THE ABSORPTION AND TRANSLOCATION OF DINITRAMINE AND PROFLURALIN IN SUSCEPTIBLE AND RESISTANT PLANTS AT VARIOUS TEMPERATURES

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CHAPTER I

INTRODUCTION

The increased use of herbicides in the United States has resulted in increased net income to farmers. Selective herbicides are usually more valuable for weed control. Many environmental factors affect the selectivity of herbicides. Among these are humidity, light, pH, and temperature. Any one of these factors may be critical in determining the effectiveness of a particular herbicide. To be effective, a systemic herbicide must enter the plant and exert phytotoxic effects after it is translocated.

Substituted dinitroaniline herbicides were introduced in 1960 and since that time many have been developed and tested. Recent investigations have indicated crop injury due to application of this type of herbicide under less than optimum temperatures (36). Increased herbicide phytotoxicity with changing temperatures could result from increased herbicide uptake, greater translocation of herbicide from root to shoot, reduced capacity of enzymes to detoxicate the herbicide, or changes in the preceding factors that would result in greater concentration of the herbicide within the plant. A temperature effect at the site of herbicide action could also explain increasing phytotoxicity.

Considering the preceding possibilities, this study was designed to determine whether temperature influence on the uptake and translocation of various herbicides could explain the observed effect on phytotoxicity. This absorption and translocation information could possibly be used to determine the optimum temperature for application of and help explain the mode of action of the respective herbicides.

CHAPTER II

LITERATURE REVIEW

Initial studies with trifluralin (α,α,α-trifluoro-2,6-dinitro-N, N-dipropyl-p-toluidine) indicated a temperature influence on phytotoxicity (33). Other herbicides of this group were obtained to determine if they exhibited the same properties. These included dinitramine (N^4,N^4-diethyl-α,α,α-trifluoro-3,5-dinitrotoluene-2,4-diamine) and profluralin (N-n-propyl-N-cyclopropyl-methyl-4-trifluoromethyl-2,6-dinitroaniline). Physical properties and chemical structure are given in Appendix A.

Since the use of the substituted dinitroaniline chemicals as herbicides is relatively new, literature concerning temperature effects on their phytotoxicity is negligible. Edaphic, absorption, translocation, light and pH factors will be mentioned to give a clearer idea as to the effects these herbicides have on crops and weed species.

Many environmental factors influence the herbicidal activity of a given chemical. Hammerton (28) has reviewed the literature concerning some of the factors. Temperature, light, humidity, and edaphic factors have a major influence on the absorption and translocation of herbicides in plants.

Temperature Effects

The temperature effect on absorption, translocation and phytotoxicity of herbicides is still not well understood. It appears to have a pronounced effect upon a plant's response to a herbicide. Different families of herbicides appear to respond differently to an increase or decrease in temperature. Agbakoba (1) found that when the temperature was increased from 75 F to 95 F, absorption and translocation of 2,4-D[2,4-dichlorophenoxy(acetic acid)] and picloram(4-amino-3,5,6-trichloropicolinic acid) was increased. Kidney beans, rye, and crabgrass were more susceptible to 2,4-D at 25 C than at 5 C. Since the physiological processes were apparently slowed down, the plants were less susceptible at the lower temperature (40). While working with fiddleneck(Amsinckia intermedia), Muzik (57) found that sensitivity to 2,4-D was greater at 26 C than at 10 C at all growth stages. He attributed this to less translocation and absorption at the lower temperature and suggested a detoxifying mechanism by which plants were able to resist the smaller amount of herbicide translocated at the low temperature. He also placed wheat plants in saturated solutions of monuron[3-(p-chlorophenyl)-1,1-dimethylurea], linuron[3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea], diuron[(3-(3,4-dichlorophenyl)-1-dimethylurea], fenuron(1,1-dimethyl-3-phenylurea), atrazine[(2-chloro-4-(ethylamine)-6-(isopropylamino)-s-triazine] and simazine[2-chloro-4,6-bis(ethylamino)-s-triazine] at 13 C and 22 C. The plants were killed at 22 C but survived at 13 C. He suggested the lower temperature may have reduced root uptake, thus reducing damage to the plant. Several workers (65, 76, 83) have indicated that the s-triazines were absorbed by roots and translocated more rapidly at high temperatures than at low temperatures. They concluded that the increased concentration of herbicide in the plant was enhanced by greater transpiration and apoplastic movement at the higher temperature. However, Thompson et al. (82) found that atrazine caused injury to maize when grown for 18-21 days at 15 and 10 C. They suggested that herbicide accumulation due to reduced translocation at the low temperature may have caused the injury. Dudek et al. (17) treated plants at 16, 24, and 32 C with propazine[2-chloro-4,6-bis(isopropylamine)-s-triazine] and terbutryn[2-tert-butylamino)-4-(ethylamino)-6-(methylthio)-s-triazine] and found the higher temperature enhanced translocation in wheat and sorghum. Singh et al. (77) reported the movement to leaves of stem injected prometryne[2,4-bis(isopropylamino)-6-(methylthio)-s-triazine] in soybean and jimsonweed was promoted by high temperature. Figuerola (20) noticed greater terbutryn(2-tert-butylamino-4-ethylamino-6-methylthio-s-triazine) toxicity to winter wheat when the plants were treated at 20 C. Only slight injury was noted at 5 C.

While working with Agropyron repens, Caseley (13) discovered greater toxic effects of paraquat (1,1'-dimethyl-4,4'-bipyridium ion), dalapon (2,2-dichloropropionic acid), and aminotriazole (3-amino-s-triazole) treatment at 26 C than at 6 C. Since most of the above herbicides were more rapidly translocated at the higher temperature of the respective studies, higher concentrations of herbicides in the stems and leaves increased phytotoxicity.

Absorption and translocation in woody species was also affected by increased temperatures. Higher daily temperatures of 24 to 40 C enhanced absorption and translocation of 2,4,5-T[2,4,5-trichlorophenoxyacetic acid] in winged elm (87). When Quercus, Acer, Carya, and Pinus trees were sprayed with 2,4,5-T and placed in temperatures of 55, 75, and 95 F, all species absorbed more 2,4,5-T at 95 F. The *Acer* species exhibited greater herbicide concentration in the roots at 55 F (10). Morton (54) noticed that when 2,4,5-T was applied to a central leaf of mesquite, it was translocated basipetally at 21 C, acropetally at 38 C, and both basipetally and acropetally at 29 C. Weatherly and Watson (86) noticed that when lengths of stem or petiole of *Salix fimenalis* were cooled to about 0 C, the transport of labelled assimilates was reduced or stopped. This indicated the exchange of labelled compounds between the sieve tubes and the surrounding cells was decreased by lower temperatures.

Other families of herbicides exhibit distinctly different temperature effects. When treated with TCA (trichloroacetic acid) at 7-10 C and 20-25 C, *Agropyron repens* had less injury at the higher temperatures (26). When soybeans were treated with dinitramine and trifluralin, greatest stand reduction was observed when temperatures remained below 60 F (50). However, Lebaron et al. (46) noticed best weed control in soybeans at higher temperatures when using profluralin. Penner (64) found a reduction in accumulation of ^{14}C-trifluralin in the roots of maize and soybean plants grown at 30 C compared to those grown at 20 C. Hill et al. (36) showed in greenhouse studies that more injury occurred when peanut seedlings were treated with trifluralin at soil temperatures at 21 and 38 C and least injury occurred at 32 C. Anderson (2) found that tomato seedlings were more sensitive to trifluralin at 13 C than at 18 C as measured by seedling dry weight. Red root pigweed (*Amaranthus retroflexus* L.), wild oat (*Avena fatua* L.), barnyardgrass (*Echinochloa crusgalli* L.), and rye grass (*Lolium multiflorum* Lam.)

seedlings were more susceptible to trifluralin and isopropalin [(4-isopropyl-2,6-dinitro-N,N-di(n-propyl)aniline] at 18 C than at 13 C. According to Probst et al. (67), trifluralin is not translocated in significant amounts to the tops of plants such as soybean (Glycine max). Trifluralin appears to act primarily in the root system, and it is possible that herbicides that do not translocate acropetally could accumulate in the roots under cool conditions. Thus it is evident that temperature effects vary with the herbicide and plant species used in the experiment.

Edaphic Factors

Temperature, soil moisture, pH, organic matter, herbicide volatility, clay content, and rate of herbicide applied all influence the performance of a herbicide.

As adsorption processes are exothermic, reduced adsorption by soil would be expected at higher temperatures. Interactions between solute and solvent as well as between solute and adsorbent determine the extent of adsorption. In soil, the adsorbed herbicide could resist leaching and yet some plant roots might secrete organic compounds that could exchange with the herbicide and allow uptake by the plant.

The adsorption of monuron, atrazine, CIPC (isopropyl-m-chlorocarbanilate), DNBP (2-sec-butyl-4,6-dinitrophenol), and simazine by bentonite was greater at 0 C than at 50 C (29). When samples were equilibrated at 0 C and then heated to 50 C, the additional herbicide adsorbed at the low temperature was released upon heating. Experiments with terbutryn have shown greater injury on winter wheat (Triticum aestivum) grown at high soil temperature than on plants grown at low soil temperature (19). High soil water content favored the uptake of terbutryn through the root system and most injury occurred under high transpiration rates. Dudek et al. (17) reported similar results when terbutryn was applied in nutrient solution to sorghum and winter wheat.

Messersmith et al. (52) observed decreased phytotoxicity of trifluralin incubated for 10 months in Sharpsburg silty clay loam and Anselmo sandy loam as the temperature increased from 15 C to 35 C. Breakdown rate of ^{14}C-trifluralin to $^{14}CO_2$ in both soils was more rapid at 1.6 field capacity than at 0.8 field capacity and at 1 ppm than at 100 ppm of trifluralin. Hawxby et.al. (33) reported greater loss of ^{14}C-trifluralin from nutrient solution at 37.8 C than at 15.6 C at the end of 24 hr. The total loss of ^{14}C-trifluralin by volatilization (^{14}C not found in the nutrient solution or plants) after 24 hr was also greater at 37.8 C than at 15.6 C. Losses were greater at 10 ppm than at 5 ppm of trifluralin.

Although soil pH influences the phytotoxicity of some herbicides, Burns et al. (11) observed that a soil pH range of 6.0-7.5 did not appear to affect performance of trifluralin for rhizomes or seedling johnsongrass control. However, Anderson et al. (4) reported greater trifluralin, benefin(N-butyl-N-ethyl-α,α,α-trifluoro-2,6-dinitro-p-toluidine), and nitralin[(4-methylsulfonyl)-2,6-dinitro-N,N-dipropyl-aniline] adsorption under acid soil than under alkaline conditions. Nitralin persistence was greater in acid than in neutral soil (69).

Organic additions to soil influenced the retention of phytotoxicity of the substituted dinitroaniline herbicides. Bardsley, et al. (6) found that increasing organic matter from 1.5 to 6% resulted in more retention of active trifluralin. Apparently increased absorptive capacity of the organic materials retained trifluralin vapors. While conducting experiments with run-off water, Sheets et al. (75) reported less than 1% of total trifluralin applied was recovered in surface run-off 5-8 months after application. Sediment filtered out of the water by suction contained 84% of the trifluralin that was detected in run-off. Greater persistence of trifluralin was noted in loam (.4 ppm) than in loamy sand (.1 ppm). This indicated that the trifluralin apparently adsorbed to the soil particles and adsorption was enhanced by increased organic matter. Schrader (70) reported slight injury in cotton from dinitramine and nitralin as organic matter was decreased from 4% to 1.2%. Soybeans were injured at all organic matter levels when dinitramine was applied at 1.12 kg/ha. These results indicated greater adsorption and less availability to plants as organic matter levels increased. From experiments with leaching of trifluralin, benefin, and nitralin in soil columns, Anderson et al. (4) concluded that organic matter content of the soil was the most important factor affecting adsorption of herbicides. They found that organic colloids had much higher adsorptive capacity than clays.

Several workers have indicated loss of trifluralin vapors due to increased concentration and soil moisture. Swann (79) attributed these results to reduced adsorption of trifluralin molecules as hydration of the soil colloids increased. He noted the injury symptoms on shoots resulting from exposure to trifluralin vapors were similar to those observed in plants grown in treated soil. Increasing the rate in soil surface treatment from 0.56 to 4.48 kg/ha resulted in substantial increase in vaporization. He concluded that the vapor phase may be responsible for the herbicidal activity of soil applied trifluralin.

Bardsley et al. (7) calculated the vapor pressure of trifluralin in an aqueous system to be 126 mm Hg at 80 C. This contrasted with a figure of 1.99 X 10^{-4} mm Hg at 29.5 C, which is for trifluralin crystals. Gaseous losses of trifluralin were greater with increased air movement, reduced barometric pressure, and lower relative humidity. Vapor loss of trifluralin was proportional to concentration. However, loss of trifluralin as a vapor on a percentage basis was much greater at lower concentrations since a correspondingly high fraction of the compound is crystalline at higher concentration. Crystals are at a lower energy level than liquids, thus vapor loss was greater as the crystals dissolved. It appears that water serves as a vehicle for promoting volatilization of trifluralin and that, in effect, a form of distillation takes place.

Because of the volatility of trifluralin and other substituted dinitroanilines, they are usually most effective when incorporated (34, 42, 45, 78). Oliver and Frans (61) reported inhibition of lateral roots of soybean and cotton was directly related to depth and method of incorporation of trifluralin. Bardsley et al. (7) found that placement of trifluralin 1.3 cm deep in sandy soil was sufficient to provide an adsorption barrier to volatilization.

Substituted dinitroaniline herbicides apparently are slowly degradable. Analysis indicates trifluralin doesn't accumulate with repeated annual application and that a steady and continuous decline in the level of trifluralin present in soil occurrs with time (62). Burnside (12) reported a phytotoxic residue of trifluralin persisted in Sharpsburg silty clay loam for one or more years, especially at above-normal rates of application. Repeated applications did not cause a significant buildup and there was little herbicide remaining in the soil during subsequent years from normal rates of trifluralin. Similar results were noted in experiments with other substituted dinitroaniline herbicides (34, 69).

Mode of Action

The morphological effect of some of the dinitroaniline herbicides, especially trifluralin, have been known for some time. The absence of lateral roots was observed when trifluralin was applied to various crops (3, 18, 33, 60, 73, 78). This was accompanied by stunting in height of the plants. In most cases growth of the tap root was unaffected. Soil experiments showed lateral root inhibition only along that portion of the taproot growing in treated soil. Several workers have indicated this injury was due to absorption of the herbicides in use through the roots (33, 48, 49). Hassawy and Hamilton (32) found that indoleacetic acid (IAA) and kinetin counteracted the trifluralin inhibition of lateral root initiation. However, when trifluralin was applied to the shoot zone of green foxtail (Setaria italica (L.) Beav. 'Empire') and prose millet (Panicum miliaceum L. 'White'), the plants were killed (43). Swann and Behrens (80) and Standifer and Thomas (78) indicated no apparent effect on plant growth due to foliar application of trifluralin.

Microscopic investigations showed many abnormalities of stem and root tissue which had been treated with substituted dinitroaniline herbicides. Most root observations showed drastic reduction in mitotic figures, accompanied by radial enlargement of the root tips (25, 44, 47, 59, 81). Behind the tip region the cells were abnormally large and thin walled. Nuclear division was disorganized and cell wall and cell plate formation were rare. This lack of differentiation of xylem cells reduced water transport and may have decreased mineral nutrient supply to the tops, resulting in stunted plants. Bayer et al. (8) noticed enlarged pericycle cells corresponding to structures studied in colchicine-treated roots of wheat by Foard, Haber, and Fishmann (21), who termed these structures primordiomorphs. The presence of many polynucleate cells led many observers to conclude trifluralin acted as a mitotic poison (47, 81). Kust and Struckmeyer (44) noticed high starch levels in xylem elements of the upper part of the tap roots and concluded trifluralin or its metabolites may have suppressed the activity or synthesis of enzymes needed for respiratory metabolism. Hendrix and Muench (35), and Hawxby et al. (33) noticed more enlarged root tips with increased temperature.

