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An aqueous solution of technical glyphosate [N-(phosphonomethyl) glycine] was applied in homogeneous sprays of 120, 180 and 300 μm droplets to seedlings of a bush type green bean (Phaseolus vulgaris L. 'OSU 1604') at a carrier rate of 20 l/ha. At each droplet size all combinations of 3 dosage levels of glyphosate, with or without the addition of a non-ionic surfactant (X-77), were compared. Plant growth decreased as glyphosate dosage increased. There was no significant effect of adding surfactant. When compared with 180 μm droplets, approximately 2.2 times and 1.6 times as much active ingredient was needed to give a 50% growth reduction with 120 and 300 μm droplets, respectively.

A preliminary study of effect of surfactant concentration on glyphosate toxicity indicated both surface tension and contact angle of the spray solution had no significant relation to the phytotoxicity of the herbicide spray. Surfactant (X-77) enhanced the phytotoxicity of glyphosate most at the concentration of 0.1% v/v.

HERBICIDAL EFFECT OF GLYPHOSATE AS INFLUENCED BY SPRAY DROP SIZE

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INTRODUCTION

The development of better spray equipment for the application of pesticides has led to the possibility of generating more uniformly sized droplets. Spray droplet size is a critical factor in determining the amount of drift from the target area and the biological performance of pesticides. The increased concentration associated with the low volume (20 to 40 l/ha) of carrier in controlled drop application (CDA) sprays increases the importance of drop size in relation to assuring suitable deposition and coverage to obtain the desired vegetation control and at the same time minimize environmental contamination.

While many studies have been conducted on the effect of spray droplet size on insect control, only a few have dealt with herbicides. Most of the previous work with drop size of herbicide sprays has been done with phenoxy herbicides and the results have been contradictory. Smith (75) reported that moderate to large droplets with the application rate of 10 to 20 ml per square yard were the most effective when the ammonium salt of 2,4-D (2,4-dichlorophenoxyacetic acid) was applied to young kidney beans (Phaseolus vulgaris L.). He suggested that the ineffectiveness of small droplets might be due to the fact that many of the droplets were "stranded" on islets on the lamina of the leaf without direct access to the conductive tissues. The results of Fisher and Young (22) also suggested that coarse droplets of 2,4,5-T (2,4,5-trichlorophenonyacetic acid) were more effective in killing mesquite (<u>Prosopis juliflora</u> L.) than the fine drops.

However, Ennis et al. (20) reported that herbicidal toxicity of an oil solution of the triethanolamine salt of 2,4-D and ethyl ester of 2,4-D increased as drop diameter decreased from 300 µm to 100 µm. Behrens (1), Way (78), Hurtt et al. (42) all showed that small drops (100 µm) of 2,4-D, 2,4,5-T and MCPA ([(4-chloro-o-telyl) oxy] acetic acid) were more effective than large drops (300 µm or 500 µm).

Behrens (1) concluded that the increased efficiency of small drops of 2,4,5-T was due to the larger leaf area covered. McKinlay et al. (54) found that the phytotoxicity of dodecyl and tetradecyl amine salts of 2,4-D increased as the droplet size decreased from 400 µm to 100 µm they also found that small drops (100 µm) of aqueous sprays of paraquat (1,1'dimethyl-4,4'-dipyridinium ion) were more phytotoxic than larger droplets (350 µm) when applied to sunflower seedlings (55). Conflicting results were obtained by Douglas (18) when he reported that optimum herbicidal effects of paraquat and diquat were achieved with 400 µm droplets. Buehring et al. (6) confirmed that the optimum result obtained with paraquat and diquat (6,7-dihydro-dipyrido [1',2'-d-2',1'-c] pyrazinediium ion) was with spray droplets in the range of 400 to 500 µm.

With a low volume application (20 l/ha), Mayes and Blanchard (53) reported that drops in the range of size 150 to 350 μm were retained equally well on wild oat (Avena fatua L.). Within this droplet size range, the spray retention would have minimum effect on the phytotoxicity of herbicides.

In the study reported here, the herbicidal effect of glyphosate [N-(phosphonomethyl) glycine], as influenced by spray drop size, was studied under laboratory conditions in which the different-sized spray droplets were retained equally well.

LITERATURE REVIEW

Herbicides are classified as "contact" or "translocated" according to whether they exert a lethal action at the site of application or in other parts of the plant. This division is to some extent arbitrary, since under certain conditions the properties of the two groups may be to a degree interchangeable. To be effective, a contact-type herbicide must at least enter the leaves, and foliar applied translocated herbicides must move from the leaf surface through the mesophyll cells and into the functional phloem tissue in amounts great enough to induce a toxic effect when transported to their sites of activity.

Factors that influence the penetration of contact or translocated herbicides are the same as factors that affect herbicide retention. They include the nature of the plant structure, physical and chemical characteristics of the spray, and environmental factors.

HERBICIDE RETENTION

Differential retention of the spray solution between crops and weeds was shown to be a major factor in the selectivity of many foliar-applied herbicides (36). Physical and chemical characteristics of the spray, environmental factors and the nature of the plant--leaf angle, leaf surface and growth habit--greatly influence the spray retention on plants.

A. Nature of the Plant Structure

1. Gross Morphology:

The gross morphology of plants may play an important role in penetration by influencing the amount of the foliar-applied chemical intercepted and retained (3). The stage of plant development, the shape and lamellar area of the leaf, the angle or orientation of the leaves in relation to the spray, and specialized features, which provide for localized accumulation, may all play an important role. Maximum retention occurs when leaves are positioned at 50° to 90° to the incidence of the spray. Leaves in a horizontal position retained more spray than those at an angle of 45° from horizontal. Also the stage of plant development, e.g. the ratio of young to mature leaves and leaf:stem ratio may markedly influence spray retention (16).

2. Specialized Structures:

Spray retention may also be influenced by specialized structures like trichomes, stomata, veins etc. (8, 30, 34, 70). Trichomes, common features on many plant surfaces, vary markedly in size, morphology frequency, distribution and function. Challen (9) showed that water retention was greater on leaves having an "open" pattern than on those having a "closed" trichomes pattern. The "open" pattern may enhance wetting due to capillary action, while the "closed" pattern could depress it by entrapment of air beneath the water droplets. Ennis (20) reported that pubescent soybean [Glycine max (L.) Merr.] leaves retained more aqueous and 0.5% Carbowax 1500 spray than glabrous soybean leaves.

3. Epicuticular Wax:

Variation in retention between species has been attributed to the hydrophobic nature of the epicuticular waxy covering (4, 23). Norris and Bukovac (62) found that the lower leaf surface has more epicuticular wax than the upper leaf surface in pear (Pyrus communis L.). An increase in waxiness decreases the adherence of aqueous spray molecules (59), while reduction of wax deposits on pea (Pisum sativum L.) leaves by soil treatment with TCA (trichloroacetic acid) increases retention, cuticle penetration and susceptibility to dinoseb (2-sec-butyl-4,6-dinitrophenol), MCPA and mecoprop (2-(2-methyl-4-chlorophenoxy)propionic acid) (65). The composition of epicuticular wax appears to be a more important factor than the degree of waxiness; some fractions of wax have a greater influence on water repellency than others (52). The highly water repellent wax of cabbage (Brassica oleracea var. capitata) contains higher quantities of paraffins and ketones than the non-repellent wax of broad beans (Vicia faba L.) which consists largely of fatty acid esters (44). Retention is also influenced by the physical form of wax, a crystalline or semi-irregular form producing strong water-repellency while an amorphous, flat or smooth form allows retention and spreading (45, 46).

