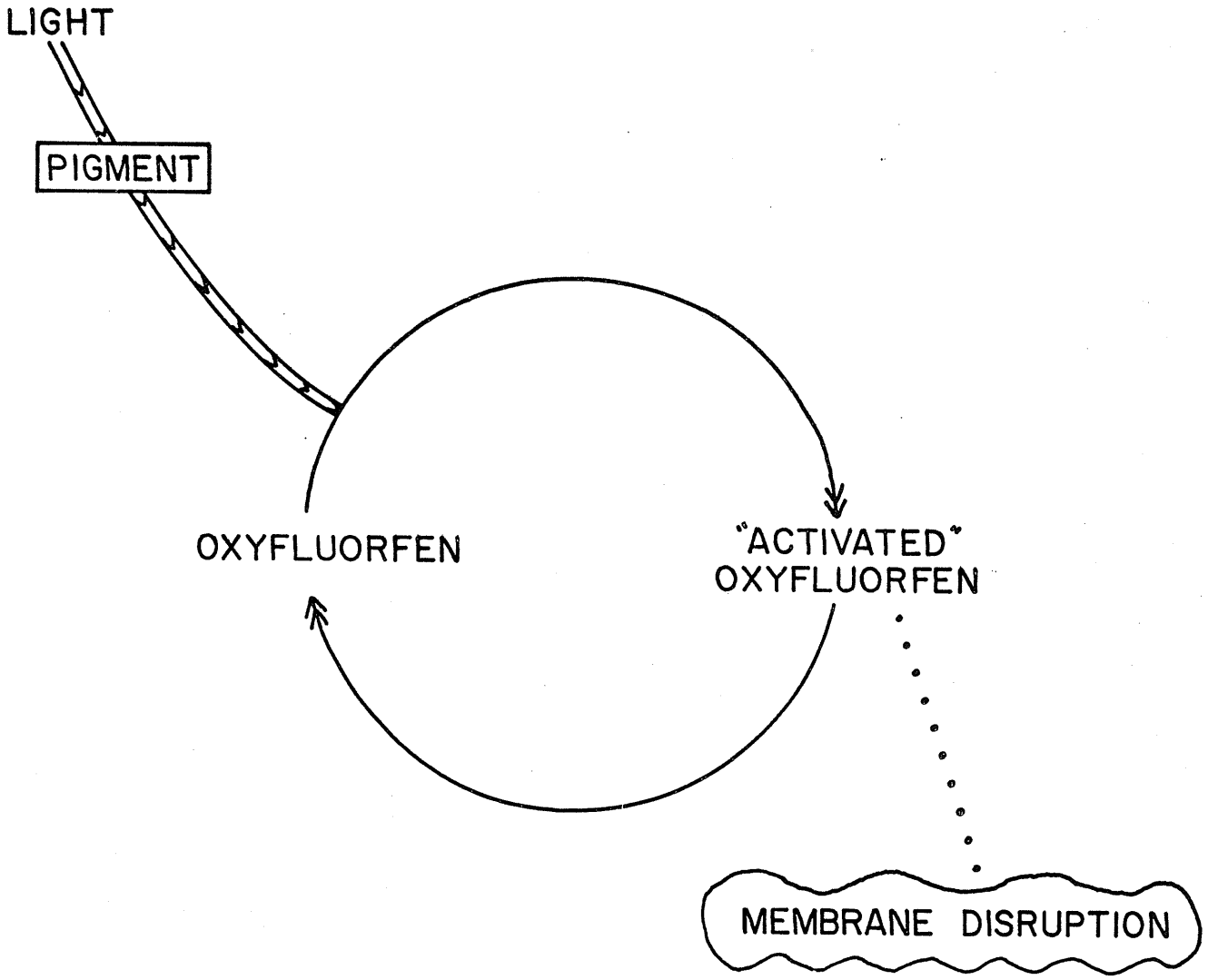
Oxyfluorfen caused cumulative destruction to buckwheat leaf discs as indicated by the increasing injury with time (FIGURE 5). This type of injury must be caused by more than a constant number of injury-causing molecules. In order to get this accelerating rate of injury "activated" oxyfluorfen would exert its herbicidal effect and return to its normal state. Oxyfluorfen could be recycled many times in the presence of light. Recycling would explain the accelerating rate of injury of Figure 5 and it would also account

for the severe injury caused by unusually low applied dosages (FIGURE 3).

