

AN ABSTRACT OF THE THESIS OF

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Title: Glyphosate Activity as Affected by Spray Drop Size and Leaf Coverage.

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Bean shoot parts that respond to glyphosate (N-(phosphonomethyl)glycine) in ways useful for bioassay were determined by applying glyphosate doses of 3.8 to 60.3 ug ae per plant to the simple leaves when the first trifoliolate leaflets were about 1 cm long. Dry weights of parts that were almost fully enlarged at treatment were greater in treated than untreated plants after two weeks. Maximum weight increase was found with the 15.1 ug dose. Growth of younger shoot parts was reduced by glyphosate, showing linear reductions with log dose from 3.8 to 30.2 ug. Growth reduction of young shoot parts was therefore concluded to be a better measure of sublethal glyphosate activity than reduction of total shoot growth.

Using the above assay, growth of bean plants from a controlled environment was evaluated after treatment with a 3.0 mM glyphosate solution applied in uniform drops of 138, 430, and 1230 um diameter. The largest drops were less effective than both smaller sizes, and no difference in activity between the two smaller sizes was found. Bean plants grown outdoors in pots responded similarly to a 12.2 mM

glyphosate solution applied in drops of 138, 240, 430, 740, and 1230  $\mu\text{m}$  diameter. There were no activity differences between the four smallest sizes, and the 1230  $\mu\text{m}$  size was less effective than all others.

To ascertain effects of leaf coverage on glyphosate activity, 1.0  $\mu\text{l}$  drops of glyphosate solution were applied to the simple leaves of bean plants and physically spread with the tip of a small, glass rod to cover areas of different size. Herbicidal activity was reduced with increased drop spread on plants grown in a controlled environment, but not with plants grown outdoors. Cuticular adsorption of glyphosate was assumed not to be a factor in reducing activity because isolated leaf cuticles from beans grown both outdoors and in a growth chamber were shaken in an aqueous solution of  $^{14}\text{C}$ -glyphosate at 25 C and showed no adsorptive tendency throughout an 83-hour period.

The hypothesis that glyphosate applied in low volumes is more effective because of reduced leaf surface contact is not supported by drop size data, and is supported only by results from controlled environment treatments showing less activity from increased drop spread.

Glyphosate Activity as Affected  
by Spray Drop Size and Leaf Coverage

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GLYPHOSATE ACTIVITY AS AFFECTED  
BY SPRAY DROP SIZE AND LEAF COVERAGE

INTRODUCTION

Glyphosate is a highly effective, systemic, broad spectrum, foliar active herbicide that is widely used. Its effectiveness has been shown to be influenced by many factors, including environmental conditions, other herbicides, surfactants and other additives (including ammonium sulfate), polyvalent metal cations, carrier volume, and spray drop size (Turner, 1985; Wills and McWhorter, 1985; Wyrill and Burnside, 1977).

Of these factors, probably the least understood is spray drop size. The work that has been published thus far in this area has been inconclusive, with variable results even from the same researchers (Buhler and Burnside, 1983A and 1987). Interactions of drop size with other factors that affect glyphosate activity, particularly spray volume, are almost a certainty. Furthermore, even with drop size alone, it is difficult to ascertain if activity changes between treatments are due to differences in spray interception and retention or to biological activity. Other difficulties in working with spray drop size include problems with generating drops of desired uniform size and correctly measuring the drops.

A general conclusion about glyphosate is that it is most effective when applied in low carrier volumes (high concentrations), with reduced activity as spray volume increases (Sandberg et al.

1978). For example, applying 1.0 kg glyphosate per ha in 50 l spray volume is usually more effective than applying 1.0 kg per ha in 400 l spray volume. Preliminary experiments have shown this effect when spray runoff to soil was not a factor. One possible reason for this effect is that the concentration gradient from the low carrier volume drops is greater, causing greater diffusion into the leaf. However, the water used as a carrier can evaporate quickly, leaving a dried deposit. Therefore, the concentration in the spray solution is not likely the direct cause.