Trifluralin has been found to inhibit the synthesis of DNA, RNA, and protein during seedling germination of corn (66, 71, 74), oats (72), and barley and Sesbania sp. (51). Shahied (74) found that cysteine reversed the effect of trifluralin on lateral roots when applied in combination with the herbicide. He postulated an effect of trifluralin on sulfhydryl groups, leading to an inhibition of cell division. Penner and Early (66) noticed inhibition of RNA synthesis and indicated possible trifluralin binding to the chromatin with subsequent reduction of template availability for transcription. However, other workers have recorded no inhibition and even some increase in RNA in trifluralin treated roots and shoots of corn and sorghum in the dark (16). Protein was higher in the treated shoots and lower in the treated roots than the check.

Negi et al. (58) reported oxygen uptake and oxidative phosphorylation were inhibited by 10^{-4} M trifluralin in isolated mitochondria of corn, sorghum, and soybeans. Phosphate uptake was reduced 50% or more. This was not related to the selectivity of trifluralin since it caused a greater reduction in oxygen and phosphate in a resistant species (soybean) than in a susceptible species (sorghum). Wang et al. (84) suggested a major mechanism of phytotoxicity of the substituted dinitroanilines seemed to be the disruption of ATP formation, either by interfering with the energy-generating mechanism or by blocking the energy transfer mechanism of mitochondria.

Harvey (30) suggested the effectiveness of dinitroaniline herbicides appeared to be related to variations in inherent phytotoxicities. Part of the differential effect on shoot growth could be due to differences in absorption and translocation. Since velvetleaf (Abutilon theophrasti Medic.) was unable to inactivate oryzalin (3,5-dinitro-N^4, N^4-dipropylsulfanilamide) and dinitramine in the same manner that it did trifluralin, detoxification processes may be responsible for the tolerance of some plants to some of the dinitroanilines. In a later study, Harvey (31) suggested that the relative effectiveness of dinitroaniline herbicides was related to their volatility and the influence of their vapors on germinating seedlings. The effective herbicide concentration in solution was influenced by adsorption to soil. Under conditions of high soil moisture, or with dinitroaniline herbicides of low volatility, differences in soil moisture affected field performance.

Degradation studies of trifluralin have shown that aerobic degradation (soil conditions of normal exposure to light, atmosphere, and moisture) proceeds through a dealkylation step followed by progressive reduction (67). Anerobic degradation (soil conditions of excessive rainfall) occurs with a preliminary reduction prior to dealkylation (67). Golab et al. (23) indicated the major conversion product of trifluralin in carrots was α,α,α-trifluoro-2,6-dinitro-N-(n-propyl)-p-toluidine. Similar results were reported when peanuts and sweet potatoes were treated with trifluralin (9).

Murray et al. (56) showed a direct correlation between phytotoxicity and selected structural changes on the dinitroaniline ring. The dinitroaniline ring substitutions in order of decreasing phytotoxicity to bioassay indicator plants were substitution in the one and five positions, one only, and four only. The combined one and four position substitution on the ring was least phytotoxic. They also concluded that selectivity was controlled by herbicide application rate.

Hall and Giam (27) proposed a model to explain the herbicidal activity of trifluralin and similar compounds. They postulated an intermediate, formed in a nucleophilic reaction of trifluralin, was the actual species responsible for herbicidal activity. The factors which influenced the formation of this intermediate also influenced herbicidal activity.

Absorption and Translocation

Herbicides are applied to the plant via the apoplast. To perform their normal function, those that act on or through the plant must pass through the apoplast and make contact with the symplast. If applied to the roots, they must pass through the apoplast of the cortex and stele; they may move from symplast to apoplast where they move rapidly to the leaves. They may then enter from the chlorenchyma into the phloem and thus may circulate in the plant; or they may largely remain immobile in the leaves (15). The ability of root cells to discriminate between 2,4-D and dalapon, retaining the former while freely transporting the latter, seemed to indicate definite protoplasmic control over root absorption. However, low mobility may result from active accumulation by living cells at points of accumulation, or from lack of an active sink. High mobility may result from lack of an active accumulation or metabolism at the point of application (14).

Absorption and translocation of a given herbicide is also dependent on inherent characteristics of the herbicide itself. Foy (22) found that prometone [2,4-bis(isopropylamine)-6-methoxy-s-triazine] and propazine moved acropetally with the transpiration stream. Basipetal translocation in soybeans, sorghum, and corn was negligible. Jones and Foy (39) found methazole [2-(3,4-dichlorophenyl)-4-methyl-1,2,4-oxadiazolidine-3,5-dione] translocation was similar to compounds which move only in the apoplast. The uptake of chloramben (3-amino-2,5-dichlorobenzoic acid), atrazine, EPTC(S-ethyl dipropylthiocarbamate), linuron, and chorpropham, increased with increasing temperature and concentration (53). Although transpiration increased as the temperature increased, herbicide uptake and transpiration were not related. However, other investigators (65, 85) noticed increased ^{14}C-atrazine accumulation in plant tops with increasing temperature. They credit part of the increased uptake to increased transpiration. Ketchersid et al. (41) found that basipetal movement of trifluralin in peanuts was greater than with benefin or nitralin, thus increasing the toxicity of trifluralin. However, translocation of the herbicide decreased as the age of the peanut seedling increased.

Light

The effect of light on dinitroaniline herbicides is still not well understood. Maksymowicz and Rieck (49) were unable to isolate photodegradation products of dinitramine. Parochetti and Hein (63) could find no significant losses from photodecomposition for trifluralin, benefin, or nitralin when comparing radiated and unradiated soil surface-treated samples. However, other workers have found trifluralin was degraded by photodecomposition (41, 52). Loss of biological activity was much greater from glass surfaces than from soil surfaces when exposed to sunlight (88).

Miscellaneous Effects

The combination of other chemicals with trifluralin may alter the effect of the herbicide. Arle (5) reported that the combination of (disulfaton)[o,o-diethyl-S-(2-(ethylthio)-ethyl)-phosphorodithioate] or (phorate)[o,o-diethyl-S-((ethylthio)-methyl)-phosphorodithioate] with trifluralin resulted in increased cotton seedling growth as compared with trifluralin used alone. Apparently the insecticide helped overcome secondary root inhibition in the zone of incorporation and thus increased the initial growth of the cotton (68). Hilton and Christiansen (37) have reported that seedling tolerance to trifluralin is dependent on stored lipid content. Grasses low in seed lipids were susceptible and broadleaf species high in seed lipids were tolerant. They found that addition of external lipids, such as tocopherol, to the soil reduced trifluralin damage to susceptible plants. They have postulated that crop seedlings might be protected by a nonphytotoxic lipoid carrier.

Animal experiments with substituted dinitroanilines are rare but Golab et al. (24) have reported that labelled trifluralin was degraded in eleven hours in septic rumen fluid medium. In ruminant animals, 99% of the ingested radioactivity from labelled trifluralin was recovered within six days in urine (17.8%) and feces (81.2%). Milk and blood contained none. The principal degradation products formed were those similar to that of anaerobic trifluralin degradation (67).

A review of the literature has indicated many factors that can influence the degree of susceptibility or resistance to dinitroanilines among plant species. Few results have been reported concerning temperature effects on the absorption and translocation of this family of herbicides. The objective of this study was to determine the influence of temperature on the absorption and translocation of certain dinitroaniline herbicides when applied to resistant and susceptible plant species.

CHAPTER III

METHODS AND MATERIALS

Bioassay Studies

Growth chamber studies were conducted to determine the effect of profluralin on soybean (variety Dare), sorghum (variety RS6-12), pigweed (Amaranthus palmeri S. Wats.), and barnyardgrass (Echinochloa sp.). Air dried Perkins sandy loam was obtained and mixed with profluralin in a 10 liter glass bottle for 15 minutes. The treated soil was then serially diluted with similar untreated soil to give profluralin concentrations of 0, 0.125, 0.25, 0.5, 1, and 2 ppmw. About 200 grams of treated soil was placed in 6 ounce, plastic cups and 5 bean seeds, 15 sorghum seeds and many pigweed and barnyardgrass seeds were placed in the respective cups. Four replications of each dilution and species were arranged in a completely randomized design in a growth chamber with 14 hr days at 15 klux at 32 C and 10 hr of dark at 27 C. A combination of fluorescent and incandescent lights were used. After planting, 25 ml of distilled water was added to each cup and the weight of each cup was maintained by the addition of ½ strength Hoagland's solution (38) every day. After one week, the soybeans were thinned to 3 plants per cup, the sorghum to 6 plants per cup, and the barnyardgrass and pigweed to 10 plants per cup. Herbicidal response of the plant species was measured by harvesting aerial parts of the plants

15 days after planting and recording fresh weight. Dry weight was obtained after placing the plants in an oven at 110 C for 24 hr.

Another experiment consisted of obtaining the upper limits of species tolerance by diluting the herbicide concentration to 0, 0.5, 1, 2, 4, and 8 ppmw. The same procedure as previously mentioned was followed.

Uptake of Profluralin and Dinitramine by Intact Plants

Soybean and sorghum seeds were germinated in paper rolls and transferred after 5 days to 80 ml of aerated, half strength Hoagland's solution in green, glass jars. Pigweed and barnyardgrass seeds were planted in Perlite, a volcanic mineral, watered with half strength Hoagland's solution, and transplanted to nutrient solution after 8 days. Preliminary experiments indicated extra iron was needed so barnyardgrass was placed in nutrient solution containing twice the normal amount of iron to prevent chlorosis. Sorghum and soybeans were treated 12 days after planting, barnyardgrass 18 days after planting, and pigweed 22 days after planting. Treatment in nutrient solution consisted of 0.4 µM (9.72 µg/70 ml) solution of uniformly ^{14}C-ring labeled profluralin (specific activity 34.3 µC/mg) or 0.4 µM (9.02 µg/70 ml) of trifluoromethyl-labeled dinitramine (specific activity 18.6 µc/mg). Plants in the treated jars were placed under continuous light (19.4 klux) at 15.6, 21.1, 26.7, 32.2, or 37.8 C and relative humidity was maintained at 60%. The plants were harvested after 24 hr by dipping plant roots in each of 4 large containers of water to remove part of the radioactivity adhering to the root surface and blotting with paper towels.

Plants were divided into tops and roots. Fresh weight of each plant part was then determined and they were quickly frozen at -40 C in closed test tubes. The volume of the nutrient solution was measured after treatment to determine water uptake during treatment time. The weighed plant parts were ground in a high-speed homogenizer in 10 ml of 95% ethanol. Small portions of the homogenate were removed and analyzed for ^{14}C by liquid scintillation counting. One-half ml samples of the nutrient solution were removed at 24 hr and assayed for radioactivity. The ^{14}C adsorbed on the glass of the culture vessel was then removed by washing with 5 ml of ethanol and counted. Analysis was done with a Packard liquid scintillation counter, model 3320. The counting solution consisted of xylene, p-dioxane, and ethanol (5:5:3 v/v) containing 80 gm of napthalene and 5 gm of PPO per liter of solution. There were 4 replications per harvest time in a randomized block design. Each experiment was duplicated.

Thin Layer Chromatography

Barnyardgrass, pigweed, sorghum, and soybean plants were used to determine whether radioactivity in various plant parts was parent chemical or metabolites. Four plants of each species were treated as described previously at 16 C and 38 C. The plant parts were homogenized in a hand homogenizer in 10 ml of 95% ethanol. Half ml samples were evaporated to 0.2 ml with a stream of dry nitrogen and spotted on Eastman Chromagram sheets. Duplicate half ml samples were placed in counting solution to indicate total radioactivity spotted. Tops and roots of two plants and a standard were placed on each sheet. Chromatograms were allowed to develop in an ethyl acetate and benzene solvent

(1:3 v/v) for 90 min. They were then air dried, covered with plastic (Saran) wrap, and placed in a packet of Kodak Ready Pack No-Screen X-ray film at -3 C for 25 days. After locating the exposed areas with the developed film, the radioactive spots were scraped into 15 ml counting solution with a glass tube connected to a suction tube. A comparison of the stock solution of labeled profluralin or dinitramine and the plant part homogenate was used to determine whether the parent chemical and/or metabolite product was being translocated to the various plant parts.

Autoradiography

Autoradiograms of whole plants were prepared as an aid in the study of the translocation and absorption of labeled profluralin and dinitramine. Plants were treated as described previously but only at 16 or 38 C. After rinsing plant roots in 4 containers of water, the plants were placed on wire screens, frozen at -40 C, and lyophilized. They were then autoradiographed according to a method described by Crafts and Yamaguchi (15). The plants were placed on white cardboard and covered with plastic (Saran) wrap. In a dark room, the mounted plants were placed in a film packet of Kodak Ready Pack No-Screen X-ray film and sealed with masking tape. The film packet was then placed between a piece of sponge rubber and plywood covered with aluminum foil. A stack of film packets, sponge rubber, and plywood was formed and bound together by 2 cotton web belts. The stack was kept at -3 C until the film was developed 25 days after treatment.

Uptake of Profluralin from Soil

A soil mixture containing 1 ppmw profluralin was obtained by combining 4000 gm of Perkins sandy loam with 1.5 mg ^{14}C-labeled profluralin and 2.5 mg unlabeled profluralin. About 300 gm of the soil was placed in 16 ounce cartons. Seeds of cotton, soybean, and peanut were germinated for 2 days at 32 C on wet paper towels, placed on top of the soil, covered with 100 gm additional soil, and watered with 60 ml half strength Hoagland's solution. Because of the small lateral roots of barnyardgrass and pigweed, these species were not used in excised root or soil uptake studies. Four seeds of each species were added to each of three cartons. After five days, the seedlings were harvested by washing off the soil, sonicating the roots for 15 seconds to remove soil, dividing into roots and tops, and determining fresh weight. Three temperature ranges were used during the experiment. After germination, some of the plants were grown at 10 hr, 32 C nights and 14 hr, 35 C days with 27 klux. A second group of plants were grown at 27 C nights and 29.5 C days and a third group were treated at 21 C nights and 24 C days under the same day length and light intensity. Analysis of the plants was the same as in the uptake of herbicides in intact plants.

Absorption with Excised Root Tips

One cm sections of lateral and tap root tips were excised from 10 day old seedlings of sorghum, soybean, cotton (variety Westburn 70), and peanut (variety Spancross 72). They were cultured for varying times at 15.6, 21.1, 26.7, 32.2, and 37.8 C in 10 ml half strength Hoagland's solution containing 0.125 ppm (0.43 uc) of profluralin in a 20 ml closed vial. Three lateral root tips and three tap root tips of each species were placed in each of five vials for each temperature, and one root tip of each kind was removed 1, 4, and 12 hr after the roots were placed in the profluralin solution. The root tips were blotted lightly, washed 30 sec in n-hexane, placed in 15 ml of counting solution and assayed for radioactivity by liquid scintillation counting. After treatment, nutrient samples were obtained from each vial and placed in counting solution for analysis. ^{14}C adsorbed on the glass of the culture vial was determined by replacing the culture solution with counting solution.and recording the radioactivity. The entire experiment was duplicated.

Statistical Analysis

Data from liquid scintillation experiments were analyzed by using an IBM computer model 360. Various programs were used to analyze the different experiments. A standard F test and/or Duncan's Multiple Range statistical testing at the 0.05 level was utilized to test significant differences within a particular experiment.

CHAPTER IV

RESULTS

Preliminary Bioassay Studies

Four species were evaluated as to their resistance or susceptibility to profluralin. The two bioassay studies were combined. Soybean was resistant at concentrations up to 4 ppmw (Figure 1). Pigweed was also somewhat resistant but was affected at concentrations greater than 2 ppmw as plants were stunted and chlorotic. Sorghum and barnyardgrass were affected at all concentrations.

The average dry weight per plant at various profluralin concentrations showed that barnyardgrass and sorghum were most susceptible. They were greatly affected at the lowest concentration tested and were stunted and chlorotic. Soybean exhibited the greatest resistance to profluralin. The dry weight of soybean was reduced and the other species tested died at profluralin concentrations greater than 4 ppmw during the time period they were tested. Sorghum leaf tips were chlorotic and plant height was reduced at concentrations greater than 1 ppmw. Pigweed was stunted at concentrations greater than 2 ppmw but it was more resistant than barnyardgrass or sorghum.