B. Physical and Chemical Characteristics of the Spray Solution

The physical properties of the spray in which the herbicide is applied may be varied in a number of ways which could affect spray retention.

1. Spray Volume:

One simple way in which the application may be varied is by changing the spray volume per unit area. The coverage of spray solution will be increased proportionally to the spray volume to the point of "run-off". Increased coverage has an effect similar to increased retention on the leaves. When the leaves are wet, additional spray tends to run-off and is ineffective. In one study, the volume retained increased with increasing volume up to 50 ml/m^2, but with no further increase in retention with higher spray volumes (3). By using different carrier volumes (47 to 281 liter/ha), Buehring et al. (6) showed increasing leaf areas covered with increasing carrier volumes of spray.

2. Spray Drop Size:

Smith (75) found that large droplet sprays applied at low pressure were retained by soybean plants to a greater degree than small droplet sprays applied at high pressure. Droplets that are too small (diameter below 150 µm) do not deposit well. Small drops tend to drift away before reachnig the target and are a potential source of problems to adjacent crops and provide less effective control in the target area. Droplets that are too large tend to bounce off the leaf or run off the leaf surface. With a fixed volume of solution per unit area, the larger the diameter of the droplet, the less will be the coverage when compared to droplets of small diameter. Conversely, excessively small droplets will quickly dry due to evaporation of the carrier. M. Hosseinipour (38) showed at $40^{o}C$ and with a relative humidity of 15%, 250 µm diameter drops of a 0.1% solution of Brilliant-sulfo-flavin lose 10% of their diameter through evaporation after falling 3 meters in air. Under the same condition drops with a 165 µm diameter lose 20% of their diameter, while 130 µm diameter drops were reduced to half of their original size after a fall of 2 m. Lake and Taylor (48) found that drops from 110 to 440 µm in diameter were equally well retained by young wild oat, and that retention was linear up to about 150 l/ha by tracing the oil phase of an emulsion of barban (4-chloro-2-butynyl-m-chlorocarbanilate). Merritt and Taylor (57, 58) reported when spray volume rate ranged from 20 to 25 l/ha, there was no retention difference between droplet size from 150 to 350 µm, but with increasing spray volume up to 100 l/ha, drops of 150 and 250 µm diameter were retained better than 350 µm droplets. If deposition is accomplished, retention is high below a certain critical droplet diameter and is low at larger diameters. The critical diameter in the case of water on pea leaves is about 100 µm and increases as the surface tension is decreased. It is highly probable that retention will vary from species to species due to differences in leaf surface characteristics (36).

3. <u>Surface Tension and Viscosity of the Spray Solution:</u>

The surface tension of a spray solution is one physical characterisitic which may be changed readily to bring about considerable variation in retention. Brunskill (4) showed that retention of spray droplets on pea leaves increased with decreasing surface tension and decreasing drop diameter. At the surface tension of water (71 to 72 dynes/cm) the pea leaves showed almost complete reflection of 250 µm and 350 µm droplets. Reducing the surface tension of water with methanol or acetic acid increased retention to a maximum when a critical value of 45 dynes/cm had been reached. The contact angle of the spray and solid surface area is decreased as the surface tension is decreased. The critical surface tension for maximizing retention differs among species. In general, lowering of surface tension reduces the volume retained by species which are easily wetted and increases the volume retained by species not readily wetted. With white mustard (Brassica alba L.) at the second and third growth stages (3 to 6 leaves), the well-developed leaves retained a large amount of spray at a spray surface tension of 62 dynes/cm. At lower surface tensions the surface was wetted so readily and completely that there was spray run-off from the leaves and down onto the stems. The same effect occurred with sunflower (Helianthus annuus L.) at all three stages of growth (1 to 3 pairs of leaves). With pea, linseed (Linum usitatissimum L.) and barley (Hordeum vulgare L.), the reduction of surface tension increased spray retention but the effect varied with stage of growth (3).

Brunskill (4) reported the effects of viscosity on retention by using glycerol in water on pea leaves. He found that with high viscosity (6.5 Poise) and small droplets (120 μm) he could achieve 90% spray retention, while with low viscosity (1.31 Poise) spray and 250 μm droplets only 11% of the spray was retained.

4. Property of Carrier and Herbicide Formulation:

In a study reported by Ennis et al. (20) there was a marked difference in the retention of aqueous droplets by the leaves of various species. Aqueous droplets bounced off the leaves of many species—peanut (*Abrus hypogaea* L.), cabbage and oat (*Avena sativa* L.), while on tobacco (*Nicotiana tabacum* L.) and coleus (*Coleus blumei* Benth.), the water drops formed a circle of numerous stellate droplets. Droplets of oil and butyl ester of 2,4-D were retained by all the species in this study. Species which retained wholly aqueous droplets likewise retained droplets of different 2,4-D formulations—ester, Na salt and Morpholine salt. Species which are difficult to wet with water also tended to repell the droplets of the 2,4-D preparations.

C. Environmental Factors Affecting Retention

The environmental conditions under which the application of a herbicide is made have considerable influence on the retention of the spray solution. Humidity, rainfall and temperature all have some effect on spray retention.

1. Humidity:

Under conditions of high humidity, the evaporation of spray solution during or after application will be slower than with low humidity, thereby increasing the retention time of the spray solution.

The approximate life of a drop of pure water at 60°F and 40% relative humidity is 4 seconds for 50 µm drops, 16 seconds for 100 µm droplets and 63 seconds for 200 µm droplets. In still air, the rate of fall of a 200 µm drop is over 2 ft/second so that large drops will evaporate very little in falling a short distance.

2. Rainfall:

The effectiveness of most foliar-applied herbicides is reduced if rain falls soon after application. Therefore rainfall is an important factor in the retention of herbicide sprays. Some herbicides which are highly soluble in water, such as dalapon and maleic hydrazide, penetrate leaf surfaces only slowly and can be washed off if rain occurs within 12 or fewer hours after application. Doran and Anderson (17) reported that simulated rainfall less than eight hours after postemergence application of the herbicide bentazon (3-isopropyl-1H-2,1,3-benzothiadiazin-4-(3H)-one 2,2-dioxide) reduced its activity on velvetleaf (*Abutilon theophrasti* Medic.) and common cockbur (*Xanthium pennsylvanicum* Walb.).