Another possible reason for lower carrier volumes giving greater effect can be related to the plant surface area covered. Low carrier volumes result in less surface area covered than high volumes, and this may cause more active ingredient to be absorbed and/or translocated by the plant. If this is the case, altering the leaf surface area covered by a given glyphosate treatment in ways independent of carrier volume should also alter activity, with greater coverage resulting in reduced phytotoxicity. Changing drop size is one way to do this. Large drops contact a smaller leaf surface area than an equal volume but larger number of small drops, and might be expected to provide greater herbicidal activity.

The objectives of this research were to:

- 1) Develop methods to accurately assess effects of spray drop size and leaf coverage on glyphosate activity;
- 2) Determine drop size and leaf coverage effects on glyphosate phytotoxicity independent of variations in other factors known to affect activity.

## REVIEW OF LITERATURE

### Spray Retention and Drop Size

Reichard (1988) used a high speed motion picture camera to record the impact and retention of spray drops 63 to 545  $\mu\text{m}$  diameter on several plant species and found that microstructure influences drop rebound more than does drop size. Over 90% of water drops of all sizes tested rebounded from broccoli and wheat leaves after initial impingement, while almost none rebounded from the pubescent, adaxial surface of apple leaf. Rebound from cabbage leaf was decreased after the cabbage leaf surface was rubbed gently, but enough to change its appearance. Adding surfactant reduced rebound, but the effect was apparently independent of drop size. Ennis et al. (1952) reported that additives, pubescence, and leaf angle were the primary factors affecting spray drop retention, and Lake and Taylor (1974) found no effect of drop size on spray retention on wild oats using a size range of 110 to 440  $\mu\text{m}$  diameter. From the above, it appears that spray drop size has little influence on spray retention by plants. Spillman (1984), however, indicated that most drops smaller than 100  $\mu\text{m}$  diameter will be retained by leaves upon impact regardless of other factors.

### Glyphosate Activity and Drop Size

In a study of glyphosate toxicity to bermudagrass, Jordan (1981) suggested that the greater herbicidal activity he observed from low carrier volumes may have been due to smaller drops. The low volumes were applied with smaller nozzles that generated smaller

drops. He indicated that smaller, more concentrated drops may increase the rate of diffusion into the leaf. As another possibility, he suggested that larger drops from bigger nozzles may have become physiologically isolated by killing or damaging the leaf cells under the drops. This seems unlikely, as the larger drops were of a more dilute glyphosate concentration.

Buhler and Burnside (1983A) also varied the water carrier volume of glyphosate sprays by changing only the nozzle size. They found greater activity with low volumes and suggested that the smaller drops were associated with increased absorption into the leaves. More recently, the same authors (1987) reported greater herbicidal effect of glyphosate with 250  $\mu\text{m}$  drops than 215 or 80  $\mu\text{m}$  spray drops on a dense stand of volunteer wheat and large crabgrass. There was more drop size response with large crabgrass than wheat. They attributed their results to greater penetration of the dense canopy by the large drops, and because large crabgrass has a prostrate growth habit, the effect would be accentuated with that species. One would expect good spray interception by a dense canopy, but measurements of spray retention were not reported, and are difficult to do.

#### Drop Size Effects on Other Herbicides

Data from the literature concerning drop size effects on other herbicides is sometimes contradictory, even for the same herbicides. Overall, more researchers conclude that small spray drops are more effective than large. Smith (1946), using aqueous sprays of 2,4-D on kidney beans, found large drops (250 to 560  $\mu\text{m}$  diameter) more

inhibitory to growth than small drops (about 30 um diameter). He attributed the difference to greater spray interception by the plants of the large drops. Hydraulic nozzles were used, and drop size changed by increasing nozzle pressure from 10 to 40 psi. Ennis and Williamson (1963) suggested the high volume and high pressure with associated turbulence Smith used to generate small drops may have caused the bean leaves to have a more acute angle, therefore intercepting less spray. These same workers ran several experiments in both greenhouse and field using three different methods for producing drops. Three herbicides were tested on six sensitive crops, but spray volumes used in field experiments were far less than normally applied. Drop sizes ranged from 100 um to 750 um diameter, equivalent concentrations and doses were used within each experiment, and equal interception and retention of drops were assumed. In all tests, small drops of 2,4,5-T butyl ester, 2,4-D butyl ester, and CIPC solutions were more inhibitory to growth of sensitive plants than were large drops. The better response from small drops was ascribed to more efficient absorption and translocation, with small drops giving more area of contact, and growing points getting more frequent and direct chemical contact. They suggested that larger drops were less effective because the high chemical concentration caused the large drops to become "physiologically isolated because of their profound effects on the leaf cells in direct contact with the substances."