From the preceding results, soybean and pigweed were chosen as resistant species and barnyardgrass and sorghum as susceptible species.

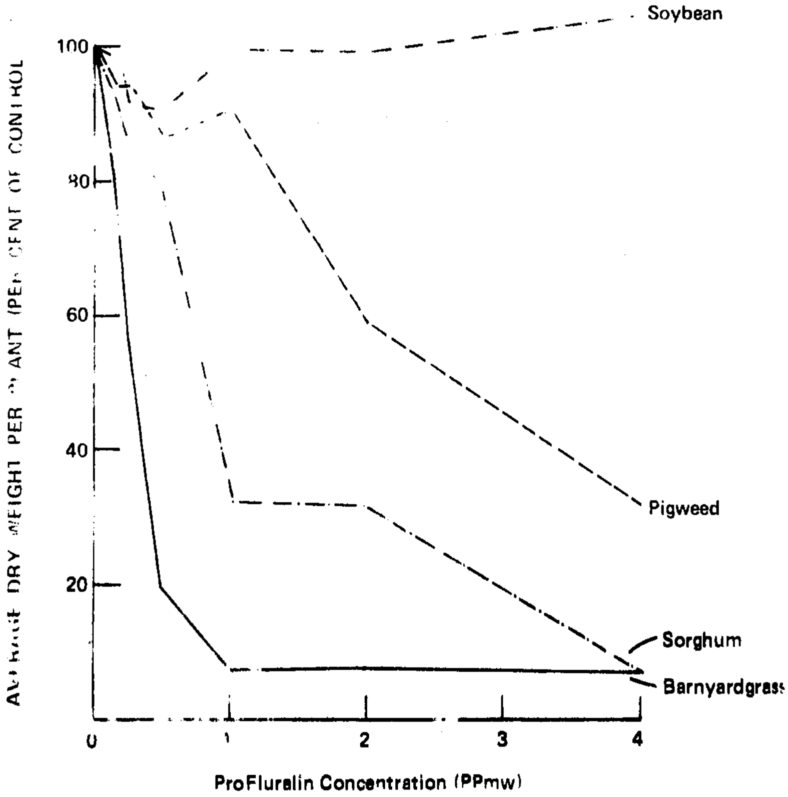


Figure 1. Plant Response to Varying Profluralin Concentrations.

The Uptake and Distribution of Profluralin and Dinitramine in Intact Plants

Barnyardgrass, pigweed, sorghum, and soybean were grown in nutrient solution and then treated at various temperatures for 24 hr with radioactive profluralin or dinitramine by adding the herbicides to the nutrient solution. At the end of the treatment period, the plants were divided into roots and tops and analyzed for radioactivity.

At the end of the profluralin treatment it was noticed that root tips of intact sorghum and barnyardgrass plants exhibited radial enlargements at all temperatures, while root tips of pigweed and soybean contained no enlargements. These knobs were especially prominent on the adventitious roots from the stems of sorghum plants. The roots of all species exhibited these enlargements when treated for 24 hr in dinitramine and nutrient solution at 26, 32, and 38 C. However, only sorghum root tips had these knobs at 15 and 21 C. It is possible that the increased surface area of the roots with the enlargements could enhance absorption of the herbicide in the nutrient solution, thus affecting phytotoxicity.

Many workers (25, 44, 47, 59, 81) have reported these abnormal roots on plants treated with trifluralin. Nuclear division was disorganized and lack of differentiation of xylem cells reduced water transport and may have decreased mineral nutrient supply to tops, resulting in stunted plants. Kust and Struckmeyer (44) have postulated that trifluralin or its metabolites may have suppressed the activity or synthesis of enzymes needed for respiratory metabolism. According to Lignowski (47), the lack of cell division in these enlargements is due to disruption of the spindle apparatus. Shahied (74) has postulated an effect of trifluralin on sulfhydryl groups, leading to inhibition of cell division, as cysteine reversed the effect of trifluralin on lateral roots.

Analysis of radioactivity indicated that the total amount and concentration of profluralin was greater in and on the roots than in the tops of all species at all temperatures (Figures 2-4). In general, the radioactivity associated with roots decreased and increased in tops with increases in temperature. When barnyardgrass was exposed to profluralin at the various temperatures, the level of profluralin radioactivity in tops increased slightly with increasing temperature (Figure 2A). ^{14}C-profluralin sorbed by roots varied but lowest amounts appeared at the higher temperatures. Profluralin radioactivity in pigweed tops increased slightly with temperature increase (Figure 3A). However, the level of profluralin radioactivity sorbed by the pigweed roots decreased by half as the temperature increased from 16 C to 38 C. Profluralin levels in sorghum tops (Figure 2B) doubled as the temperature increased from 16 C to 38 C. However, sorghum roots showed a decrease in profluralin amounts as temperature increased. ^{14}C-profluralin amounts in soybean tops (Figure 3B) doubled as the temperature increased from 16 C to 38 C. A decrease of herbicide level associated with the soybean roots was noted as the temperature increased. Penner (65) reported soybeans grown at 30 C concentrated less ^{14}C-trifluralin in their roots than soybeans grown at 25 C.

On a percentage basis, it appears that soybean and pigweed roots sorbed the most profluralin. This may have been due to differences in the size of the plants. When expressed on a per gram fresh weight

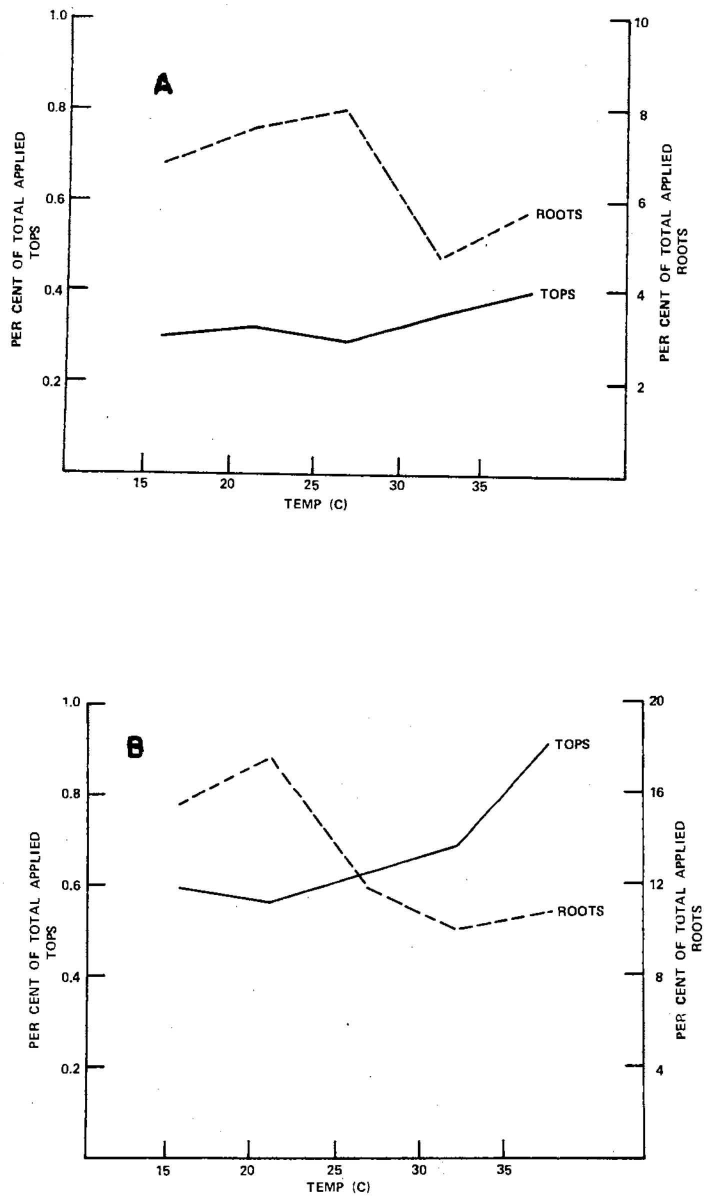
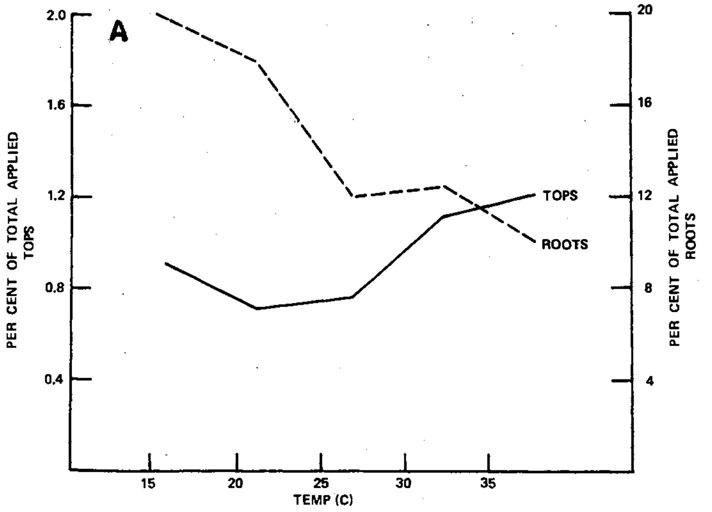


Figure 2. The Effects of Temperature on the Accumulation of ^{14}C-Profluralin in Barnyardgrass (A) and Sorghum (B) 24 hr After Treatment. The LSD (95% Level of Confidence) for Tops was 0.59 and for Roots was 4.75%.



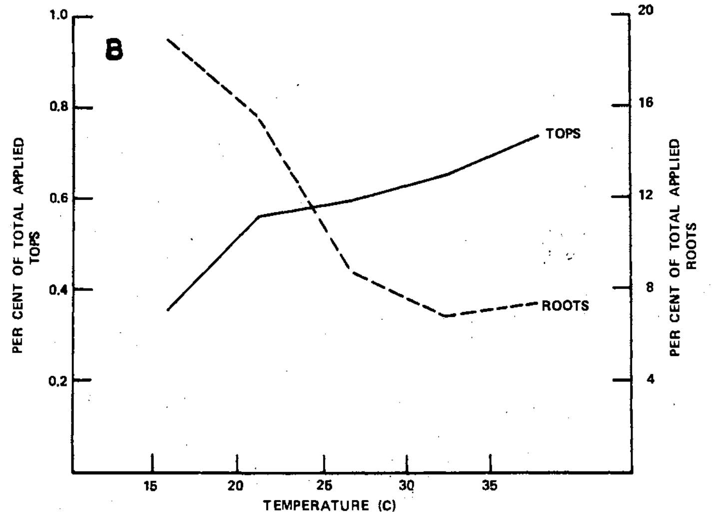


Figure 3. The Effects of Temperature on the Accumulation of ^{14}C-Profluralin in Pigweed (A) and Soybean (B) 24 hr After Treatment. The LSD (95% Level of Confidence) for Tops was 0.59 and for Roots was 4.75%.

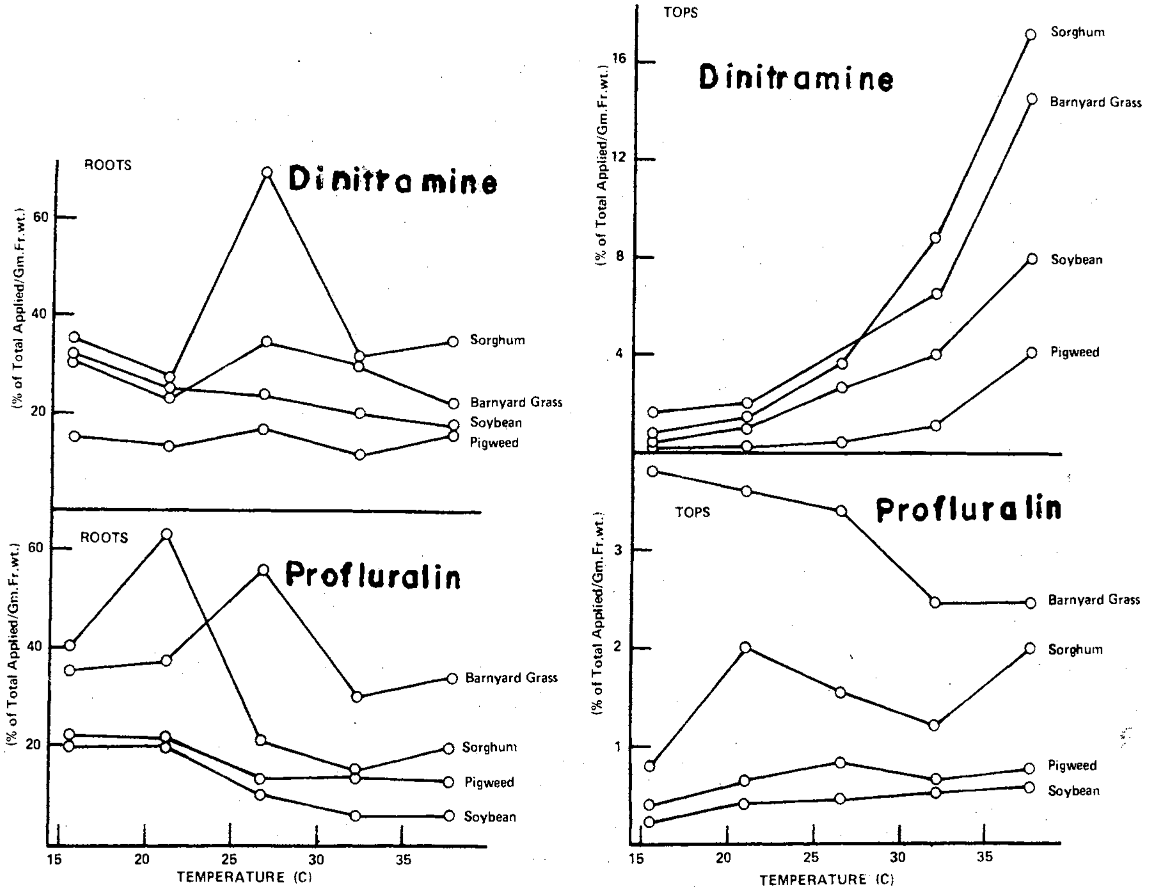


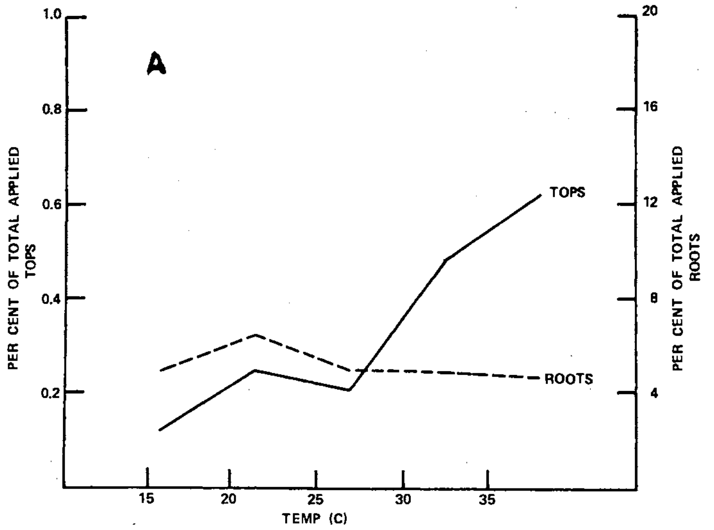
Figure 4. The Effects of Temperature on the Accumulation of ^{14}C-Profluralin and ^{14}C-Dinitramine in Plants 24 hr After Treatment.

basis, barnyardgrass and sorghum roots sorbed greater concentrations of profluralin than soybean or pigweed roots (Figure 4). Sorghum and barnyardgrass tops also accumulated higher concentrations of profluralin when expressed on a per gram fresh weight basis (Figure 4). This may be responsible for the phytotoxicity of the herbicide to susceptible species. However, very little profluralin was translocated to the tops of any of the species. Analysis of variance did indicate significant differences at the 95% level in profluralin concentration between temperature treatments in and on roots of pigweed, sorghum, and soybean.