3. Temperature:

Temperature may play an indirect role in the spray retention by influencing the deposition of the spray. Temperature lapse refers to the change in air temperature between one level and another above the ground. The normal lapse rate is for higher temperature near the ground and is equal to 0.1°F between points eight foot and

32 feet above the ground. As the lapse rate increases (i.e. the eight foot level becomes warmer) the vertical mixing or turbulence becomes greater and particles in the air are rapidly mixed and moved. A temperature inversion exists when the eight foot level is cooler than the 32 foot level. This puts a cap of warm air over the cooler ground and tends to produce stable, non-turbulent air. A spray-laden cloud could be carried under this warm air cap for a great distance and is a very hazardous situation if drift is a potential danger as from aerial spraying of brush.

4. Environment Prior to Spray Application:

The environmental conditions under which the plant developed before a herbicide is applied affect the plant morphology, thickness of epicuticular wax, and wax deposition and components, thereby influencing the retention of the spray on the surface of the plant (37, 80). Within the temperature range of 15 to 27°C, wax structure of the leaf surface varied from a pattern of single upright rodlets to one of flat, overlapping dentritic platelets parallel to the leaf surface. Overlapping wax form had less spray retention than the upright rodlet form.

HERBICIDE PENETRATION

Many factors connected with the nature of the plant, environment, composition of the spray solution, and methods of application have been found to influence penetration. The amount of herbicide penetrating the leaf, as a proportion of that deposited upon its surface, is often low. There is a general presumption that if the amount of herbicide penetrating a species is increased, then the biological effect is increased or that a similar biological effect can be obtained with a lesser amount of herbicide applied.

A. Nature of the Plant Structure

1. Gross Morphology:

A species which can retain more of the spray solution will have a chance for more spray to penetrate into the plant. In other words, the gross morphological features which influence retention would be expected to indirectly affect penetration as well. Gross morphology is an important factor in foliar penetration in so far as it my influence spray retention. When the plant surface could retain more of the spray solution it would have more chance to penetrate through the surface of the leaf to the site where it could exert its herbicidal activity.

2. Cuticular Penetration:

The permeability of intact cuticle depends upon such factors as cuticle thickness, the nature of wax deposits, the degree of hydration of cutin, and the presence of ectodesmata. The amount of wax may be the factor most inhibiting the penetration of non-polar compounds (50, 61). It has been shown that young actively expanding leaves absorb greater quantities of a foliage-applied growth substance than do mature leaves (67). This has been attributed to the increase in the age of cutin wax deposits, and therefore the cuticle thickness. Absorption is enhanced by the presence of cracks in the cuticle of older leaves and by insect punctures. Rapid penetration, possibly due to the presence of ectodesmata in the epidermal cells, may also take place through the thin cuticle associated with veins, particularly the midrib (25). The surface of the vein is composed of thin-walled parenchymatous cells (bundle sheath) which extend to the upper and lower epidermis and are concerned with conduction (21).

There seems to be no doubt that the cuticle acts as a barrier to the movement of herbicide molecules but there is some controversy as to its importance in determining herbicide selectivity. Holly (35) concluded that differences in penetration rate play a minor role in the selectivity of some herbicides by studying the penetration of MCPA, 2,4-D and 2,4,5-T in resistant and susceptible species. Crafts (11) suggested that there might be specific pathways through the cuticle. He found that lipid-soluble compounds penetrated the cuticle more readily than water-soluble chemicals, and the dissociated molecules of weak organic acids also penetrated and induced a response.

He proposed that lipid-soluble compounds followed a lipoidal route and water-soluble compounds an aqueous pathway.

3. Penetration through Specialized Structures:

In addition to influencing wetting and retention, trichomes are more directly involved in foliar absorption (49). Leaf hairs, depending on their morphology, may provide a microclimate which can alter the drying time of aqueous sprays and thus the absorption pattern. Glandular hairs associated with major veins on *Phaseolus* leaves became labelled before other epidermal cells when the leaf was treated with silver nitrate. The greater permeability around the base or foot cells of trichomes may be related to delayed development of the cuticle in this region.

Stomata appear to play a two-fold role in foliar penetration. First, under certain conditions, the aqueous spray solution may move *en masse* through the stomatal pore and diffuse through the air space of the leaf (19, 71). Secondly, the cuticle over the guard cells may be more permeable and then these structures may serve as the preferred site of entry (27).

Guard cells are known to be more permeable to urea, glycerine and fluorochrome dyes than the other epidermal cells. It was also found by Goodman and Addy (29) that guard cells of open stomata became labelled earlier than those of closed stomata. There is evidence that the cuticular membrane remains the first barrier to entry of leaf cells even though entry of the stomatal pore is effected. Electron micrographs reveal that stomatal openings are not perforations, but only invaginations of the cuticular membrane (29, 30, 81). This inner cuticle is thinner, more permeable and more hydrated than the cuticle covering of the outer surface and the risk of applied sprays drying is diminished (15). Consequently, molecules readily penetrate the thin cuticle lining and the high humidity inside the air space of the leaf ensures the presence of a water continuum on the cell surfaces (13). It would appear that the main role of stomata in spray penetration is to modify the ease of cuticular penetration rather than to act as portals of entry.

The existence of micropores has been indicated by electron micrographs (72) and by the occurrence of cuticular transpiration from leaves with closed stomata (14). Sargent (69) suggested that pores postulated by Crafts may be similar to the ectodesmata demonstrated by Franke (25). However, Franke (27) did not believe that materials enter pores before they reach the cellulose walls, since ectodesmata extend from the walls of epidermal cells to the cuticle which they do not perforate. He suggested that the hydrophilic properties of the wall faciliated the passage of water-soluble substances, whereas the lipophilic substances penetrated via ectodesmata.

B. Physical and Chemical Characteristics of the Spray Solution

1. Herbicide Formulation:

It is generally believed that an inverse relationship exists between polarity and the efficiency of penetration of a compound through the lipid portion of the cuticle. A suitable balance of the polar-apolar groups of the molecule is essential for efficient penetration. In a study of the penetration rates of a series of chloro-substituted phenoxy acetic acids, it was found that increasing chlorination favored penetration into the lipid portion of leaf discs of *Phaseolus*, but significant differences also existed in the capacity of the petiole to transport certain original acids (70).

In commercial practice, phenoxy-acid herbicides are normally formulated as esters, amines or other salts. Of these formulations, it had been reported that the isopropyl ester of 2,4-D was more efficiently absorbed by the leaves of soybean and corn (*Zea mays* L.) plants than the amine or sodium salts (33). However, light alkyl esters penetrated the cuticle, but did not partition out of it into the vascular system, thereby killing the leaves by contact injury and destroying the mechanism of translocation. Heavy esters of 2,4-D and 2,4,5-T, formed by alcohols such as butoxyethanol having both water and fat solubility, are more effectively translocated than the low molecular weight ester (11). There is evidence to suggest that conversion of the ester to the free acid is necessary for expression of herbicidal activity. Crafts (12) reported that esters of 2,4-D and 2,4-DB were hydrolyzed during penetration *in vivo*. From barley leaves, the acid portion of the isopropyl ester of 2,4-D was translocated to other parts of the plant, the ion moving via the aqueous route to the symplast, whereas the alcohol portion remained in the treated area.