The following hypothesis (FIGURE 9) on the mode of action of oxyfluorfen is consistent with our experimental results and those of other workers. The main points are:

1. Light for activation is received by a carotenoid or xantho-phyll-protein comples.
2. The energized pigment complex forms an "activated" oxyfluorfen by charge separation.
3. "Activated" oxyfluorfen disrupts membrances.
4. "Activated" oxyfluorfen returns to normal state and may be recycled in the light.

FIGURE 9. Hypothesis for light activation and mode of action of oxyfluorfen


LIGHT
PIGMENT
OXYFLUORFEN
"ACTIVATED"
OXYFLUORFEN
MEMBRANE DISRUPTION

SUMMARY AND CONCLUSIONS

Root uptake of nitrofluorfen and oxyfluorfen occurs readily in grassy and broadleaf species. The concentration of herbicide in solution adjacent to the roots regulates the amount of herbicide absorbed by the plant. The herbicides are translocated to the shoots in small amounts. Limited translocation to the site of action may explain why pre-emergent applications of nitrofluorfen and oxyfluorfen are most effective without soil incorporation.

Nitrofluorfen and oxyfluorfen are relatively stable within the plant. Metabolism is too slow to act as a protective mechanism against injury or to form a herbicidally-active metabolite chiefly responsible for injury.

Oxyfluorfen depends upon light for activation as do other ortho and/or para-substituted diphenyl ethers. Light energy impinging upon the plant surface is received by a carotenoid-protein chromophore having maximum absorption in the 565-615 nm region of the spectrum (FIGURE 8). The acquired energy "activates" the oxyfluorfen molecule probably through charge separation. The "activated" oxyfluorfen disrupts cell membranes, thereby causing injury. Rapid, continuous injury occurs from low concentrations of oxyfluorfen, thus suggesting that oxyfluorfen and "activated" oxyfluorfen are continuously recycled in light.

Electrolytic conductivity has been a useful measure of herbicidal injury throughout this study. This technique is well suited for measuring rapid injury by contact herbicides.

SUGGESTIONS FOR FURTHER WORK

Uptake studies could be furthered by using the split-pot technique (Prendeville *et al.*, 1967) to determine the optimal site of uptake from the soil. Uptake by leaves and translocation to other aerial parts should be studied as it relates to post-emergent activity.

Additional work is necessary to determine the sequence in which photosynthesis, membrane permeability and respiration are affected by nitrofluorfen and oxyfluorfen. Use of the Gilson Respirometer would enable a co-ordinated approach to this problem.

It would be useful to follow a time course of conductivity after treatment by vacuum infiltration to show conclusively whether membrane damage is regulated by metabolism.

The groundwork has been laid for further studies on light activation of diphenylether herbicides. This subject should now be approached by a photobiologist in an attempt to identify the pigment involved in activation and to determine its mechanism of involvement. Positive identification will be difficult since the chromophore is probably a pigment-protein complex which would be labile under the conditions of extraction. Comparing the absorbance spectra of viable and non-viable herbicide-treated cell suspensions

would indicate whether there is a protein fraction in the pigment.

A second phase of light activation deserving attention is identification of "activated" oxyfluorfen. The presence of the charged state of oxyfluorfen may be confirmed by using spectral-sensitive absorbance techniques now in use for studying activated pigment form. Another approach would be to compare the electronegativity of oxyfluorfen within the plant system under light and dark conditions.

A few specific suggestions for further studies on light activation are as follows:

1. Test the effect of light intensities higher and lower than those already tested.
2. Test the effect of light intensity at various herbicide concentrations.
3. Test the effect of combinations of various wavelengths.
4. Test the effect of various wavelengths of light at an intensity of 1000 $\mu W/cm^2$ or greater.

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