In greenhouse work with 2,4-D and 2,4,5-T, Hurt et al. (1969) found that tree seedlings were affected more by smaller herbicide drops at each of three doses. They used micropipettes to treat tree

seedlings, and a spinning cup apparatus to generate drops of 125, 250, and 500  $\mu\text{m}$  diameter to treat bean plants. Five fold greater activity occurred with drops of 125  $\mu\text{m}$  diameter than with 500  $\mu\text{m}$  drops. The 250  $\mu\text{m}$  size appeared intermediate in activity but was not significantly different from the smaller size. They calculated dose by counting the number of drops on the bean leaves and multiplying by the volume of the particular drop size used. Significant errors can result from this method because a 10% error in determining drop diameter causes a 33% error in drop volume calculation. It is doubtful, however, that a five fold error in estimating dose occurred. Brady (1972) showed that uptake of 2,4,5-T-isooctyl ester into leaves of six hardwood species was inversely related to drops size. Fisher and Young (1950) found that moderately coarse to course drops of 2,4,5-T spray were generally better for controlling mesquite when applied by airplane. Their observations did not include deposition data. Lettuce response to sub-lethal doses of MCPA in different drop sizes was evaluated by Way (1969), who found consistent trends for the 100  $\mu\text{m}$  diameter drops to produce greater effects than 500  $\mu\text{m}$  drops.

Homogeneous sprays of small drops of 2,4-D in diesel oil were found to be more effective than sprays consisting of larger drops when applied to sunflower seedlings (McKinlay et al., 1972). It took three times and six times as much active ingredient for equal effect when applied in drop sizes of 200 and 400  $\mu\text{m}$  diameter, respectively, than in drops 100  $\mu\text{m}$ . Two factors were suggested for the increased activity from small drops. One is that the area per volume covered is greater with small than large drops, giving an

increased rate of penetration. The other is that large, concentrated drops of 2,4-D damage cells, as Ennis and Williamson (1963) proposed, causing reduced translocation of 2,4-D. The latter possibility was supported by microscopic observation of leaf tissue in which cells beneath only the 400  $\mu$ m drops were seen to be damaged. Whether the damage was from 2,4-D or the diesel oil carrier was not known. They did find that dose could be increased equally well by increasing the number of drops of a given size and herbicide concentration, or by increasing the concentration while maintaining the same drop size and number. They inferred that reduced penetration due to smaller area covered by the equal number of more concentrated drops was balanced by the effect of higher concentration. But it also provides evidence that damage from the largest drop size was due to something other than active ingredient.

Behrens (1957) indicated that low-volume applications of 2,4,5-T must employ sufficiently small drops to achieve adequate frequencies of drop deposition. He concluded that drop spacing is of major importance in herbicidal effectiveness, having found that a drop spacing of 3.1 mm, equal to 72 drops per square inch, is the maximum allowable to maintain good herbicidal effectiveness of 2,4,5-T on cotton and mesquite. Though drop spacing may be more important than size, they are, of course, directly related when a given volume of spray is applied. Stevens and Bukovac (1987), on the other hand, demonstrated that uptake efficiency of daminozide and 2,4-D-triethanolamine in field bean was not correlated with concentration, carrier volume, drop size, drop frequency, or leaf coverage. The quantity taken up by a plant was increased only by

increasing the applied dose, regardless of other factors.

Spray drop size had significant effects on paraquat, diuron, and fluometuron in work by Buehring and Santelmann (1969), with large drops less efficacious than small ones. Drop size effect was masked as dose increased. The herbicidal activity of both amitrol and MSMA were unaffected by drop size.

With aqueous paraquat, McKinlay et al. (1974) observed greater phytotoxicity to sunflower seedlings with uniform drops of 100 than 350  $\mu\text{m}$  diameter. They also compared treatments applied with standard hydraulic nozzles and found the standard method to be as effective as the small, uniform sized drops. Cooke et al. (1984) also found that a broad spectrum of drop sizes produced by hydraulic nozzles at 200 l/ha provided better biological control than a variety of alternative spray application techniques when applying fungicides and herbicides.