2. Herbicide Concentration:

Sargent and Blackman (67) showed that penetration is linearly related to the concentration (within the range 0.01 to 100 mg/l of 2,4-D) of the external herbicide when measured directly from uptake by the leaf disc. However, the relationship between concentration and penetration of translocated-type herbicides, when measured by plant responses, may deviate from linearity. This was because at high concentrations of herbicide, physiological changes might be induced in the uptake and transport process, thus altering subsequent penetration (39).

3. Surfactant:

Surfactants may enhance, depress, or have no effect on herbicide activity (7, 24). Generally, surfactants facilitate and accentuate the emulsifying, dispersing, spreading, wetting, solubilizing and surface-modifying properties of herbicidal formulations to bring about enhancement of foliar penetration and herbicidal action. The interaction among surfactants, herbicides, and plants is complex. It was first thought that the surfactant reduced the surface tension of the herbicide solution, thus enhancing leaf wettability and cuticle penetration (5). However, Weintranb et al. (79) reported that the addition of Tween 20 enhanced phytocidal activity of alcoholic solutions of 2,4-D with no effect on surface tension. Maximum reduction in surface tension occurs in the concentration range from 0.01 to 0.1%. At high concentration, in the critical micelle concentration (CMC) range, the surface tension remains constant, but the micelles may solubilize the cutin with subsequent removal of the cuticular wax. In contrast, herbicidal effectiveness is often maximal at a concentration 10 times, or greater, of the CMC (24). So, in addition to lowering surface tension and thus increasing retention, the swelling of cuticle by surfactant should have a more lasting effect since the large surfactant molecules would tend to prevent complete contraction of the cuticle as water evaporates after treatment. An observation of Staniforth and Loomis (76) provided some support for this hypothesis. They found that the enhancing effect of a surfactant on the action of phenoxy herbicides could be achieved by applying surfactant and herbicide as separate sprays as well as in a single application.

4. Spray Volume and Drop Size:

Increasing the spray volume would increase the retention of the spray solution within a certain range (6) and thus increases the chance for herbicides to penetrate the epidermal cells. However, while the spray volume is increased, the concentration of the spray solution is decreased, consequently, the rate of herbicide penetration is reduced (67). Also, more energy would be required to apply a large spray volume. The concept of low volume spray (20 to 40 l/ha) with controlled drop application should have a promising future.

A suitable droplet size is critical and important for the biological performance of the herbicide spray. It will provide adequate contact area and sufficient moisture for herbicide penetration before drying of the spray solution. Drop size had a significant effect on the phytotoxicity of paraquat, diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) and fluometuron [1,1-dimethyl-3-(a,a,a-trifluro-m-tolyl) urea] on cotton (6). McKinlay et al. (55) by using an oil formulation of dodecyl and tetradecyl amine salts of 2,4-D on sunflower seedlings, found that for the dosage of herbicide, the stem curvature increased as the droplet size decreased from 400 µm to 100 µm. They also reported that smaller droplets of paraquat were more phytotoxic than larger droplets on sunflower seedlings (54).

5. <u>pH of the Spray Solution:</u>

Acidity represses ionization of some penetrants such as weak acids, dissociation of the free acid groups and long-chain aliphatic acids in the cuticle, and amino acid residues in the protein portion of the plasmalemma, thus influencing the polarity of the cuticle and penetrants. For herbicides in which the hydrogen ions has little or no effect on dissociation (amine, ester, etc.), the pH of the spray solution has little effect on penetration (13, 32). However, hydrogen ion concentration plays a significant role in the penetration of weak, organic acid-type, foliar-applied herbicides. The weak acids like 2,4-D are the most toxic and penetrate most efficiently at low pH. Under such conditions they remain as undissociated molecules which are more likely to partition onto portions such as wax and the lipoids of the plasmalemma (2, 73). Penetration through pear leaves generally followed closely, but not absolutely, the dissociation curve for NAA (22). A similar relationship has been reported for penetration of 2,4-D into bean leaves (67).

Orgell and Weintraub (63) and Patten (64) reported that the enhanced penetration of 2,4-D at neutral or alkaline pH values was found to be influenced by certain cations—ammonium, ammonium phosphate and ethanolammonium ions, supplied either as salts of 2,4-D or as buffer cations. In alkaline solutions, induced responses approached those observed with acid solutions. Inorganic ions may influence membrane swelling and the altering of their permeability.

C. Environmental Factors Affecting Absorption

The effect of environmental factors on foliar absorption is very complex. The environmental factors--light, temperature and humidity, may exert their effect during the absorption process or they may influence plant development prior to absorption, resulting in either an increase or a decrease in sensitivity of the plant to a given herbicide dose. Further, control of these three factors at the same time is extremely difficult so that the effects of these environmental factors have often been confounded to varying degree in earlier reported studies.

1. Effect of Environment Prior to Treatment:

The environment under which the plant develops may markedly affect the absorption of a chemical subsequently applied to the foliage. The light intensity will affect the gross morphology of plants. Under high light intensity the leaf angle will be smaller than that under low light intensity. Also, the plant surface structure will be greatly influenced by light intensity. The thickness of cuticle and epicuticular wax will be increased under high light intensity, the protective layer formed thus reducing the absorption of sprays applied to the leaf surface. Whitecross et al. (80) reported that under conditions of 60% and 40% of full greenhouse light there was an apparent reduction in surface wax deposition under 40% light when compared to 60% light. They also observed marked changes in plant wax ultrastructure within the temperature range of 15 to 27°C. Wax structure varied from a pattern of single upright rodlets to one of flat, overlapping, dentritic platelets parallel to the leaf surface. The wax structure was consistent at any particular temperature irrespective of light condition. It is expected that spray retention and penetration would vary with the different wax type and the amount of wax.

Plants grown under water stress conditions do not necessarily have a thick cuticle layer, but the cell will be more compact, have more dense cytoplasm, and will absorb more herbicide because of high water tension inside the plant (37). Hull et al. (41) found there was no significant differences in most response categories associated with high humidity (76 to 82%) or low humidity (28 to 34%) grown velvet mesquite (*Prosopis juliflora* L.) treated with picloram (4-amino-3,5,6-trichloropicolinic acid). However, low root temperature and moisture stress have been shown by Skoss to reduce absorption (74).

2. Light:

As for the absorption process, one of the effects of light on penetration of some herbicides is related to the persistence of the applied dose on the plant. Some organic compounds are rapidly photodegraded and hence the dosage available for uptake decreases with time. It has been generally found that light enhances uptake, and relatively low intensities (5,000 to 15,000 lux) are adequate for maximal response (32, 68). Sargent and Blackman (68) reported on the complexity of the light effect on 2,4-D penetration with Phaseolus. Penetration continued at a steady state in the dark. In light, penetration was slow and steady for about 4 hours; thereafter, a striking increase in rate occurred. This surge in penetration was observed at neither low 2,4-D concentration (100 mg/1) nor from applications to the adaxial leaf surface. Interruption of the light, alternation of the temperature, irradiation with ultraviolet light and the use of metabolic inhibitors adversely affected penetration rate. Light enhanced uptake of 2,4-D (68) and NAA (31) was negated by inhibitors of the Hill reaction, indicating that this uptake is dependent upon a supply of ATP.