Nutrient samples taken at the end of the 24 hr experiment indicated a decline of profluralin concentration as the temperature increased in all species. The average amount (per cent of total applied) left in the nutrient solution for all species at 16 C was 12%, while the average amount left at 38 C was 7%. There was a decline in the average percentage left in solution for all species as the temperature increased from 16 C to 38 C. Possibly the high volatility of the herbicide was a factor in the final concentration of profluralin in the nutrient solution. Since profluralin volatility increased with temperature, plants were exposed to less herbicide with time at higher temperatures, and absorption and translocation were less. Swann and Behrens (80) indicated that volatility of trifluralin was an important factor in herbicide loss from nutrient solutions and soil. They reported increased volatility with increasing temperature and soil moisture. They have also reported the vapor phase may be responsible for herbicide activity of soil applied trifluralin.

All species exposed to dinitramine appeared to accumulate more total radioactivity and higher concentration of radioactivity on a per gram fresh weight basis and in and on roots than in tops. In general, there were large increases in concentration and total dinitramine label in tops while there were no changes or only slight decreases in radioactivity sorbed in roots with increases in temperature (Figures 5 and 6). When barnyardgrass was exposed to dinitramine in nutrient solution at the various temperatures, the total herbicide or metabolites increased five times in the plant tops as the temperature increased from 16 C to 38 C (Figure 5A). Dinitramine radioactivity associated with barnyardgrass roots was almost constant as the temperature increased over the same range. The dinitramine radioactivity in pigweed tops also increased by a factor of five as the temperature increased from 16 C to 38 C (Figure 6A). Dinitramine radioactivity associated with pigweed roots was variable but was lowest at 38 C. The amount of dinitramine label in sorghum tops (Figure 5B) increased about three times as temperature increased over the given temperature range. The highest dinitramine level in any of the species was observed in and on sorghum roots at 16 C and amounts associated with the roots decreased as the temperature increased. Soybean plants responded much like the other species (Figure 6B). Soybean plant tops showed an increase in ^{14}C label of about five fold as temperature increased from 16 C to 38 C. Dinitramine levels in and on soybean roots decreased as the temperature increased from 16 C to 38 C. Analysis of variance showed differences (significant at the 95% level) between temperature treatments associated with roots of soybean and sorghum and in tops of pigweed and soybean.

Analysis of nutrient samples indicated a higher percentage of dinitramine than profluralin was left in the solution at the end of 24 hr. The average percentage left in solution for all species at 16 C



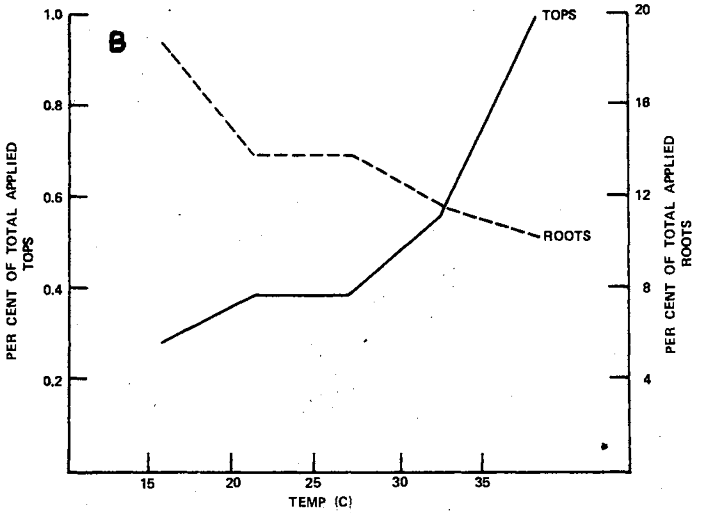
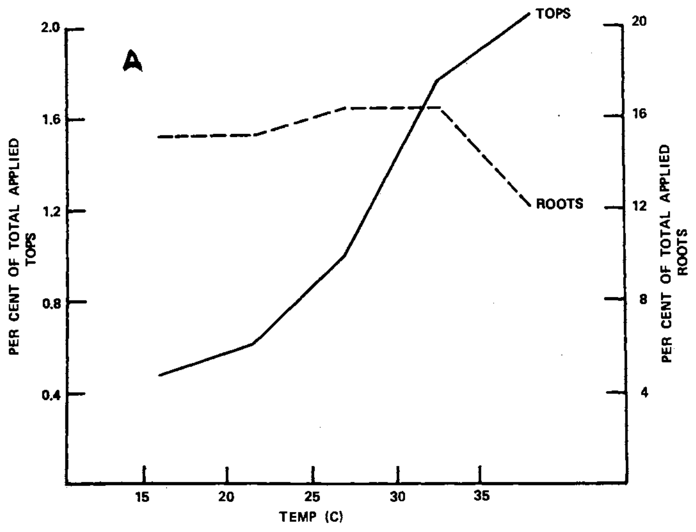


Figure 5. The Effects of Temperature on the Accumulation of ^{14}C-Dinitramine in Barnyardgrass (A) and Sorghum (B) 24 hr After Treatment. The LSD (95% Level of Confidence) for Tops was 0.59 and for Roots was 4.75%.



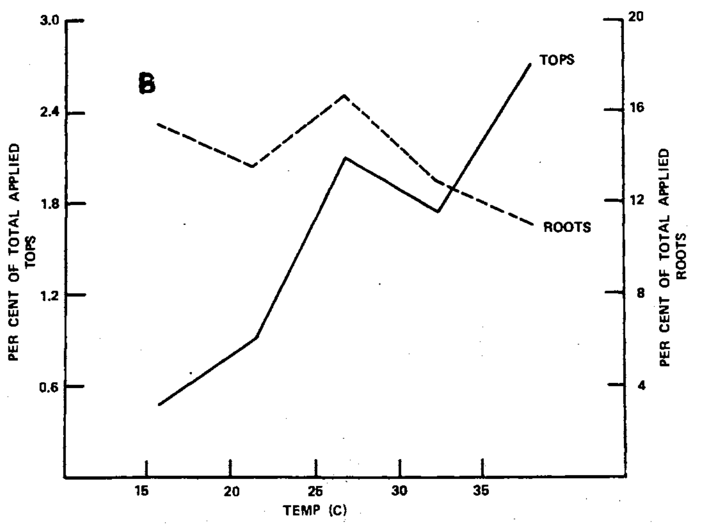


Figure 6. The Effects of Temperature on the Accumulation of ^{14}C-Dinitramine in Pigweed (A) and Soybean (B) 24 hr After Treatment. The LSD (95% Level of Confidence) for Tops was 0.59 and for Roots was 4.75%.

was 55% and at 38 C the average percent for all species was 41%. There was a decline in the amount left in solution for all species as the temperature increased from 16 C to 38 C. However, dinitramine was not as volatile in water solution as was profluralin. Root sorption again was greatest in pigweed and soybean, but, when expressed on a per gram fresh weight basis, sorption of dinitramine was highest in barnyardgrass roots and sorghum roots (Figure 4). Sorghum tops and barnyardgrass tops also accumulated greater concentrations of dinitramine when expressed on a per gram fresh weight basis (Figure 4). Translocation of dinitramine to the plant tops was greater with higher temperatures and this may indicate a certain degree of apoplastic movement of the herbicide with the transpiration stream.

Water Uptake Studies

To determine the effect of transpiration on movement of herbicide applied in the nutrient solution, the volume of the nutrient solution in the culture bottles was measured after the 24 hr experiment with profluralin and dinitramine and intact plants. Bottles with no plants were also measured to determine the effect of aeration on water loss. Water loss was expressed on a per gram fresh weight basis. Analysis of variance of the corrected totals indicated significant differences in water loss by transpiration between barnyardgrass, pigweed, sorghum, and soybean plants at the various temperatures. These results are indicated in Figures 7 and 8. On a plant top weight basis, the susceptible species (barnyardgrass and sorghum) lost more water due to transpiration than did the resistant species (soybean and pigweed). Water loss showed an increase in a linear pattern with increasing

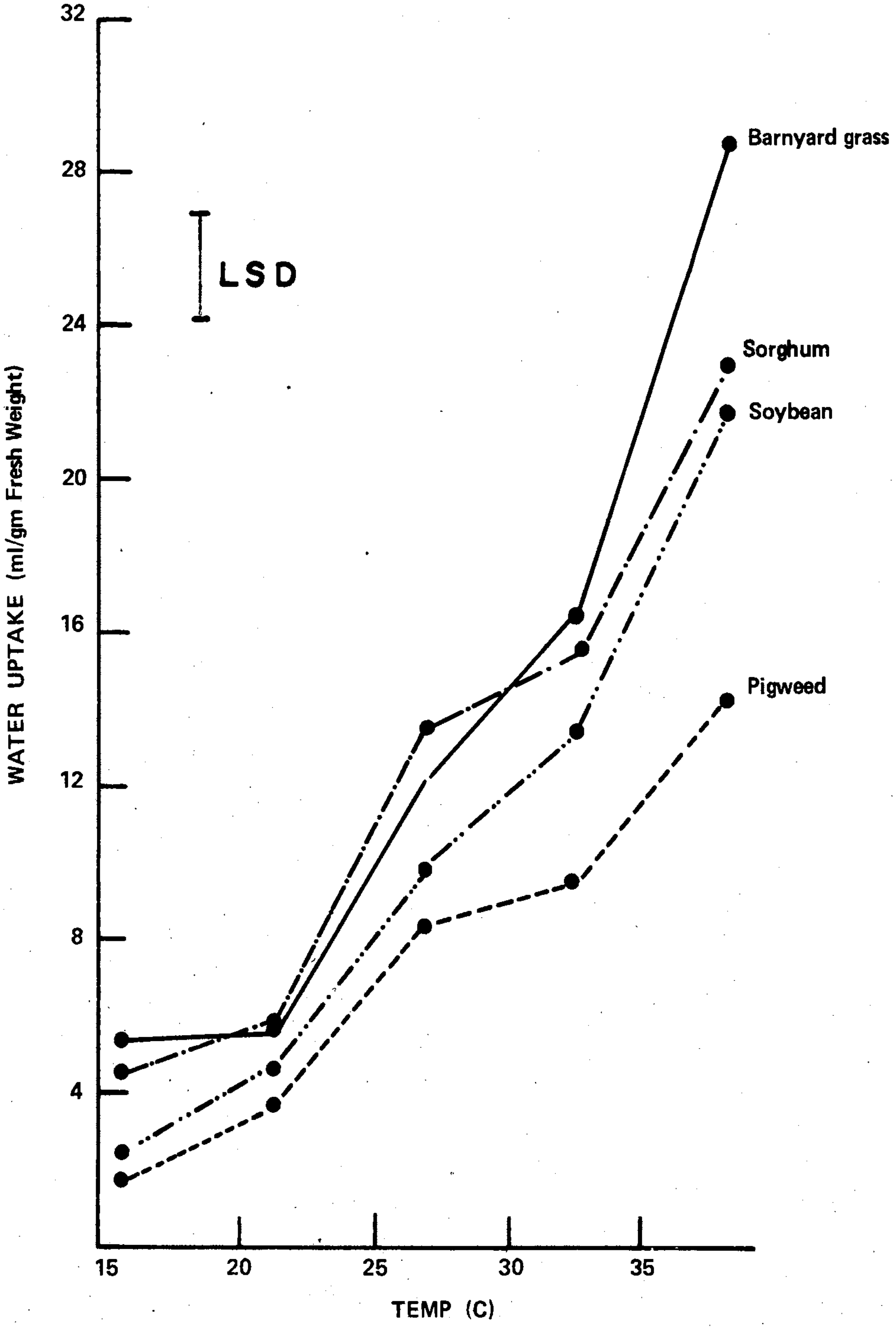


Figure 7. The Effects of Temperature on Transpirational Water Loss by Plants Cultured for 24 hr in Nutrient Solution Containing ^{14}C-Profluralin.

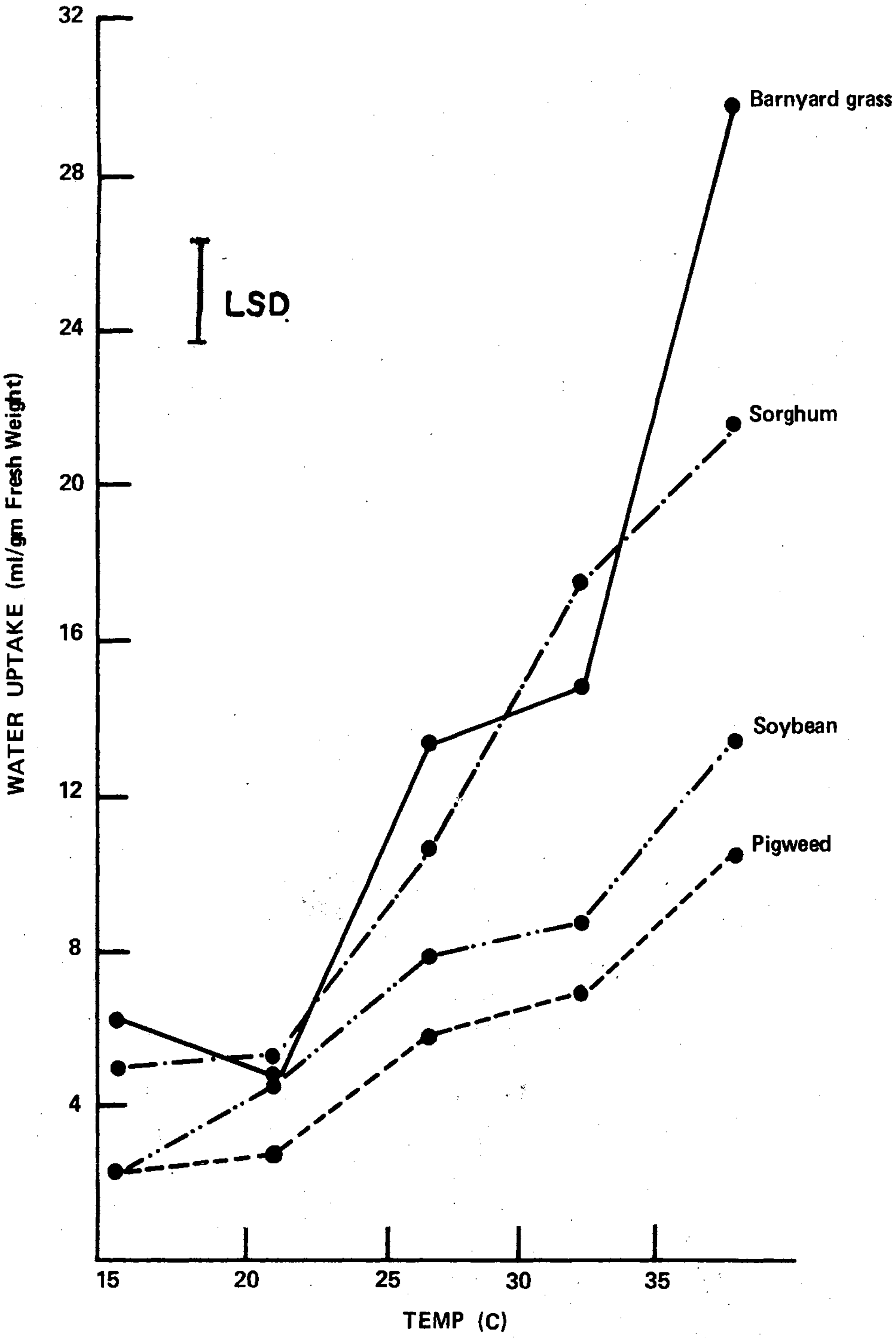


Figure 8. The Effects of Temperature and Transpirational Water Loss by Plants Cultured for 24 hr in Nutrient Solution Containing ^{14}C-Dinitramine.

temperature for plants cultured in both dinitramine and profluralin. This increase in transpiration with temperature may be responsible for an increase in herbicide concentration in the leaves and stem of plants and may lead to plant injury at higher temperatures. Sheets (75) and Vostral et al. (83) concluded that the increased concentration of s-triazines in plants was enhanced by greater transpiration at higher temperatures.