Douglas (1968) treated broad bean leaves with aqueous diquat and paraquat drops of 250 to 1000  $\mu\text{m}$  diameter. An increase in size above 250  $\mu\text{m}$  increased herbicidal efficiency to an optimum of about 400  $\mu\text{m}$  for paraquat and between 500 and 550  $\mu\text{m}$  for diquat at all three concentrations evaluated. The technique he used was interesting in that he collected only two or three drops on each leaf. Because diquat and paraquat are contact herbicides, the active ingredient in each drop killed the tissue immediately below and around it, creating a necrotic lesion. By measuring the diameter of the lesions formed, and knowing the size and concentration of drops from which they formed, he calculated efficiency of the herbicides as the area of lesion formed per  $\mu\text{g}$

active ingredient.

Barban was more effective on wild oat in drops of 110  $\mu\text{m}$  than in drops 220 or 440  $\mu\text{m}$  diameter (Lake and Taylor, 1974). At low volume rates of 5 l/ha for difenzoquat and chlorfenprop-methyl, and 10 l/ha for barban, a drop size of 150  $\mu\text{m}$  was more effective than 250  $\mu\text{m}$  diameter (Merritt and Taylor, 1977). At 15 l/ha, no activity difference from drop size was found with difenzoquat. With oil solutions of barban and chlorfenprop-methyl at 20 and 45 l/ha, respectively, the larger drop size was more effective. Factors suggested as possibly causing these results are factors that influence control and dispersion of the active ingredients over a leaf, such as spray retention and drop spreading. Merritt (1982) found that low volume/high concentration applications of difenzoquat caused localized scorch in wild oats and reduced herbicide uptake, an effect to which large drops contributed the most.

Buhler and Burnside (1984) applied given doses of fluazifop-butyl, haloxyfop-methyl, and sethoxydim to sorghum plants in single drops of 1, 2, 4, 8, 16, and 24  $\mu\text{l}$  (a spherical drop of 1.0  $\mu\text{l}$  is 1240  $\mu\text{m}$  in diameter). Smaller, more concentrated drops were more phytotoxic than the larger, more dilute drops. They also reported greater activity with these compounds in the field when applied in low carrier volumes and suggested that the smaller drops produced from smaller orifice nozzles used with the low volumes may have been a reason. They indicated the smaller drops may have given better distribution over the leaf surface, resulting in more herbicide absorption.

It is obvious from the above that few definite conclusions

regarding the role of spray drop size on herbicide activity can be reached. Reports conflict, and data is lacking to allow explanation of the diverse data on a chemical, physical, or physiological basis.

#### Production of Monosize Drops

Researchers have used various techniques for producing drops of uniform size. Probably most commonly used has been a spinning disk, or rotary atomizer. Gebhardt (1988) recently reviewed the history and operation of this device. Basically, liquid is metered onto the surface of a rotating disk or cup where centrifugal forces move it to the periphery. Droplets are produced either singly, from ligaments, or from sheets of liquid flung from the periphery. Which type of drop formation occurs is a function of liquid metering rate, liquid viscosity and surface tension, disk size, and rotation speed as well as modifications that may have been made to the disk. Commercial rotary atomizers usually have grooves or teeth on the disk to facilitate ligament drop formation at higher metering rates than would be possible if the disk were smooth. Single drop formation produces drops of most uniform size; sheet drop formation produces a drop size spectrum that could be compared to commercial, hydraulic nozzles; and ligament drop formation produces a narrow drop size spectrum, predominately composed of two main sizes of drops, the sizes depending on the liquid and disk properties. Even with sheet drop formation, uniform drop sizes can be obtained from a rotary atomizer in a fixed, horizontal position because drops automatically sort themselves out in flight into a spectrum of sizes, in which all of a given diameter settle in one region. Way

(1969), McKinlay (1969), Lake and Taylor (1974), and Taylor et al. (1976) all used rotary atomizers in research on drop size.

Ennis and Williamson (1963) initially used a device somewhat similar to a rotary atomizer but later used what they called a "glass droplet sizer." This consisted of a glass tube, the tip of which was drawn to a capillary, through which liquid was forced. The tube was contained inside a larger tube through which air was pumped. Liquid forced from the inner tube was drawn off by the air flow in consecutive drops of uniform size.