3. Temperature:

Foliar absorption of herbicide is temperature dependent and specific with plant species (69). Kempen and Bayer (47) found that increasing the temperature and light intensity lessened the time required to give 50% necrosis of Johnsongrass (Sorghum halepense L.) foliage treated with MSMA (monosodium methanearsonate). It seems that temperature exerts an indirect effect on absorption by influencing cytoplasmic viscosity, accumulation, binding, metabolic conversion, and translocation of the molecule under consideration, regulating the processes which influence the concentration gradient across the surface layers. Greater penetration of NAA was obtained with an increase in temperature from 5 to 35°C, with a Q_{10} value of 2.4 to 5.6 (60). The temperature effect was completely reversible and was demonstrated in isolated cuticles free from the epicuticular wax (61). The high Q_{10} values observed for the penetration of NAA through isolated cuticle are thought to be related to the lipoidal nature of the cuticle.

4. Humidity:

High relative humidity is generally favorable for foliar absorption of herbicides (56). Prasad et al. (66) concluded that the greater absorption and translocation of dalapon (2,2-dichloropropionic acid) under high humidity conditions is largely due to effects on the rate of droplet drying and stomatal opening. Relative humidity was the most important environmental factor in the absorption of maleic hydrazide, three to five times more being absorbed at 100% relative humidity than 50% RH (75). The enhanced permeability under high moisture conditions may be attributed to swelling of hydrophilic groupings of the cutin matrix pushing the apolar wax units apart (77). Jordan (43) found that visible injury to Bermudagrass [*Cynodon dactylon* (L.) Pers] with 0.56 kg/ha glyphosate was greater at 100% than at 40% RH. At constant temperature an increase in relative humidity from 40 to 100% resulted in a 3 to 6-fold increase in translocation of ^{14}C glyphosate in cotton (*Gossypiom* sp.).

5. Time Course:

When drops of aqueous solution are applied to a leaf, there is an initial high rate of penetration which falls off with time, the progressive reduction being associated with the rate at which the drops evaporate (40, 51). Penetration apparently continues from the residue on the leaf surface, since there is a slight positive slope to the absorption curve after the drops have dried (62). Continued penetration from the residue is more pronounced if the chemical of the spray is added to the spray droplets (37).

Herbicidal Effect of Glyphosate as Influenced by Spray Drop Size[1]

A. T. Chyau and G. D. Crabtree[2]

Abstract. An aqueous solution of technical glyphosate [N-(phosphonomethyl) glycine] was applied in homogeneous sprays of 120, 180 and 300 µm droplets to seedlings of a bush type green bean (Phaseolus vulgaris L. 'OSU 1604') at a carrier rate of 20 l/ha. At each droplet size all combinations of 3 dosage levels of glyphosate, with or without the addition of a non-ionic surfactant (X-77), were compared. Plant growth decreased as glyphosate dosage increased. There was no significant effect of adding surfactant. When compared with 180 µm droplets, approximately 2.2 times and 1.6 times as much active ingredient was needed to give a 50% plant growth reduction with 120 and 300 µm droplets, respectively.

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[1] Received for publication ________________ 1981.

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Materials and Methods

The influence of herbicide concentrations, spray drop size and surfactant on the growth of a bush-type green bean (*Phaseolus vulgaris* L. 'OSU 1604') was studied in this experiment. Plants were grown in a growth chamber with a 16 hour day (23°C, light intensity 330 uE · M^{-2} · S^{-1}) and an 8 hour night (18°C).

Four bean seeds were sown in a 11 cm pot in a peat vermiculite medium (Jiffy Mix Plus). Three days after seed emergence, plants were thinned to one per pot. An attempt was made to select uniform-sized plants during thinning. Treatments were applied at 10 days after seedling emergence, when the first primary leaves were open and the internode below the first trifoliate leaf did not exceed one centimeter in length.

Technical glyphosate[1] was used at three concentrations—1250, 2500 and 6250 ppm w/v (i.e. 0.05, 0.1 and 0.25 l/ha of 0.5/ml glyphosate) in a total spray volume of 20 l/ha. The recommended rate for control of annual weeds is 0.34 to 1.12 kg/ha.

A non-ionic surfactant, Multi-Film X-77[2] (a mixture of alkylarylpolyoxyethylene glycols, free fatty acids and propan-2-ol) was added to the spray solution for treatments that included

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[1] From Monsanto Company.

[2] From Chevron Company.

surfactant. The surface tension for each herbicide and surfactant combination was measured by using a surface tension meter (Surfactant Tension Apparatus[1]) (Table 1).

Relatively uniform droplets were produced by a rotary atomization sprayer (Micromax[2]). An attempt was made to generate droplets with diameters of 120, 180 and 300 μm by varying the speed of the disc (6900 rpm for the 120 μm and 2700 rpm for the 180 and 300 μm droplets) and by placing the target at different distances from the spray source (20 cm for the 120 μm, 30 cm for the 180 μm and 80 cm for the 300 μm droplets). Droplet diameters were measured with an ocular micrometer on slides coated with a mixture of vaseline and light mineral oil, as described by Fuchs and Petrijanoff (28) and the actual drop sizes were found to be 121.6 ± 9.6, 178.8 ± 12 and 302.8 ± 7.8 μm respectively. By using a red dye and herbicide solution mixture on a coated slide, the drop densities for each droplet size, when determined from counts made under a microscope, were found to be in the close agreement with the theoretical densities (Table 2).

Plants were removed from the growth chamber for application and drying of sprays, then returned to the growth chamber until harvest.

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[1] From Central Scientific Company.

[2] From Micron West INC.

The two primary leaves of the bean plant were supported on a flat, level surface during application. Acetate film masks restricted the treated area to 20 cm^2 for each plant. The spray volume (20 µl per plant) was controlled by varying the spraying time--18 seconds for the 120 µm droplets, 32 seconds from the 180 µm droplets and 69 seconds for the 300 µm droplets. The flow rate to the spinning disc (75 ml/min) was kept constant with a needle valve and monitored with a flow meter.

Three concentrations of glyphosate, with or without surfactant, were tested to give six treatments at each droplet size.

The growth parameter used to assess plant response was the fresh weight of the aerial parts of the plant 10 days after treatment.

Results and Discussion

The surface tensions of each spray solution (Table 1), would indicate a minor effect of glyphosate concentration but the addition of surfactant (0.06% w/v) to the solution reduced surface tension to a minimum.

The fresh weight of aerial parts of the plant was measured and expressed as percent of growth of untreated plants. The datas were subjected to an analysis of variance using the least significant difference test. The treatment means of percent of growth of untreated plants is shown in Table 3.