Metabolism and Accumulation of ^{14}C-profluralin and ^{14}C-dinitramine in Barnyardgrass, Sorghum, Pigweed, and Soybean at Low and High Temperatures

Two extremes of temperature used in the previous study (16 C and 38 C) were selected and plants were treated with ^{14}C-profluralin and ^{14}C-dinitramine for 24 hr. The tops and roots were homogenized and analyzed by thin layer chromatography to determine whether the radioactivity detected in the previous studies was the parent chemical or a metabolite and whether the effects of temperature on accumulation were effects on a metabolite or the intact herbicide. Small portions of the herbicide were also counted by liquid scintillation and this data was used to compare the accumulation in roots and tops with the previous studies. Autoradiograms of the intact plants were made and also used as a comparison to the data obtained in the previous studies.

Some of the radioactive material on the chromatograms appeared to have been lost, either during development or during the drying of the chromatogram. Only 85% of the profluralin standard and 51% of the dinitramine standard could be accounted for after chromatography.

A very small portion of the standards appeared as metabolites on the chromatograms (0.20% for profluralin and 4.9% for dinitramine). Since there was some loss of radioactivity, the data are expressed as a percentage of the total radioactivity found on the chromatogram. Profluralin or dinitramine ^{14}C-radioactivity that did not move from the point of application or that occurred in a number of spots on the chromatogram other than the initial spot were interpreted as metabolites. They were pooled and analyzed together.

All species translocated more total ^{14}C-dinitramine radioactivity (Tables II and III) and more radioactivity per gram fresh weight (Table I) at 38 C than at 16 C as was shown in the previous study. Of the four species, the order of total ^{14}C translocation was: soybean > pigweed > sorghum > barnyardgrass at 16 C and 38 C (Tables II and III). This was also indicated in the autoradiograms of the intact plants (Figures 9-12). However, the two susceptible species, barnyardgrass and sorghum, contained more label on a per gram fresh weight basis (Table I). In general, the level of dinitramine as well as metabolites in the tops of the plants increased as the temperature increased (Tables II and III), but there was a greater percentage of breakdown products at the high temperature. The level of metabolites in roots increased with temperature in all species except soybean, but the level of parent chemical in roots decreased or remained constant in all species as temperature increased. The percentage of dinitramine radioactivity in barnyardgrass, pigweed, and sorghum roots at 16 C was greater than at 38 C as was the case for tops. Thus the accumulation of parent chemical in the roots at low temperature was high and breakdown was low and this may be responsible for plant injury. However, the percentage of metabolites in

TABLE I

UPTAKE OF ^{14}C-PROFLURALIN AND ^{14}C-DINITRAMINE BY FOUR PLANT SPECIES AT 24 HOURS AFTER TREATMENT AT TWO TEMPERATURES

Treatment, Temperature, and Herbicide	Barnyardgrass		Pigweed		Sorghum		Soybean	
	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots
Profluralin			(Nanograms ^{14}C-herbicide/gm Fr. Wt.)					
16 C	23.4a[1]	258.0a	4.3a	40.9a	5.3a	51.5a	1.1a	34.2a
38 C	5.0b	32.7b	1.2a	6.3b	1.8b	9.8b	0.8a	5.0b
Dinitramine								
16 C	14.1a	219.8a	3.1a	73.9a	12.1a	166.9a	12.7a	157.3a
38 C	51.2b	172.5a	14.5b	80.7a	51.5b	148.0a	40.1b	93.6b

[1] Values for a single plant part, herbicide, and species followed by the same letter are not significantly different at the 0.05 level.

TABLE II

METABOLISM OF DINITRAMINE IN TOPS AND ROOTS OF BARNYARDGRASS AND SORGHUM AT 24 HOURS AFTER TREATMENT AT LOW AND HIGH TEMPERATURES

	Barnyardgrass				Sorghum			
	Tops (16 C)	Roots (16 C)	Tops (38 C)	Roots (38 C)	Tops (16 C)	Roots (16 C)	Tops (38 C)	Roots (38 C)
Total Radioactive Herbicide Spotted (ng)	4.18	38.06	20.67	32.26	5.76	92.48	28.02	68.74
Metabolites (ng)	0.63	6.99	3.67	7.44	0.89	10.27	8.30	14.64
Dinitramine (ng)	2.06	11.52	7.77	7.32	1.89	40.66	12.71	22.51
Total Radioactive Herbicide Detected After Development (%)	64.90	48.72	48.38	45.75	48.35	55.07	75.00	54.04
Metabolites (%)[1]	23.43	37.71	32.21	50.43	32.17	20.17	39.51	39.41

[1] The metabolites are expressed as a per cent of the total radioactivity found on the chromatogram after development. The standard dinitramine showed 4.9% breakdown products at the time of treatment and 51.4% of the standard was recovered on the chromatogram after development.

TABLE III

METABOLISM OF DINITRAMINE IN TOPS AND ROOTS OF PIGWEED AND SOYBEAN AT 24 HOURS AFTER TREATMENT AT LOW AND HIGH TEMPERATURES

	Pigweed				Soybean			
	Tops (16 C)	Roots (16 C)	Tops (38 C)	Roots (38 C)	Tops (16 C)	Roots (16 C)	Tops (38 C)	Roots (38 C)
Total Radioactive Herbicide Spotted (ng)	7.79	58.11	44.58	67.69	27.68	114.67	98.25	87.56
Metabolites (ng)	0.42	3.07	3.09	5.51	2.33	15.64	8.84	6.29
Dinitramine (ng)	3.96	21.68	12.93	16.87	6.41	29.77	31.01	28.67
Total Radioactive Herbicide Detected After Development (%)	56.20	42.60	35.96	33.05	31.16	39.81	40.56	39.94
Metabolites (%)[1]	9.57	12.41	19.32	24.62	26.69	34.43	22.19	18.00

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[1] The metabolites are expressed as a per cent of the total radioactivity found on the chromatogram after development. The standard dinitramine showed 4.9% breakdown products at the time of treatment and 51.4% of the standard was recovered on the chromatogram after development.

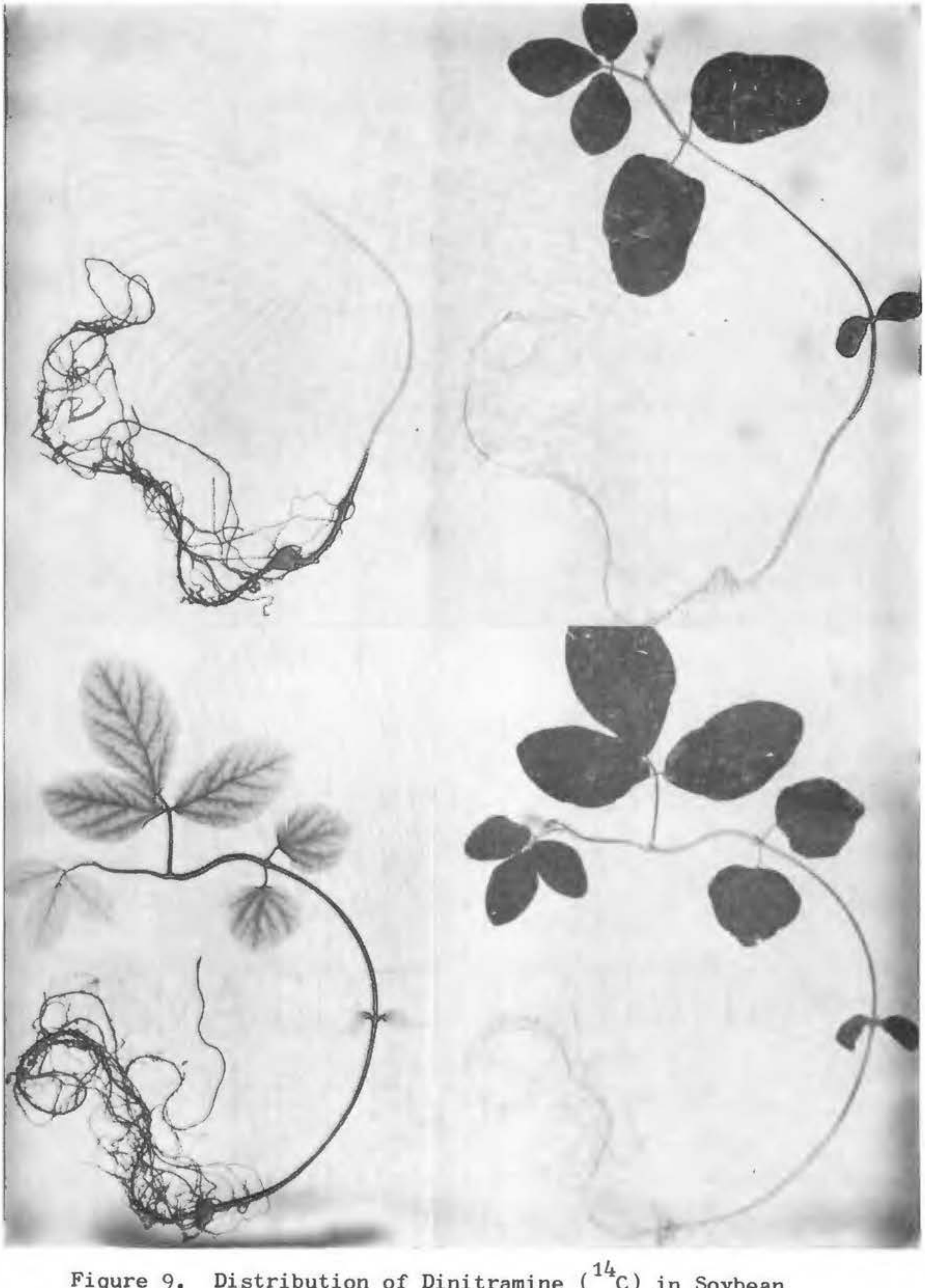

Figure 9. Distribution of Dinitramine (^{14}C) in Soybean 24 hr After Application to the Nutrient Solution at 16 C (Top) and 38 C (Bottom). Left: Autoradiogram. Right: Plant in Light.

Figure 10. Distribution of Dinitramine (^{14}C) in Pigweed 24 hr After Application to the Nutrient Solution at 16 C (Top) and 38 C (Bottom). Left: Autoradiogram. Right: Plant in Light.

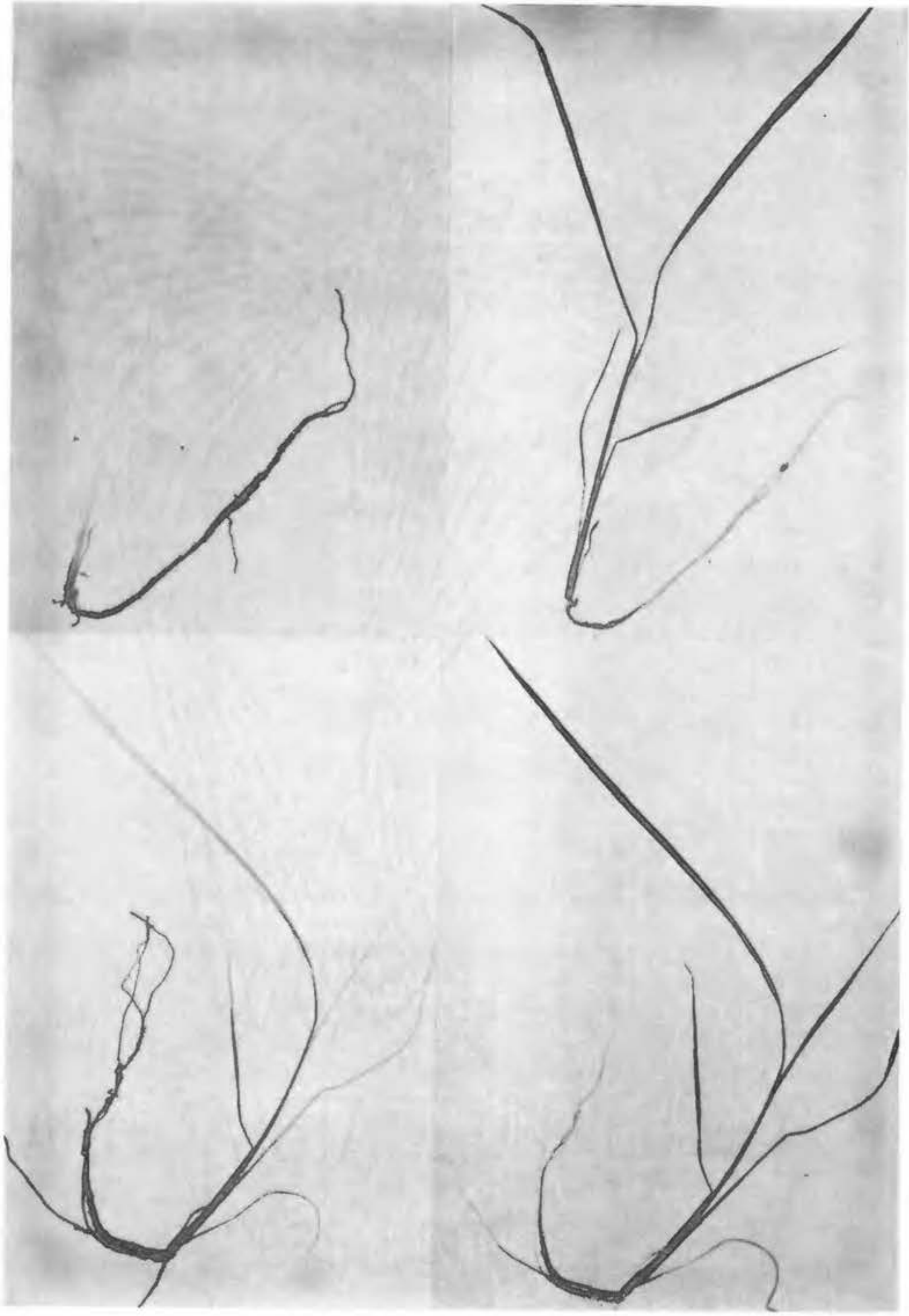

Figure 11. Distribution of Dinitramine (^{14}C) in Barnyardgrass 24 hr After Application to the Nutrient Solution at 16 C (Top) and 38 C (Bottom). Left: Autoradiogram. Right: Plant.