A vibratory apparatus for producing uniform drops was developed by Davis (1959). Liquid forced from a capillary at constant rate was broken into a constant stream of drops by a flattened wire which contacted the exuding liquid 60 times per second, forming a single drop at each contact. The device was used with oil solutions and could generate uniform drops between six and 140  $\mu\text{m}$  diameter. Rayner and Haliburton (1955) developed a rotary device to form uniform drops in the range of 50 to 700  $\mu\text{m}$  diameter. A horizontally rotating blade detached drops in a steady stream from a stabilized liquid mass fed under constant head through a stationary capillary. For his high speed motion picture records of drop impingement, Reichard (1988) used a vibrating monodisperse aerosol generator to produce drops of uniform sizes. A piezoelectric ceramic vibrating at a given frequency caused liquid forced through an orifice to break into uniform drops between 63 and 545  $\mu\text{m}$  diameter.

Merritt and Drinkwater (1977) described a device which they used to produce single drops of uniform size from 160 to 490  $\mu\text{m}$  diameter. A sharp needle withdrawn from a liquid at high speed

produces a drop from the liquid that adheres to the needle. The drop breaks away from the needle tip and travels upwards a short distance before falling, at which point it can be intercepted. Drop size is dependent on the needle size and depth it is inserted into the liquid, the speed with which it is withdrawn, and the liquid properties. By use of a hand cranked cam which pushed a metal arm under spring tension with an attached needle, they could generate a drop with each turn of the crank.

#### Measurement of Drop Size

Several methods of measuring the size of individual drops have been used, with two general categories: absolute methods, in which drops are collected in a spherical condition and measured either directly or from a photograph; and indirect methods, in which the drops are allowed to fall on a prepared surface and the resulting marks are measured. The indirect methods must be calibrated against an absolute method (Busvine, 1971).

Probably the simplest absolute method for aqueous drops is with a mixture of vaseline and paraffin oil in ratios of about 1:4, respectively. Drops collected in this medium remain spherical and can be measured under a microscope with a calibrated ocular micrometer. Fuchs and Petrijanoff (1937) used this method for capture and measurement of fog drops. Matthews (1979, page 69) warned that drops collected in this way must be covered quickly with oil to prevent evaporation reducing their size. Lake and Taylor (1974) covered the mixture with a lighter hydrocarbon, liquid paraffin containing 55% by volume of hexane, immediately before use,

apparently so the drops would be immediately covered and not suspended at the surface where they could evaporate.

Tate (1961) collected aqueous drops in a transparent cell filled with kerosene and reported that when drops sink they remain suspended as practically perfect spheres which can be measured directly or photographed. Gebhardt and Bode (1967) used a similar cell, but used two immiscible oils, pump oil and kerosene, to collect aqueous drops. The drops would remain suspended at the oil/kerosene interface where they could be measured. Daum et al. (1968) described media for collecting oil drops, and one for aqueous drops made of a hardened ester of castor oil (0.5 to 1.0%) in mineral spirits.

May (1950) described a method that has been widely used for determining drop size that requires microscope slides freshly coated with magnesium oxide. Drops impacting in the magnesium oxide leave a crater, the diameter of which is 1.16 times as large as the drop diameter. This ratio holds over a broad drop size range and is independent of the spray solution used.

Other indirect methods involve using a dye in the spray solution and collecting them on clean, glass slides or cards of various kinds and measuring the diameters of dried deposits (Lewis, 1969; Yeomans, 1949). Turner and Huntington (1970) gave instructions on making water sensitive paper, thereby eliminating the need for dye in the spray solution. They said stain size was linearly related to drop diameter when plotted on a log-log scale. Davis (1949) photographed oil spray drops collected on microscope slides that had been coated with an oleophobic film. Drops were

counted and sized from the projected image of the negative. It was necessary to know the spread factor (ratio of spread diameter to actual drop diameter) of the oil drops on the slides. Actual diameter was calculated from focal length of the drop on the slide (measured with a microscope), the spread diameter, and the index of refraction of the drop solution.

There are specialized laser particle sizing devices available that are fast and precise but very expensive. These are very useful in work such as was done by Yates et al. (1984) in determining drop spectra from nozzles from aerial application equipment.