The phytotoxicity of glyphosate was significantly affected by dosage and spray drop size, but no significant effect was attributed to surfactant. A regression model was designed to assess the significance of these results. The regression equation is:

$$Y = 208.909 - 45.59\ X_1 - 13.05\ X_2$$

where Y is the fresh weight of the bean plant expressed as a percent of untreated plants, X_1 is the $\log_{10}$ dosage of glyphosate in ppm (w/v), and X_2 is the $\log_{10}$ of droplet diameter (um). The r^2 for glyphosate dosage is 0.78, and r^2 for the droplet diameter is 0.02.

The regression lines assigned to each of the droplet size groups are as follows (Figure 1):

$$Y_{120} = 231.97 - 50.28\ X \qquad r^2 = 0.95$$

$$YY_{180} = 170.34 - 36.42\ X \qquad r^2 = 0.89$$

$$Y_{300} = 214.45 - 46.94\ X \qquad r^2 = 0.94$$

where X is the $\log_{10}$ dosage of glyphosate measured in ppm (w/v), and Y is the percent of growth.

The herbicidal mechanism of action of glyphosate is probably via the inhibition of biosynthesis of aromatic amino acids--phenylalanine, tyrosine or tryptophan. Plants treated with sufficient amount of glyphosate turn yellow and eventually die. The biological effect measured in this experiment was the fresh weight and expressed as a percentage of growth of the untreated check plants, 10 days after treatment. By this standard the 180 µm droplets were certainly more effective than those 120 µm and 300 µm in diameter. Though the smaller droplets at a given volume of spray contacted a larger portion of the leaf area, the retention and absorption of the spray might be influenced by other factors such as environmental conditions. A smaller droplet will dry more quickly and leave the herbicide on the leaf surface in a form that could less readily penetrate the leaf, while the larger (300 µm) droplets had much less contact area for penetration into the leaf. Also, Ennis et al. (20) suggested that larger drops were less effective because they might become physiologically isolated. Hull (39) concluded that in the uptake and transport process, higher concentrations of herbicide might cause physiological changes which would alter subsequent penetration. Although these might not be the only factors involved, the 180 µm droplets apparently gave adequate contact area of the leaf and also enough time for the herbicide to penetrate through the epidermal cells before the spray solution completely dried.

The rate of diffusion of herbicide into the leaf also will be affected by the dosage of herbicide applied. This is apparent from the fact that at any given size of droplet the response increased as the dosage increased.

The lack of resopnse to adding surfactant to the herbicide solution might be due to the low surfactant concentration, even though the surface tension was reduced to the critical point. At this low concentration (0.06% v/v) the surfactant molecules probably were not present in sufficient amount to facilitate the penetration of the herbicide and thus enhanced the phytotoxicity. Minimum surface tension and contact angles occurred at 0.01% surfactant concentration for most of the surfactants. However, maximum herbicidal activity was observed at 10 times these levels or greater (24). Thus herbicidal enhancement with surfactant concentration above 0.01% was not correlated with surface tension lowering, contact angle, observed wettability, or initial toxicity of the surfactants.

The practical application of the results of this study indicated that 2.24 times as much active glyphosate ingredient is needed to produce a 50% growth reduction when the herbicide was applied in homogeneous sprays of 120 µm, when compared to 180 µm size droplets. The amount of herbicide needed to be increased 1.6 times when 300 µm drops were used instead of 180 µm droplets (Figure 1).

Table 1. Surface Tension for Solution of Surfactant and Glyphosate Combinations (dyne/cm).

Surfactant[a]	Glyphosate Concentration 1250 ppm (w/v)	2500 ppm (w/v)	6250 ppm (w/v)
	(dyne/cm)		
-	76.0[b]	73.3	66.8
+	35.3	35.3	35.3

[a] + indicates addition of 0.06% v/v of non-ionic surfactant (X-77); - means no surfactant was added.

[b] Values are means of 4 observations.

Table 2. Drop Density for each Droplet Size Group.

	Drop Density		
	Drop Size		
	120 um	180 um	300 um
	(#/cm^2)		
Theoretical	138	41	9
Observed	140.5 ± 11[a]	41.6 ± 2.5	9.1 ± 0.4

[a] Values are means and standard error of 5 observations.

Table 3. Biological Performance of Bean Seedlings to Varying Spray Droplet Size, Surfactant, and Herbicide Concentration. (% of growth of untreated plants).

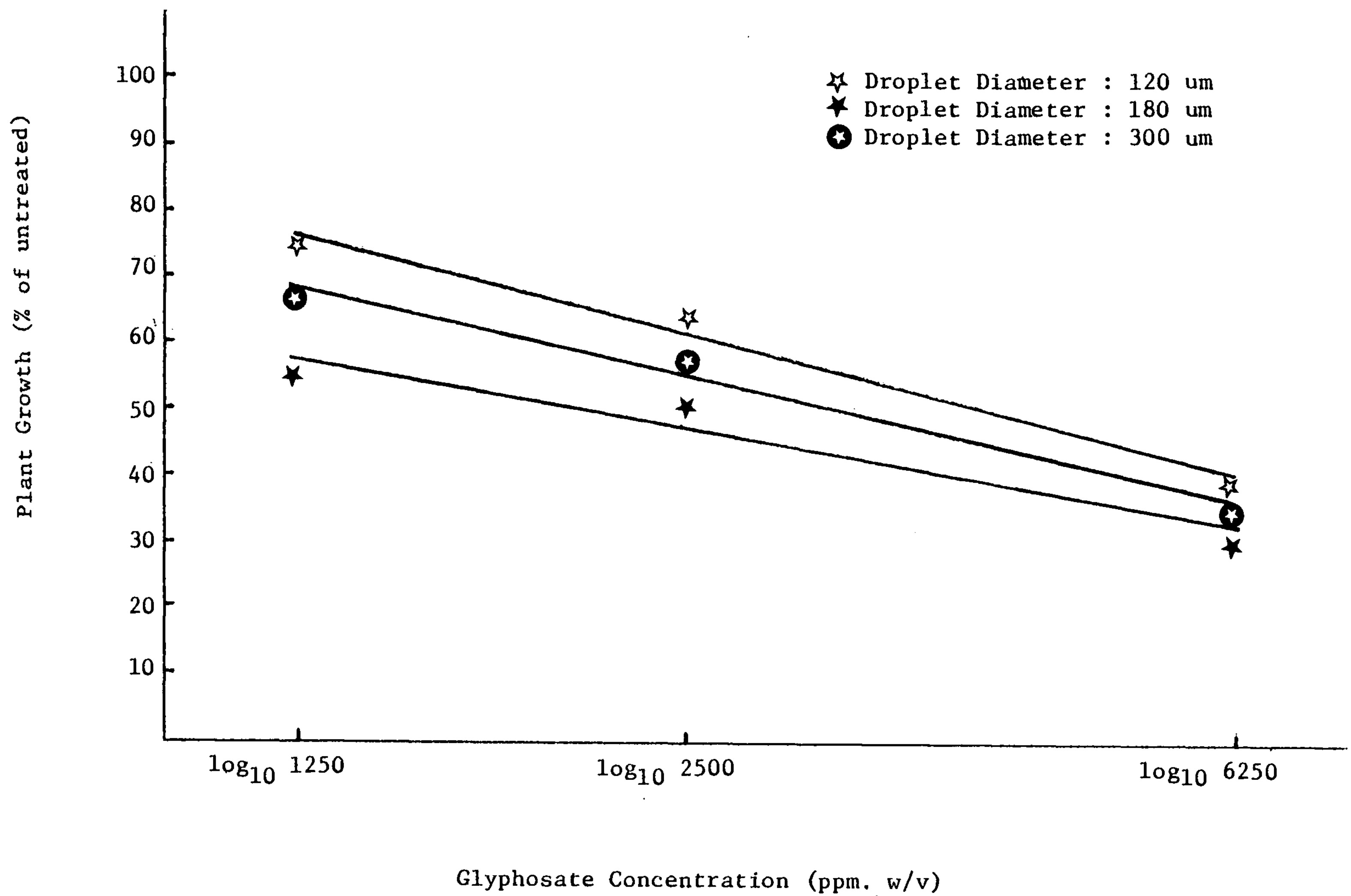
Glyphosate conc. (ppm. w/v)	Surfactant*	Plant Growth Droplet Size 120 um (%)	180 um	300 um
1250	-	75.55	56.31	68.99
1250	+	74.11	53.90	66.79
2500	-	63.55	51.06	57.24
2500	+	63.61	50.53	56.70
6250	-	40.15	30.59	35.36
6250	+	38.29	28.92	33.84