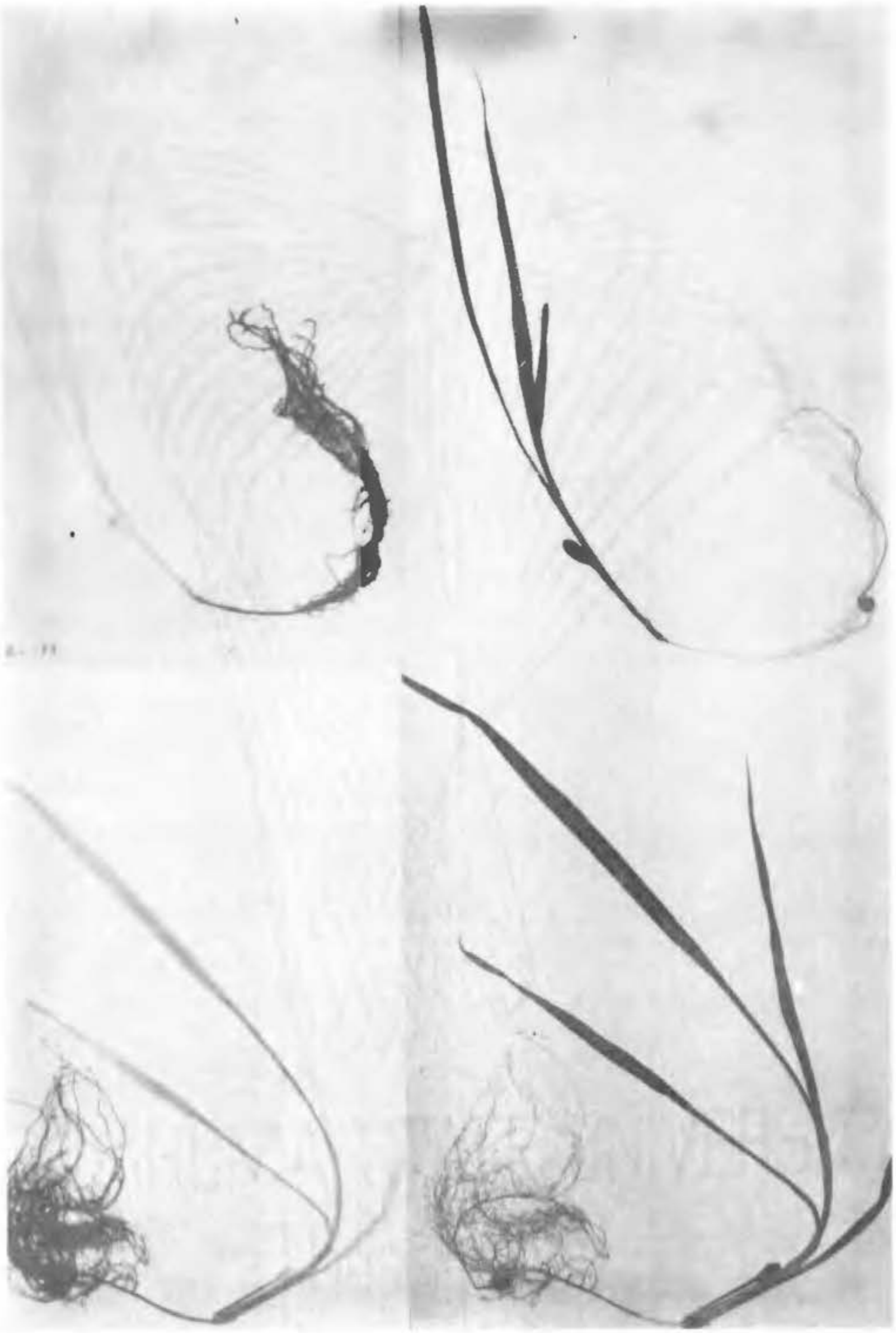

Figure 12. Distribution of Dinitramine (^{14}C) in Sorghum 24 hr After Application to the Nutrient Solution at 16 C (Top) and 38 C (Bottom). Left: Autoradiogram. Right: Plant in Light.

susceptible species (barnyardgrass and sorghum) was more than in the resistant species (pigweed and soybean), especially in the roots at 38 C. The accumulation of metabolites at higher temperatures could possibly be responsible for injury to susceptible plants. Biswas et al. (9) has reported that degradation products of trifluralin exhibited herbicidal properties, some greater than those of the parent compound. However, since much more translocation occurred at 38 C than at 16 C (Figures 9-12), the accumulation of greater parent chemical as well as metabolites in the tops of plants at higher temperatures may be responsible for dinitramine phytotoxicity. Penner (65) indicated increased trifluralin transport to plant shoots at high temperature increased phytototoxicity.

The data shown in Tables I, IV and V and Figures 13-16 further illustrate the uptake pattern of ^{14}C-profluralin radioactivity at low and high temperatures. Greater amounts of ^{14}C were found in and on the roots of all species treated at 16 C than those grown at 38 C, indicating greater absorption or greater adsorption at low temperatures (Tables IV and V). The concentration on a fresh weight basis was higher for barnyardgrass and sorghum, the susceptible species, than for pigweed and soybean, the resistant species (Table I). Only small amounts of ^{14}C were found in the tops of each species at either temperature. Thus, in contrast to dinitramine, profluralin appears to be translocated only very slightly from the roots to the tops of these species. Table I indicates that some accumulation of ^{14}C did occur in the tops of plants and that this was higher in the plants treated at the low temperature. This effect may appear to be a contradiction of the data shown in the autoradiograms in Figures 13-16. The autoradiograms indicate that there was a temperature effect where high temperatures caused an increased

TABLE IV

METABOLISM OF PROFLURALIN IN TOPS AND ROOTS OF BARNYARDGRASS AND SORGHUM AT 24 HOURS AFTER TREATMENT AT LOW AND HIGH TEMPERATURES

	Barnyardgrass				Sorghum			
	Tops (16 C)	Roots (16 C)	Tops (38 C)	Roots (38 C)	Tops (16 C)	Roots (16 C)	Tops (38 C)	Roots (38 C)
Total Radioactive Herbicide Spotted (ng)	0.91	6.53	----	0.97	2.33	18.68	0.52	2.08
Metabolites (ng)	0.12	0.49	0.11	0.16	0.10	0.90	0.09	0.44
Profluralin (ng)	0.57	3.49	0.63	0.64	1.84	10.58	0.27	0.61
Total Radioactive Herbicide Detected After Development (%)	75.81	60.93	----	82.51	83.35	61.42	70.00	50.28
Metabolites (%)[1]	17.40	12.30	14.90	20.05	5.20	7.90	25.80	41.90

[1] The metabolites are expressed as a per cent of the total radioactivity found on the chromatogram after development. The profluralin standard showed 0.20% breakdown products at the time of treatment and 84.6% of the standard was recovered on the chromatogram after development.

TABLE V

METABOLISM OF PROFLURALIN IN TOPS AND ROOTS OF PIGWEED AND SOYBEAN AT 24 HOURS AFTER TREATMENT AT LOW AND HIGH TEMPERATURES

	Pigweed				Soybean			
	Tops (16 C)	Roots (16 C)	Tops (38 C)	Roots (38 C)	Tops (16 C)	Roots (16 C)	Tops (38 C)	Roots (38 C)
Total Radioactive Herbicide Spotted (ng)	3.53	7.48	1.19	1.20	1.85	29.54	2.13	3.95
Metabolites (ng)	0.21	1.13	0.13	0.54	0.11	1.95	0.34	0.86
Profluralin (ng)	2.10	3.05	0.60	0.42	1.29	13.88	0.39	0.67
Total Radioactive Herbicide Detected After Development (%)	65.40	55.90	78.20	80.00	76.20	53.60	34.30	38.70
Metabolites (%)[1]	9.10	27.10	35.50	66.20	8.50	12.30	46.60	66.20

[1] The metabolites are expressed as a per cent of the total radioactivity found on the chromatogram after development. The profluralin standard showed 0.20% breakdown products at the time of treatment and 84.6% of the standard was recovered on the chromatogram after development.

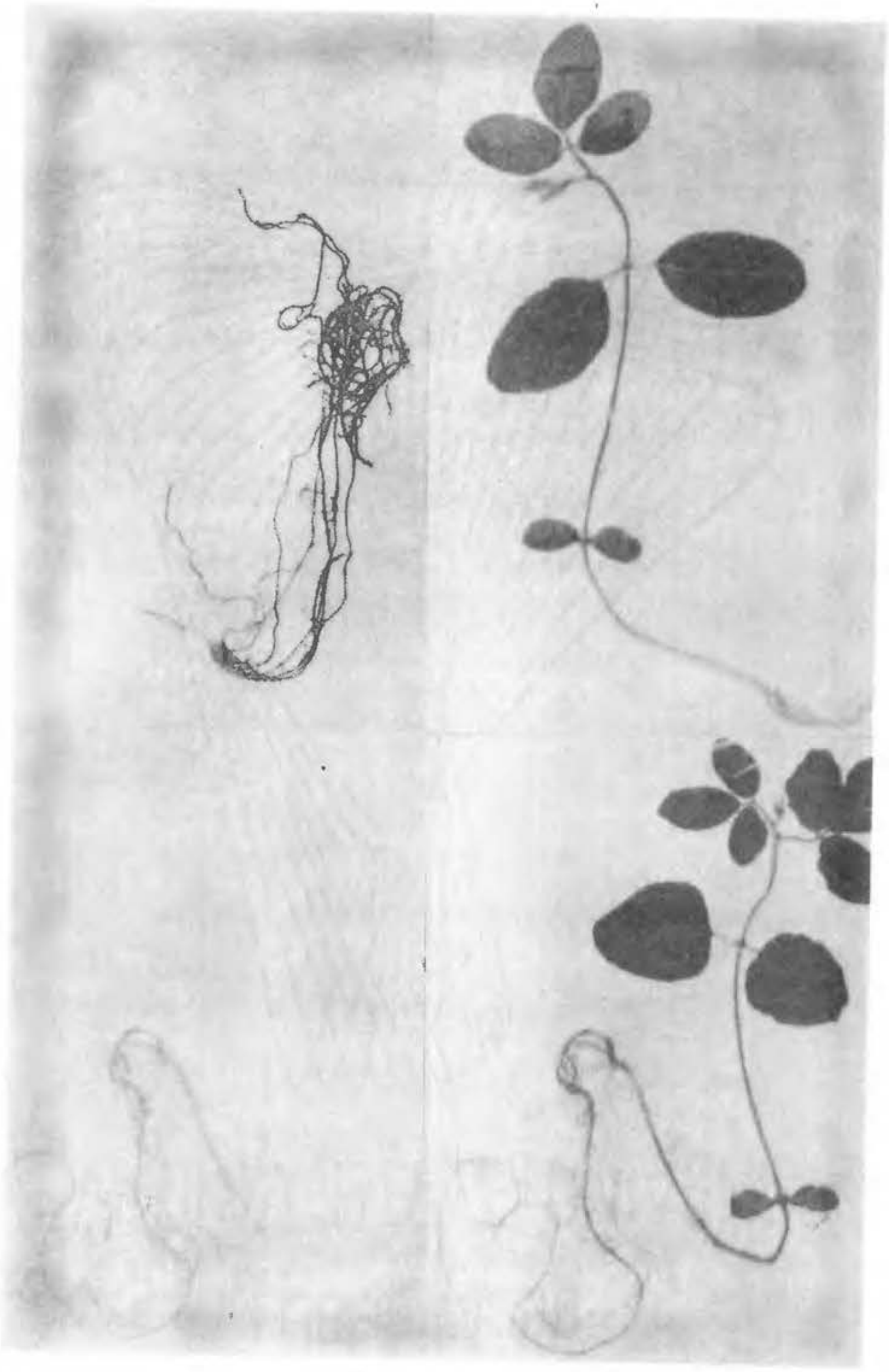

Figure 13. Distribution of Profluralin (^{14}C) in Soybean 24 hr After Application to the Nutrient Solution at 16 C (Top) and 38 C (Bottom). Left: Autoradiogram. Right: Plant in Light.

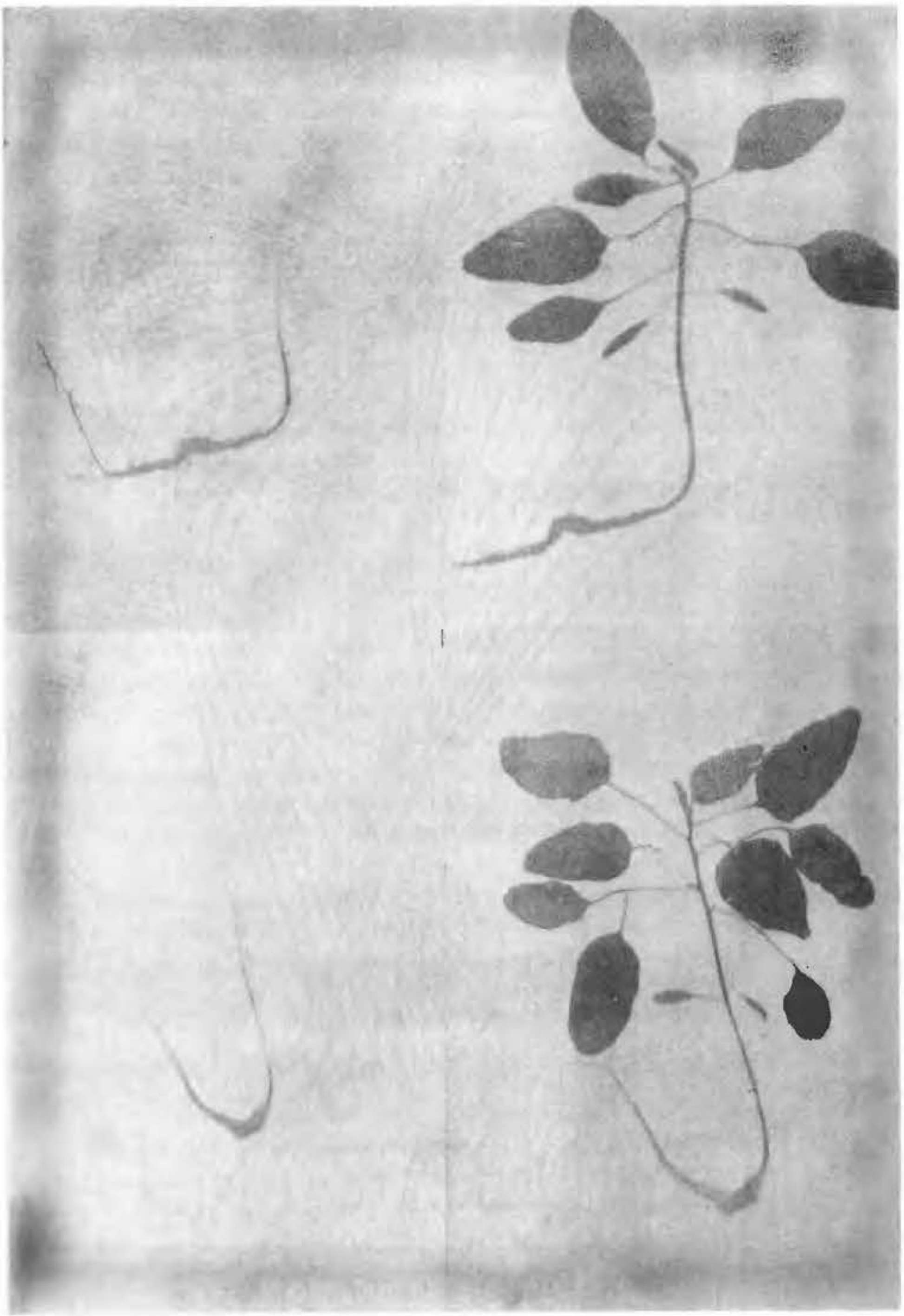

Figure 14. Distribution of Profluralin (^{14}C) in Pigweed 24 hr After Application to the Nutrient Solution at 16 C (Top) and 38 C (Bottom). Left: Autoradiogram. Right: Plant in Light.

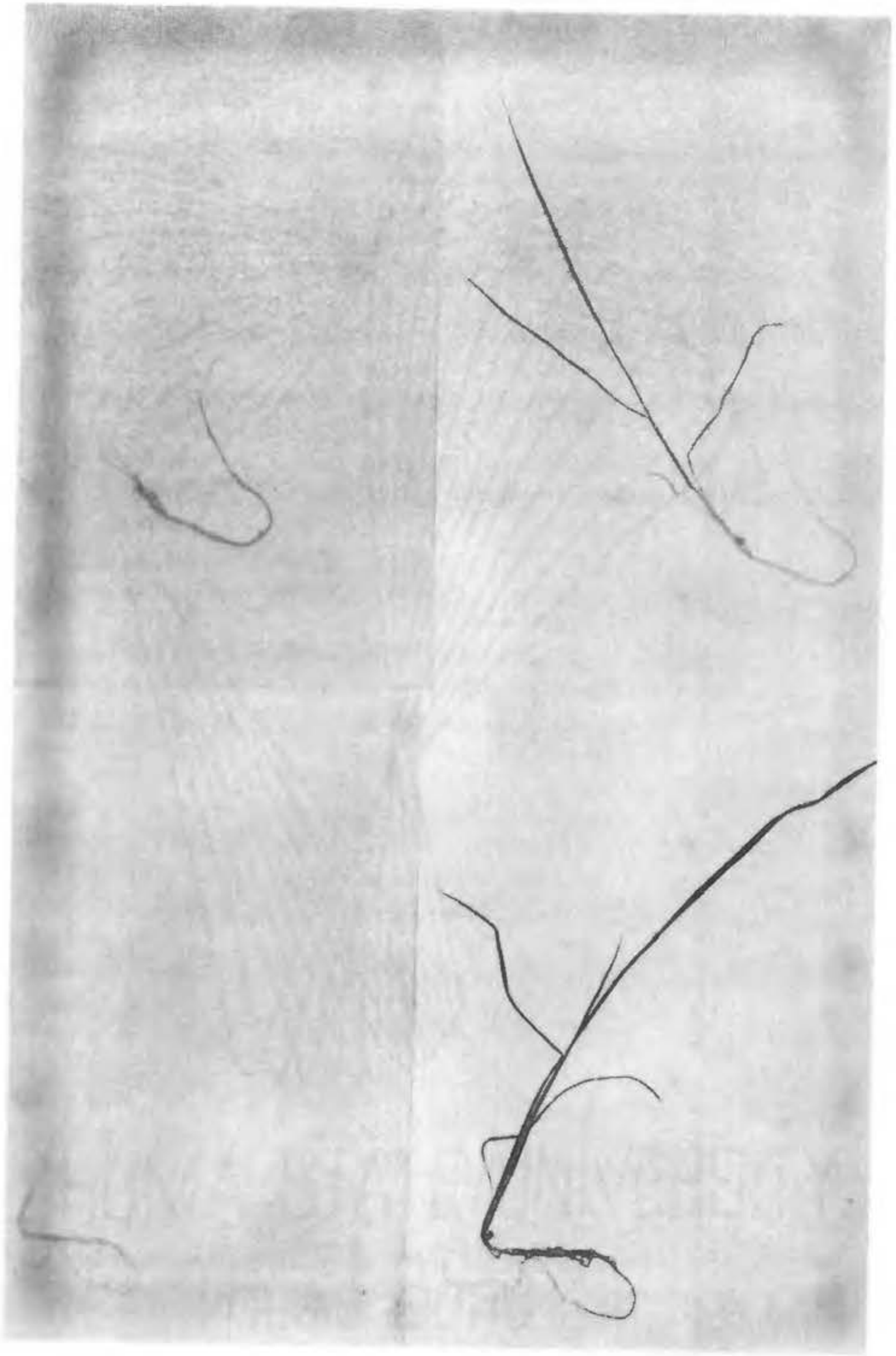

Figure 15. Distribution of Profluralin (^{14}C) in Barnyardgrass 24 hr After Application to the Nutrient Solution at 16 C (Top) and 38 C (Bottom). Left: Autoradiogram. Right: Plant in Light.