## BEAN SHOOT GROWTH AS A BIOASSAY FOR GLYPHOSATE ACTIVITY

### Introduction

Sublethal herbicide doses are commonly used in investigations of factors that affect herbicide phytotoxicity, in part because activity differences between lethal doses are often more difficult to detect. Inhibition of plant growth is often used as the measure of herbicidal activity. After a plant is treated with a sublethal dose of a particular herbicide, however, it seems reasonable to assume that the growth rates of various plant parts may be relatively different. Preliminary studies of factors affecting glyphosate activity in bean plants showed that the growth response of whole bean shoots was an inconsistent measure of glyphosate dose. The objective of this study, therefore, was to determine the parts of bean shoots that provide the most accurate and sensitive growth response to low doses of glyphosate.

### Materials and Methods

Seeds of snap beans (Phaseolus vulgaris L., 'Oregon 91G') were germinated in wet perlite in a growth chamber with a 13:11 hr light:dark cycle at 26:17 C, respectively. Relative humidity was not controlled and photosynthetic photon flux (PPF) was  $320 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ . Uniform seedlings were transplanted to a peat/vermiculite (1:1, v/v) potting mix in square 6.3 cm pots after 6 days when secondary roots were beginning to develop.

Plants were treated 9 days after transplanting when simple leaves appeared fully expanded and leaflets of the first

trifoliolate leaves were about 1 cm long. An aqueous 11.2 mM glyphosate solution, prepared from a commercial formulation ('Roundup,' from Monsanto Company) of the isopropylamine salt that contains a cationic surfactant, was applied to adaxial surfaces of the simple leaves in 1.0 ul drops with a syringe equipped with a repeating dispenser. Treatments were 2, 4, 8, 16, and 32 drops (3.8 to 60.4 ug glyphosate acid equivalent) per plant. Equal numbers of drops were placed on each half of each simple leaf (except in the lowest dosage treatment which had only one drop per leaf). Drops formed at the syringe needle tip were touched to and adhered to the leaf. Major veins were avoided.

Just before treatment, plants were sorted by size for uniformity within replications. Treatments were arranged as a randomized complete block design with six replications. Plants were harvested 13 days after treatment except replications with the largest and smallest plants, these were harvested after 12 and 14 days, respectively.

At harvest, leaf areas were measured by tracing each leaf outline on paper of uniform density, cutting out the paper figures with scissors, weighing the figures, then dividing the weight by the paper weight per  $\text{cm}^2$ . For each plant, total trifoliolate leaf area was measured separately from total simple leaf area. Each plant was then separated into 6 parts: lower stem (simple leaf node and below), simple leaf petioles, simple leaf blades, upper stem (above simple leaf node), trifoliolate leaf petioles, and trifoliolate leaf blades. All parts were dried in a forced air oven at 52 C for at least 72 hr before weighing.

Data were subject to analysis of variance, and least significant differences were determined at the 5% probability level. Coefficients of determination ( $r^2$ ) were calculated only for measurements that appeared to have a linear response to log dose.

#### Results and Discussion

Increasing glyphosate dose to 30.2 ug caused corresponding growth reductions in totaled weights of upper bean shoot parts (trifoliolate leaf blades and petioles plus upper stem) as shown in Figure 1. The decrease in dry weight with log dose from 3.8 to 30.2 ug glyphosate was linear, with an  $r^2$  value of greater than .99. In contrast, totaled weights of lower shoot parts (simple leaf blades and petioles plus lower stem) of treated plants were greater than corresponding parts of untreated plants, with maximum weight gain at the 15.1 ug dose. Growth of lower shoot parts was not expected to be greatly reduced due to glyphosate treatment because those parts appeared fully enlarged at treatment time, however, the significant growth increases were unexpected. With weights from all shoot parts combined, the increased growth from lower parts offset reduced growth from upper parts over the 3.8 to 15.1 ug dose range, resulting in insignificant weight changes in the shoot as a whole. For bioassay purposes, growth inhibition of upper shoot parts, or new growth, is clearly a better indicator of glyphosate injury than inhibition of total shoot growth.

Individual lower shoot parts differed from each other in degree of response to glyphosate dose, but not in pattern, showing large weight increases with dose to 15.1 ug glyphosate (Figure 2).