$LSD_{.05} = 1.32$

$LSD_{.01} = 1.76$

* + indicates addition of 0.06% v/v of non-ionic surfactant (X-77); - means no additional surfactant was added.

Figure 1. Growth of Bean Seedlings at 3 Spray Droplet Sizes and 3 Dosage Levels of Glyphosate with Calculated Regressions.



Plant Growth (% of untreated)
100
90
80
70
60
50
40
30
20
10
Droplet Diameter : 120 um
Droplet Diameter : 180 um
Droplet Diameter : 300 um
log10 1250
log10 2500
log10 6250
Glyphosate Concentration (ppm. w/v)

Figure 1.

Preliminary Experiment

"Effect of Surfactant Concentration on Glyphosate Toxicity"

Effect of Surfactant Concentration on Glyphosate Toxicity

INTRODUCTION

Surfactants are commonly used in herbicide formulations to impart desired properties to the formulation and the ultimate spray mixture by facilitating the emulsifying, dispersing, spreading, wetting, solubilizing and surface-modifying characteristics of the herbicidal formulations. The addition of surfactants to herbicide formulations and spray solutions may increase, decrease or have no effect on herbicidal phytotoxicity (7, 24).

In aqueous solutions, surfactants alone may be phytotoxic when applied to plants. The phytotoxicity of surfactants may adversely affect the activity of the primary herbicide by limiting its penetration and translocation in the plant.

The concentration of the surfactant is important in determining the phytotoxicity of a herbicide spray. At low surfactant concentrations, the reduction of surface tension and contact angle of droplets of the spray tends to increase the retention of the spray solution on the weed and subsequently increase the absorption of the herbicide. As the surfactant concentration increases, the surface tension will be reduced to a minimum. Surfactant concentrations beyond the critical micelle concentration (CMC) will result in no further reduction in the surface tension of the mixture. The CMC is usually low, the CMC of X-77 in distilled water was about 0.05% v/v, and for glyphosate (0.05 kg/ 20l) was about 0.06% v/v. Generally, the phytotoxicity of the herbicide increases as the surfactant concentration increases, although maximum effectiveness concentrations are reached for most herbicides.

It was reported (24) that with 0 to 1.0% w/v concentrations of different surfactants, in most cases, increased concentrations resulted in increased phytotoxicity, although some exerted an adverse effect on phytotoxicity as the concentration of surfactant reached 1.0%. Hull (39) found that the phytotoxicity of translocated, foliar-applied 2,4,5-T was reduced due to morphological changes in the plant tissue and the subsequent effect of these changes was to reduce uptake and transport processes.

In this experiment, the interaction of different levels of X-77 (a non-ionic surfactant) with the herbicidal activity of glyphosate on bean seedlings was studied to determine at what level of surfactant is glyphosate most effective under laboratory conditions.

Materials and Methods

Four seeds of a bush type green bean (*Phaseolus vulgaris* L.[1] 'OSU 1604') were sown in a 11 cm pot in a peat vermiculite medium (Jiffy Mix Plus). Three days after seed emergence, seedlings were thinned to two plants per pot. At thinning, an attempt was made to select uniform plants.

Technical glyphosate at 1250 or 2500 ppm (w/v) was used alone or with 0.01, 0.06, 0.1, 0.6 and 1.0% (v/v) of a non-ionic surfactant—Multi-Film X-77. Twenty 1 µl drops were applied with a microsyringe to the primary leaves of 7 day old bean plants.

Three days after treatment a visual rating of contact injury was made according to the following scale: 0- no injury, 1-3 varying degree of chlorosis (spots), 4-6 varying amounts of leaf tissue necrosis. Plants were harvested 7 days after treatment and the fresh weight of aerial parts of the plant was the biological response measured.

The surface tension of each solution was measured by a surface tension meter (Surface Tension Apparatus). The contact angles of droplets on the leaf surface were determined by cutting 5 mm wide leaf strips perpendicular to the midrib. Five 1 µl drops were slowly and carefully built up by a microsyringe and then put into a cuvette with saturated humidity. From values obtained by projecting an elevation view of the droplets on the leaf surface the contact angle was calculated with the equation $\theta = 2 \arctan (h/r)$, where "h" is the height of the drop at the apex, and "r" is the radius at the interface of droplet and leaf surface.

Results and Discussion

The visual rating indicated that higher concentrations of X-77 (0.6 and 1.0% v/v) and glyphosate mixture produced spotting and some leaf tissue necrosis while lower concentrations did not give any visible changes in the leaf surface. The means of the fresh weight, as measured at the end of the experiment, and the rating scores are presented in Table 4.

Both herbicide concentration and surfactant concentration had a significant effect on phytotoxicity, when response was measured by fresh weight. There was also a strong interaction between the herbicide concentration and surfactant concentration effect on phytotoxicity. The greatest phytotoxic effect occurred at the concentration 0.1% (v/v) of X-77 at both high and low glyphosate concentrations.

It is suggested that at low surfactant concentrations, there was not enough surfactant to facilitate penetration or cause phytotoxicity in itself. And at the higher concentrations of X-77, the tissue at the treated spot was killed and the process of penetration was thus blocked, so plants with some necrosis on the leaf could still survive and continue growing. At the higher concentration of herbicide, there was a significant difference between higher (0.6 and 1.0%) and lower (0.01 or 0.06%) concentrations of X-77. This might have resulted from the fact that some of the herbicide penetrated the cuticle and entered the cells before the tissue was killed.

The surface tensions of each spray solution is given in Table 5. The addition of small amounts of X-77 greatly reduced the surface tension of both 1250 and 2500 ppm (w/v) glyphosate solution, but concentrations of X-77 beyond 0.06% did not further reduce the surface tension of glyphosate solution.