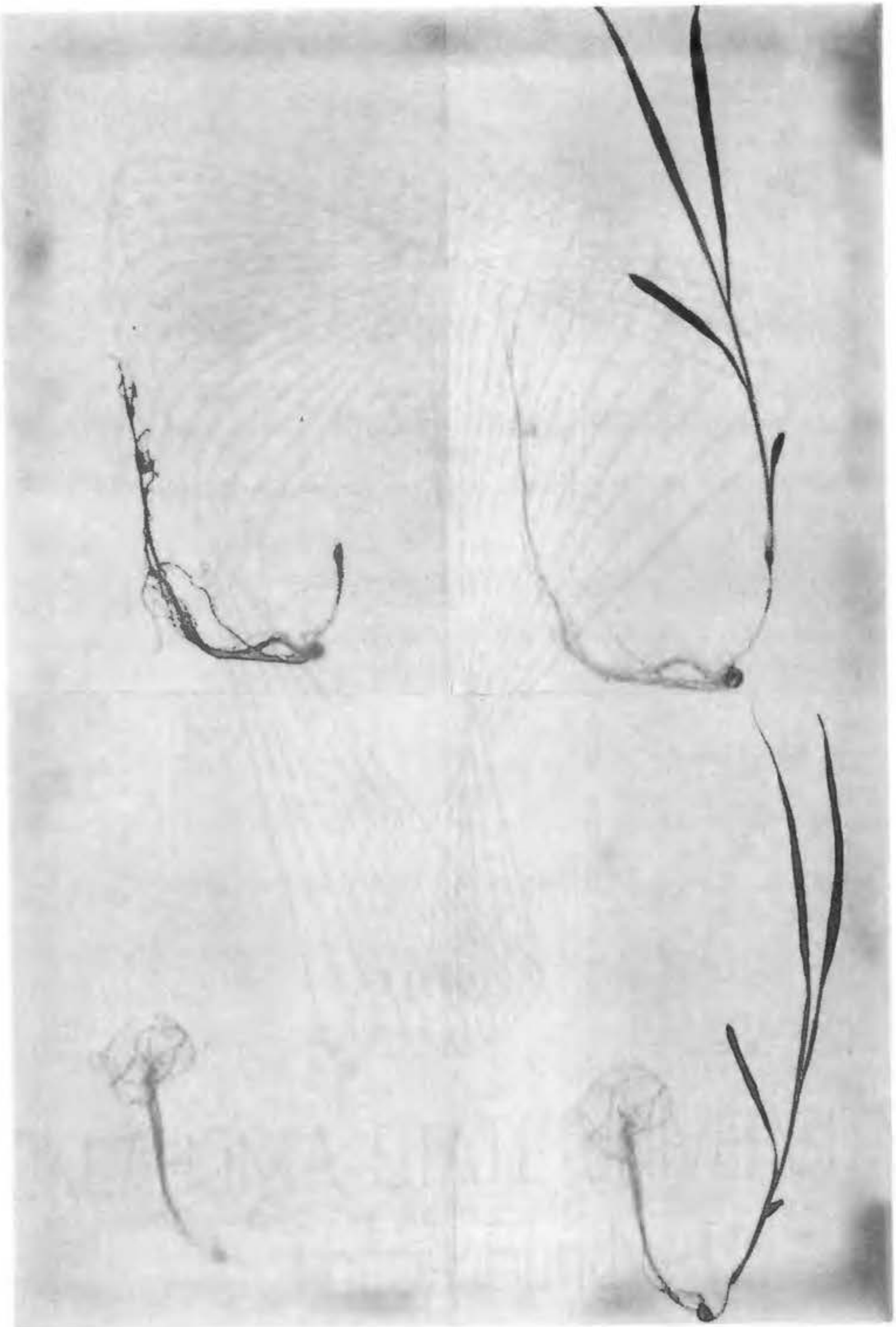

Figure 16. Distribution of Profluralin (^{14}C) in Sorghum 24 hr After Application to the Nutrient Solution at 16 C (Top) and 38 C (Bottom). Left: Autoradiogram. Right: Plant in Light.

movement of profluralin radioactivity, especially into the upper leaf tissue of soybean, sorghum, and barnyardgrass. The lower stem tissue appears to have been labeled more heavily at low temperatures. This increased labeling of lower stem tissue may have been the result of accumulation directly from the nutrient solution, since the lower stems were placed somewhat below the surface of the nutrient solution during treatment. The nutrient solution maintained at the low temperature retained more of the herbicide during treatment than those at high temperature and would thus be a source of label. The roots of plants in the low temperature cultures were heavily labeled and would also be an ample source of label for the lower stem if translocation occurred to any appreciable extent. The high degree of accumulation of ^{14}C-profluralin radioactivity in the entire tops of plants at low temperatures shown in Table I was probably the result of increased labeling in the lower stem. High temperature caused some movement into the upper leaf tissue in some species that was not noticed at the low temperature (Figures 13-16), and, as can be seen by analyzing the data in Table I, a higher percentage of the radioactivity absorbed by roots at high temperatures was translocated to the tops than in plants at low temperatures. The total amounts of ^{14}C-profluralin radioactivity translocated to tops was very low in all cases as compared to ^{14}C-dinitramine translocation.

The metabolism of ^{14}C-profluralin was affected by temperature as was the case for ^{14}C-dinitramine. High temperature caused an increased breakdown of ^{14}C-profluralin in the roots of all species and in the tops of all species except barnyardgrass (Tables IV and V). In general, metabolism in roots was greater than in the tops and was greater in the resistant species than in the susceptible species, especially at the high temperature.

Uptake of Profluralin from Soil

Plants grown in ^{14}C-profluralin treated soil were studied to give an idea of the uptake in soil versus uptake from nutrient solution. The uptake of profluralin label from the soil by cotton, soybean, and peanut plants after five days of culture indicated a species difference to the herbicide. The accumulation of ^{14}C-profluralin was greater in roots than in tops in all species at the temperature ranges studied (Figure 17). This was also indicated in the autoradiograms of plants exposed to ^{14}C-profluralin (Figures 13-16). The concentration of ^{14}C-profluralin in peanut and cotton roots increased with temperature while a small decline was noted in ^{14}C-profluralin concentration in and on soybean roots as temperature increased. ^{14}C-profluralin concentration in peanut and soybean tops declined slightly as temperature increased but tops of cotton reflected an increase in ^{14}C-profluralin concentration from 21-27 C and a decrease from 27-32 C. Greater herbicide uptake in peanut and cotton roots with increasing temperature may be due to reduced soil adsorption, thus releasing more herbicide. As adsorption processes are exothermic, reduced adsorption would be expected at higher temperatures. Parochetti and Hein (63) reported the volatility of trifluralin and benefin increased with increasing temperature and soil moisture. Since trifluralin vapors are very toxic and appear to be absorbed by roots and shoots as the vapor (80), perhaps profluralin vapors exhibit similar properties and thus affect an increased absorption into plants at increased temperatures. Plant

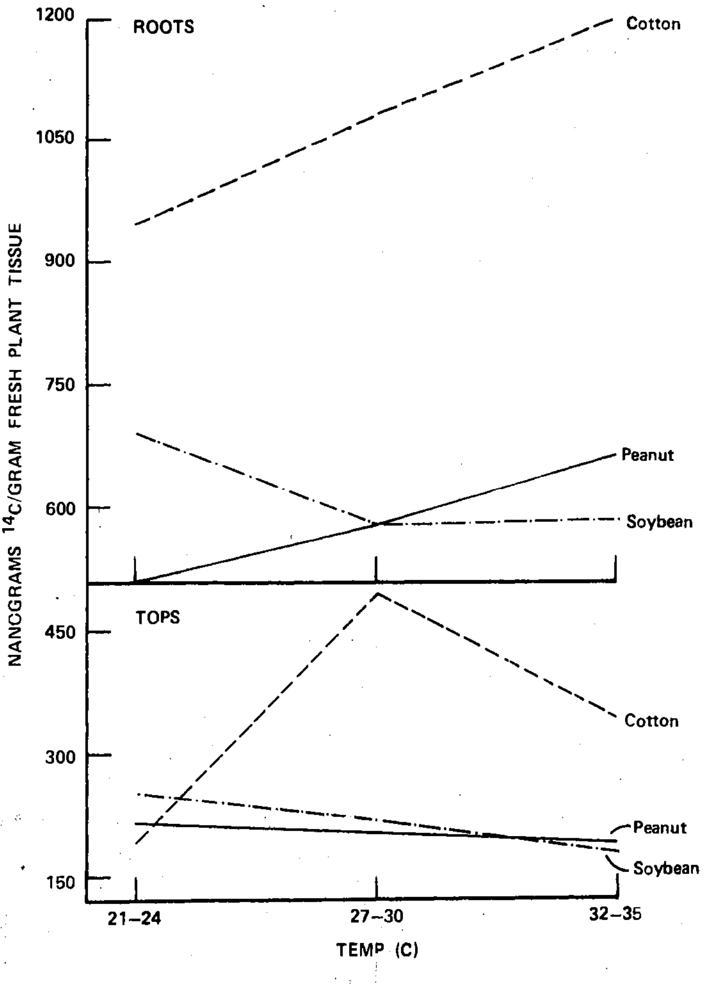

Figure 17. The Effects of Various Day-Night Temperature Ranges on the Accumulation of ^{14}C-Profluralin in Cotton, Peanut, and Soybean During 5 Days of Growth.

roots, growing in nutrient solution, would not be exposed to such vapors. This may explain why the roots did not absorb more profluralin and dinitramine at high temperatures in the nutrient solution studies.

Absorption Studies with Excised Root Tips

The previous studies indicated that there was a greater accumulation of ^{14}C-profluralin at low temperatures in the roots of intact plants. Studies were initiated to determine whether these effects could be observed in the uptake of profluralin by excised root tips. The rate of absorption of profluralin in excised roots of sorghum, soybean, cotton, and peanut for times up to 12 hr at several temperatures was determined (Figures 18 and 19). The data represent averages of five replications for each temperature and species. The initial average fresh weight for lateral roots was: sorghum, 0.51 mg; cotton, 0.57 mg; peanut, 1.29 mg; and soybean, 1.19 mg. The initial fresh weight (average) for tap roots was: sorghum, 1.42 mg; cotton, 3.86 mg; peanut, 12.0 mg; and soybean, 3.76 mg. The experiment was repeated. Analysis of variance showed differences (significant at the 95% level of confidence) in tap roots of cotton at 12 hr, tap roots of peanut at one and four hr, and tap roots of soybean at one and four hr. Differences (significant at the 95% level of confidence) were also noted in lateral roots of sorghum at one and four hr, lateral roots of cotton at one and four hr, and lateral roots of peanut at all treatment times.

In general, the amount of sorption by tap roots at the various temperatures was as follows: sorghum > soybean > cotton > peanut. At each time period studied, there was a general trend toward greater accumulation at higher temperatures. Tap roots continued to sorb

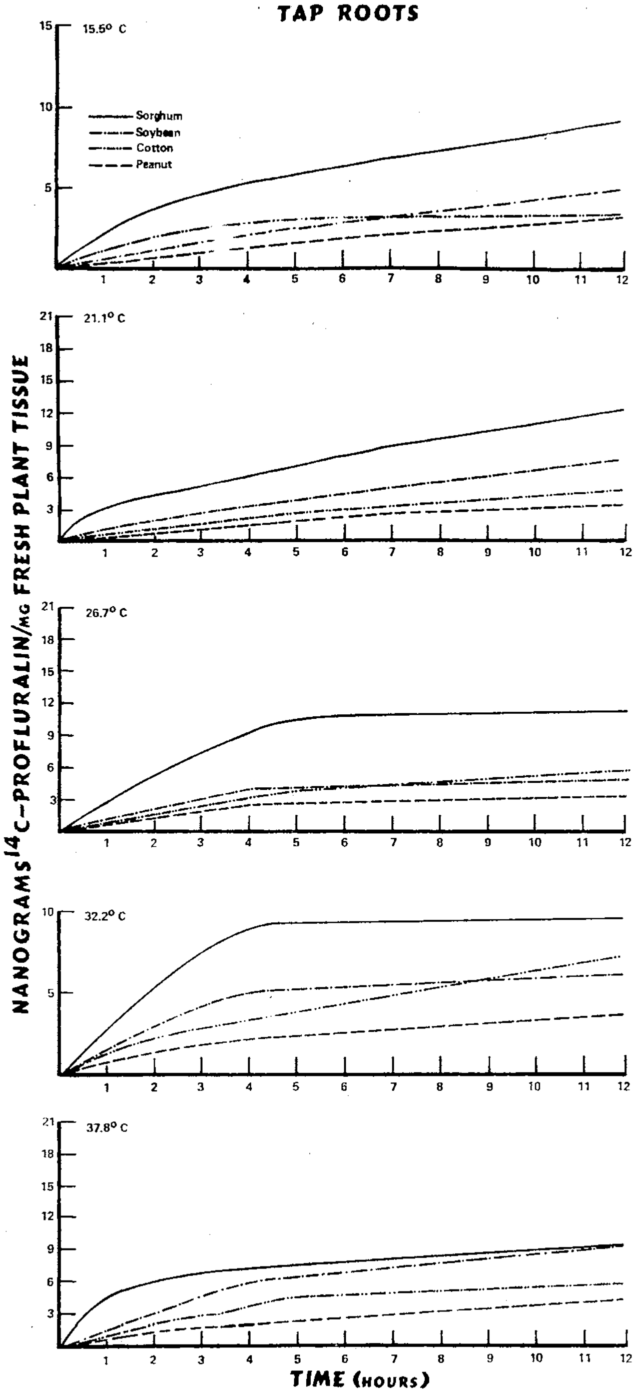

Figure 18. Rate of Sorption of ^{14}C-Profluralin in Excised Primary Roots.

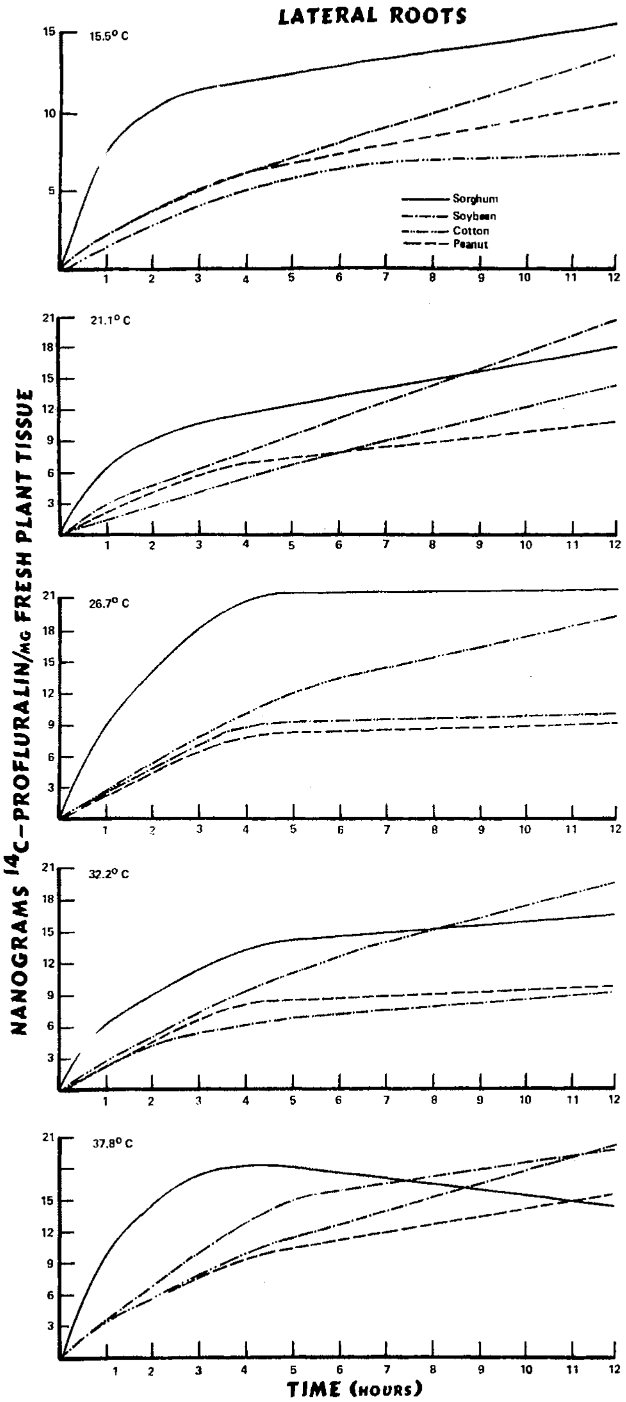

Figure 19. Rate of Sorption of ^{14}C-Profluralin by Excised Lateral Roots.

profluralin during the 12 hr experiment and in general had not reached equilibrium at 12 hr (Figure 18). The total amount of herbicide accumulated at the end of one hr by tap roots increased almost linearly with temperature in cotton, soybean, and peanut (Figure 20). At the end of four hr, there was again a linear increase of profluralin concentration with temperature in cotton, soybean, and peanut tap roots. At 12 hr cotton and peanut tap roots tended to accumulate more profluralin with increasing temperature. Thus, under these conditions, there were no indications that excised tap roots showed increased accumulation at low temperature as was noted associated with the root system of intact plants.

Lateral roots of all species had higher initial rates of absorption than tap roots (Figure 19). Initial rates of absorption were greater than those at later times. Only sorghum lateral roots reached equilibrium at the end of 12 hr and then only at higher temperatures. The lateral roots of the other species continued to accumulate more profluralin with time. Accumulation of herbicide appeared to increase with time and temperature. Moody et al. (53) reported the rate of herbicide uptake was greatest during the first hour. Uptake increased as temperature increased from 5 to 30 C.

The total absorption of profluralin at 12 hr was not proportional to the initial rates of absorption (Figure 20). This may reflect the effects of temperature on level at equilibrium rather than temperature effects on rates of absorption.

In comparing rate of uptake in lateral and tap roots of the various species, it was apparent that uptake in sorghum lateral and tap roots was greater than in the other species (Figures 18 and 19). Higher

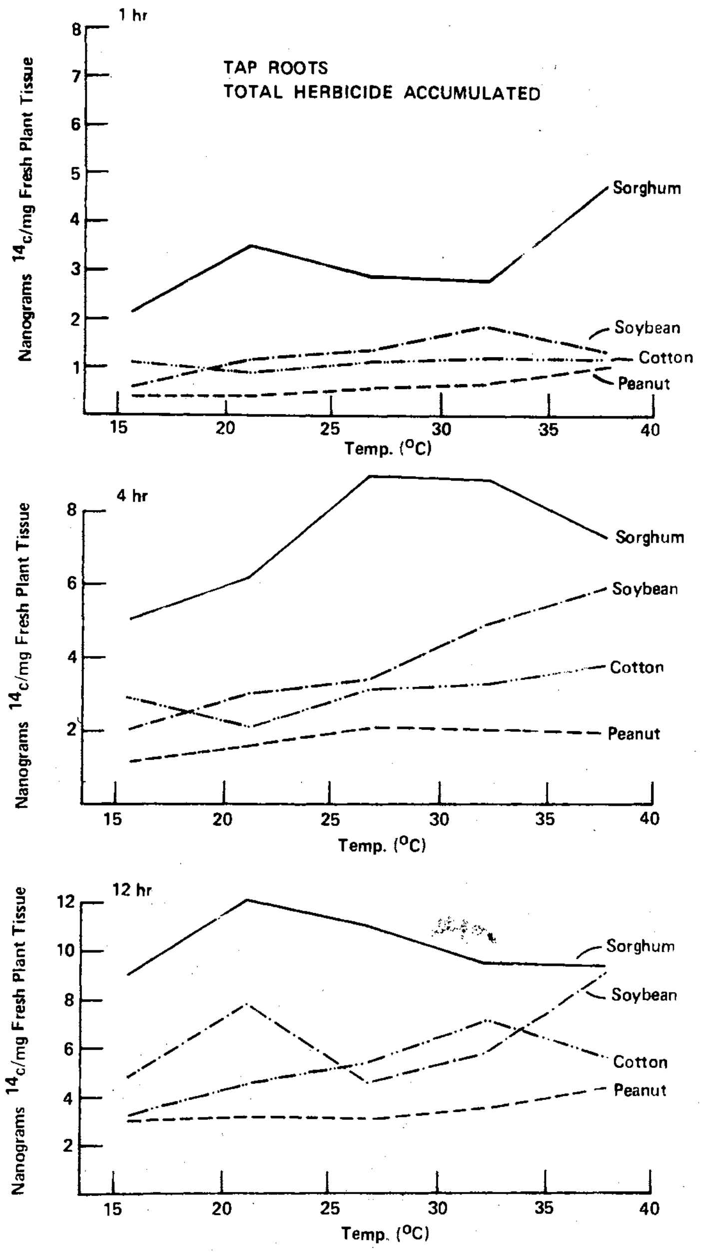

Figure 20. Total ^{14}C-Profluralin Accumulated by Tap Roots at 1 hr (Top), 4 hr (Middle), and 12 hr (Bottom).

uptake may indicate greater accumulation in sorghum and would contribute to greater profluralin phytotoxicity in the species. Although tap roots apparently are not affected as much by dinitroaniline herbicides as are lateral roots (4, 60), the rapid uptake of profluralin by lateral roots may be responsible for phytotoxicity in some species. Ketchersid et al. (42) indicated that less herbicide was taken up by tap roots than lateral roots since less trifluralin was taken up per gram of root tissue when young plants lacking lateral roots were transplanted to trifluralin-treated soil than when older seedlings with abundant lateral roots were used.

Discussion

From the experimental evidence, the following observations can be made concerning the effects of temperature on the translocation of profluralin and dinitramine and/or metabolites in the species studied.

In general, translocation of ^{14}C-profluralin radioactivity to plant tops was very limited. Pigweed translocated the greatest amount at 38 C but soybean showed the greatest increase in translocation as temperature increased. Dicot species (pigweed and soybean) appeared to translocate more profluralin radioactivity with increases in temperature than monocot species (barnyardgrass and sorghum). In general, accumulation of ^{14}C-profluralin in roots showed a decreased level with increasing temperature in all species tested. The level of profluralin radioactivity found in roots of all species was greater than that in plant tops at all temperatures. Analysis of nutrient solutions at 24 hr indicated very little herbicide still remained. Apparently the volatility of the herbicide was similar to that of trifluralin (33), and plants were exposed to decreased herbicide concentration with time. This might explain reduced uptake of profluralin, especially at higher temperatures.

Autoradiographs of plants exposed to ^{14}C-profluralin also indicated little translocation of the herbicide from the root system. Accumulation of the ^{14}C-profluralin radioactivity in roots appeared greater at 16 C than at 38 C (Figures 13-16). Some translocation was observed in the tops of sorghum, soybean, and barnyardgrass at 38 C but no translocation was observed to tops of the species studied at 16 C. Thus, the sorption of herbicide in roots may be responsible for the phytotoxicity of profluralin, especially at low temperatures. Penner (65) noticed that corn grown at 20 C accumulated a higher concentration of ^{14}C-trifluralin in both roots and shoots than corn grown at higher temperatures. Only small amounts of the ^{14}C was translocated to the shoot.

Bioassay studies indicated that accumulation of profluralin in pigweed and soybean roots was not as harmful as it was to barnyardgrass and sorghum. Barnyardgrass has rather slow germination and initial growth and would thus be exposed to a treated soil zone for some time. Sorghum apparently has little inherent tolerance to profluralin. Soybean and pigweed also metabolized a greater percentage of ^{14}C-profluralin at higher temperatures than at lower temperatures (Table V). This may be a form of detoxification and may be responsible for the tolerance of the plants to the herbicide.

Translocation of dinitramine radioactivity to plant tops, in contrast to profluralin, showed a marked increase with increasing temperature. Pigweed and soybean had higher levels in plant tops than did barnyardgrass or sorghum. Increases in ^{14}C-dinitramine levels with temperature in sorghum and pigweed was almost linear (Figures 5B and 6A). Amounts of dinitramine radioactivity in plant roots did not decrease as rapidly with temperature as did profluralin. Greatest amounts of ^{14}C were found in sorghum roots at low temperatures. Greater amounts of dinitramine remained in the nutrient solution after 24 hr, suggesting that volatility of dinitramine in water solutions was apparently not as great as that of profluralin. This may partially explain the higher levels of herbicide radioactivity in plants exposed to dinitramine. However, autoradiograms indicated greater translocation at higher temperatures with dinitramine radioactivity than with profluralin radioactivity (Figures 9-12). Perhaps root cells of the plants can more readily transfer the herbicide molecules from cortex to stele at higher temperatures, thus increasing acropetal translocation. This may indicate a mechanism of selectivity. The greater translocation of ^{14}C-dinitramine or metabolites to plant tops at high temperatures may be responsible for phytotoxicity of dinitramine.

Many workers (65, 75, 83) have indicated that movement of herbicides in plant apoplast tissue was enhanced by greater transpiration at higher temperatures. Experimental evidence obtained in this study indicated greater water loss per gram in barnyardgrass and sorghum (susceptible species) than in soybean and pigweed (resistant species) when treated with profluralin. The greater movement of water in barnyardgrass and sorghum may also be responsible for translocation of profluralin to the plant tops, as is shown in Figures 13-16. Little profluralin radioactivity was observed in pigweed tops, which also exhibited least water translocation (Figure 7). Similar transpiration results were recorded when plants were exposed to ^{14}C-dinitramine

(Figure 8). Therefore, greater translocation of dinitramine radioactivity to plant tops via the transpiration stream may lead to greater phytotoxicity in some species. Autoradiograms of dinitramine radioactivity (Figures 9-12) indicates greater translocation to plant tops with increasing temperature. The water uptake study supports these data.

When excised tap root tips were exposed to ^{14}C-profluralin for 12 hr, there was a general trend toward greater accumulation of ^{14}C in the roots at higher temperatures. Most had not reached equilibrium at 12 hr.

Excised lateral roots of all species had higher initial rates of absorption than tap roots. Initial rates of absorption were greater than at later times. Lateral roots of all species continued to accumulate more profluralin with time, but the total absorption of profluralin at 12 hr was not proportional to the initial rates of absorption (Figure 20). These excised root tip results are very similar to those observed when excised peanut roots were exposed to trifluralin (33). The rapid uptake of profluralin by lateral roots may be responsible for phytotoxicity in some species.

Experimental evidence indicated the transpiration stream of the plants studied probably was responsible for translocation of the herbicides. The volatility of profluralin, especially at high temperatures, may make the chemical unavailable for plants. This would reduce absorption, translocation, and thus phytotoxicity of profluralin. Absorption, adsorption, and accumulation of profluralin in plant root systems may be responsible for plant injury at low temperatures, as little translocation was observed. A study of trifluralin uptake in peanuts yielded similar results (33). Greater uptake in excised lateral roots was observed at 21 C and 38 C than at intermediate temperatures. High levels of trifluralin radioactivity in roots of intact peanut seedlings cultured at low temperatures was observed.

Evidence presented indicates lower volatility and greater translocation of dinitramine to plant tops at high temperatures, especially in susceptible species, may be a factor in the phytotoxicity of the herbicide.

Summary

Exposure to various concentrations of profluralin revealed tolerance differences among species tested. Barnyardgrass was most susceptible, followed by sorghum, pigweed, and soybean.

Labeled profluralin and dinitramine studies with intact plants indicated a trend toward greater uptake in plant tops with increasing temperature and less uptake in roots with increasing temperature. Analysis of nutrient samples indicated greater loss (through absorption, adsorption, or volatility) of profluralin than dinitramine with increasing temperatures.

Thin layer chromotography revealed translocation differences among species treated. The order of ^{14}C-dinitramine translocation was: soybean > pigweed > sorghum > barnyardgrass at 16 C and 38 C. Autoradiograms of intact plants indicated similar translocation patterns. The concentration of dinitramine metabolites and parent chemical in the tops of the plants increased as the temperature increased. This accumulation due to increased translocation may be responsible for plant injury. Uptake of profluralin in roots of all species appeared to be greater at 16 C than at 38 C. Autoradiograms of intact plants exposed to ^{14}C-profluralin showed the same trend. Little translocation of profluralin was observed. Since profluralin was more volatile than dinitramine, profluralin absorption was less, especially at higher temperatures. However, a greater proportion of the absorbed ^{14}C-profluralin was translocated at 38 C than at 16 C.

Uptake of profluralin from soil indicated greater root absorption with increasing temperatures. This was probably due to reduced herbicide adsorption and increased herbicide volatility with increasing temperatures.

Absorption of ^{14}C-profluralin in excised root tips of sorghum, soybean, and cotton showed a general trend toward greater accumulation of herbicide with increasing temperature. Similar results were recorded in peanut seedlings treated with ^{14}C-methazole (33). This may indicate apoplastic movement of the herbicide with the transpiration stream. This evidence was supported by water uptake studies, which showed more transpiration per gram in susceptible species than in resistant species.

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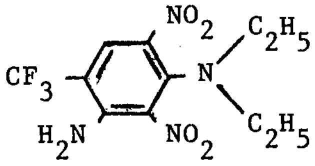
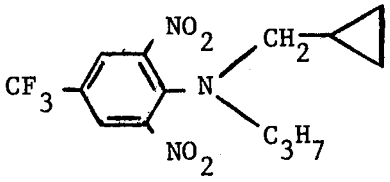
APPENDIX A

STRUCTURE AND PHYSICAL PROPERTIES OF DINITRAMINE AND PROFLURALIN



Dinitramine (N,N-diethyl-4-trifluoromethyl-2,6-dinitro-toluidine-5-amine)

Molecular weight	322.3
Melting point	98-99 C
Physical state and color	Yellow-orange, solid
Vapor pressure (mm Hg @ 20-25 C)	3.6 x 10^{-6}
Solubility in water (20-25 C)	1.1 ppm



Profluralin (N,n-propyl-N-cyclopropyl-methyl-4-trifluoromethyl-2,6-dinitroaniline)

Molecular weight	347.3
Melting point	33-36 C
Physical state and color	Yellow-orange, solid
Vapor pressure (mm Hg @ 20-25 C)	
Solubility in water (20-25 C)	0.1 ppm

VITA √

Keith William Hawxby

Candidate for the Degree of

Doctor of Philosophy

Thesis: THE ABSORPTION AND TRANSLOCATION OF DINITRAMINE AND PROFLURALIN IN SUSCEPTIBLE AND RESISTANT PLANTS AT VARIOUS TEMPERATURES

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