The contact angle of each spray solution on bean leaf is given in Table 6. The trend is the same as for surface tension, with the concentration of X-77 beyond 0.06% not significantly affecting the contact angle. In this study, neither surface tension nor contact angle of the spray solution was significantly related to the phytotoxicity of the herbicide spray.

Table 4. Effect of Surfactant Concentration of Glyphosate Phytotoxicity to Bean Seedlings. (Fresh weight and visual rating.)

Surfactant (X-77) conc. (% v/v)	Glyphosate Concentration			
	1250 ppm	2500 ppm	1250 ppm	2500 ppm
	(gms)		(scores)	
0	1.97	1.80	0	0
0.01	1.95	1.76	1	1.2
0.06	1.69	1.64	1.2	1.2
0.10	1.32	0.67	1.4	1.2
0.60	1.64	1.48	5.0	5.2
1.00	2.10	1.47	6.0	6.0

$LSD_{.05} = 0.25$

$LSD_{.01} = 0.28$

Table 5. Effect of Surfactant Concentration on Surface Tension.

	Surface Tension					
	Surfactant (X-77) conc. (% v/v)					
Glyphosate conc. (w/v)	0	0.01	0.06	0.10	0.60	1.00
(ppm)	(dyne/cm)					
1250	61.3[a]	37.2	34.8	34.8	34.4	34,3
2500	61.1	37.2	34.75	34.7	34.3	34.3

[a] Values are means of 4 observations.

Table 6. The Contact Angle of Drops of Spray Solution on Bean Leaf Surface (degrees).

	Contact Angle*					
	Surfactant (X-77) conc. (% v/v)					
Glyphosate conc. (w/v)	0	0.01	0.06	0.10	0.60	1.00
(ppm)	(degrees)					
1250	42.2[a]	25.2	14.8	14.0	12.6	12.6
2500	42.3	24.5	14.4	13.3	12.5	12.4

* Values are means of 5 observations.

[a] Non-ionic surfactant X-77.

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APPENDIX

Appendix A. Response of Bean Seedlings to Varying Spray Droplet Size, Surfactant, and Herbicide Concentration. (Fresh weight of aerial part).

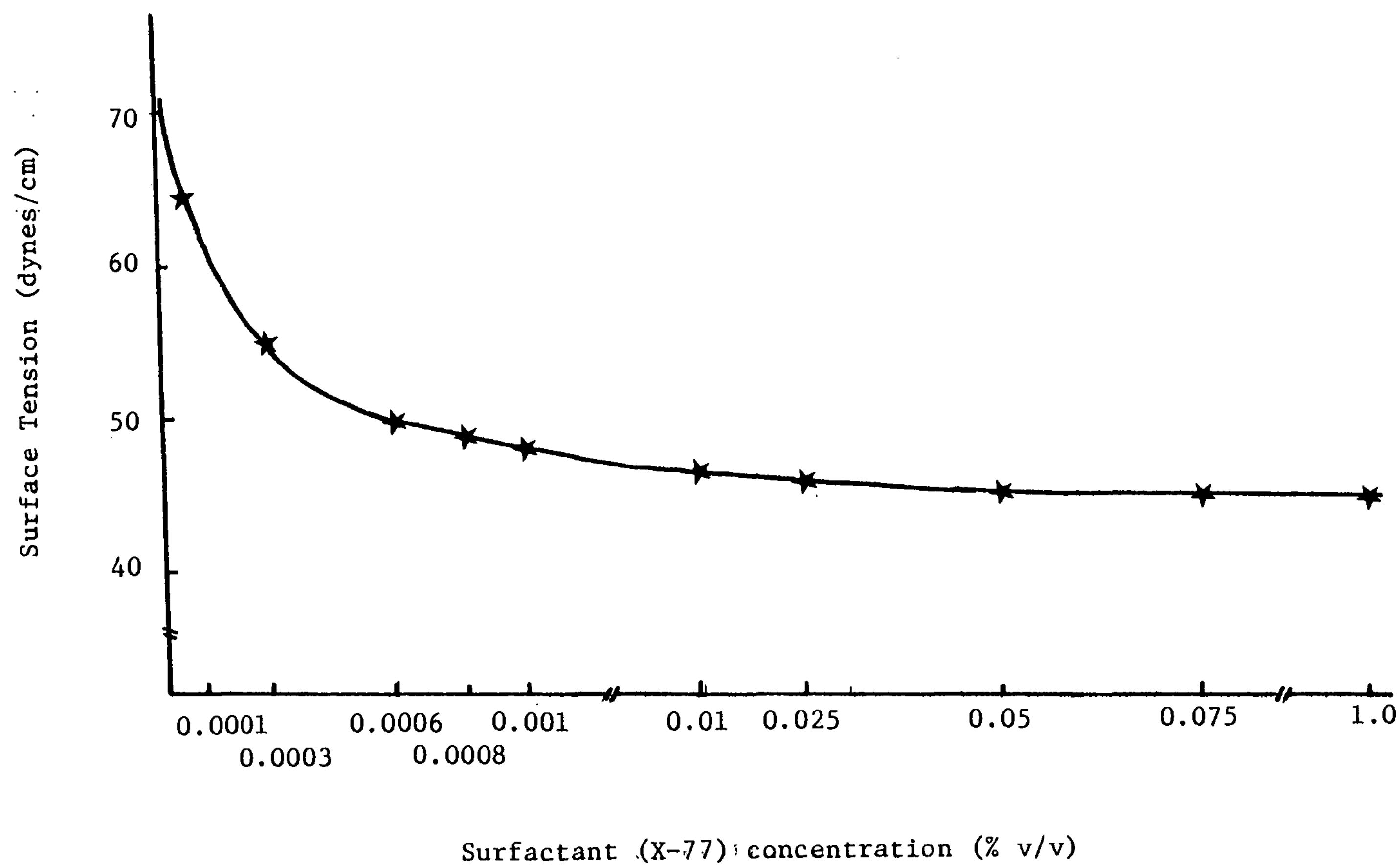
		Plant Growth		
		Droplet Size		
Glyphosate conc. (ppm. w/v)	Surfactant[a]	120 um	180 um	300 um
		(gms)		
1250	-	14.11	10.48	12.86
1250	+	13.84	10.02	12.45
2500	-	11.83	9.51	10.67
2500	+	11.86	9.41	10.56
6250	-	7.48	5.69	6.56
6250	+	7.12	5.36	6.27

$LSD_{.05} = 0.38$

$LSD_{.01} = 0.50$

[a] + indicates addition of 0.06% v/v of non-ionic surfactnat (X-77); - means no surfactant was added.

Appendix B. Surface Tension of Distilled Water and X-77 Mixture. (dynes/cm)


70
60
50
40
Surface Tension (dynes/cm)
0.0001
0.0003
0.0006
0.0008
0.001
0.01
0.025
0.05
0.075
1.0
Surfactant (X-77) concentration (% v/v)