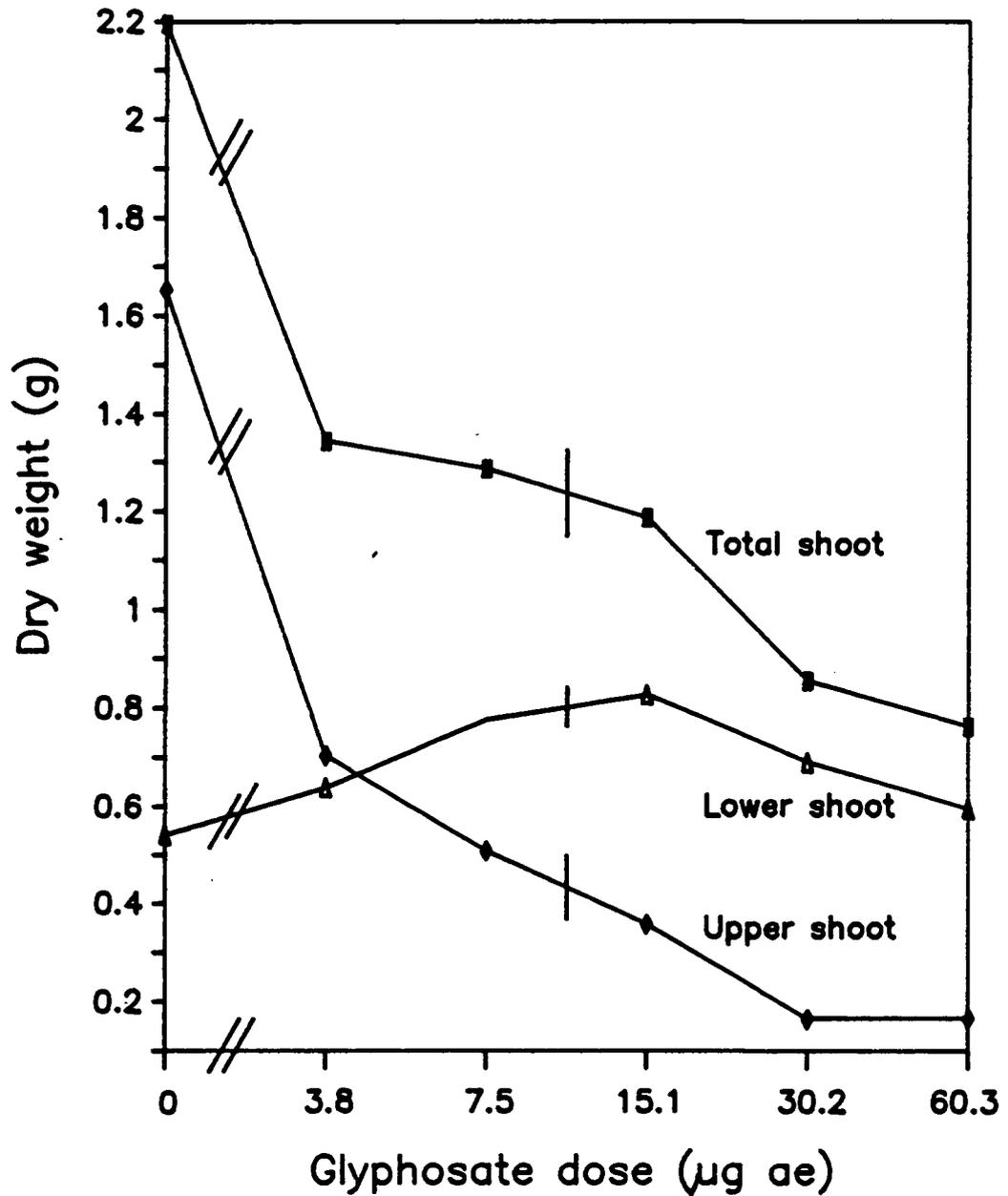


Figure 1. Bean shoot dry weights as affected by glyphosate dose 12 to 14 days after treatment. Lower shoot parts include the simple leaves, the simple leaf petioles, and stem below and including the simple leaf node. Upper shoot parts include all other shoot material. Vertical bars represent LSD .05.

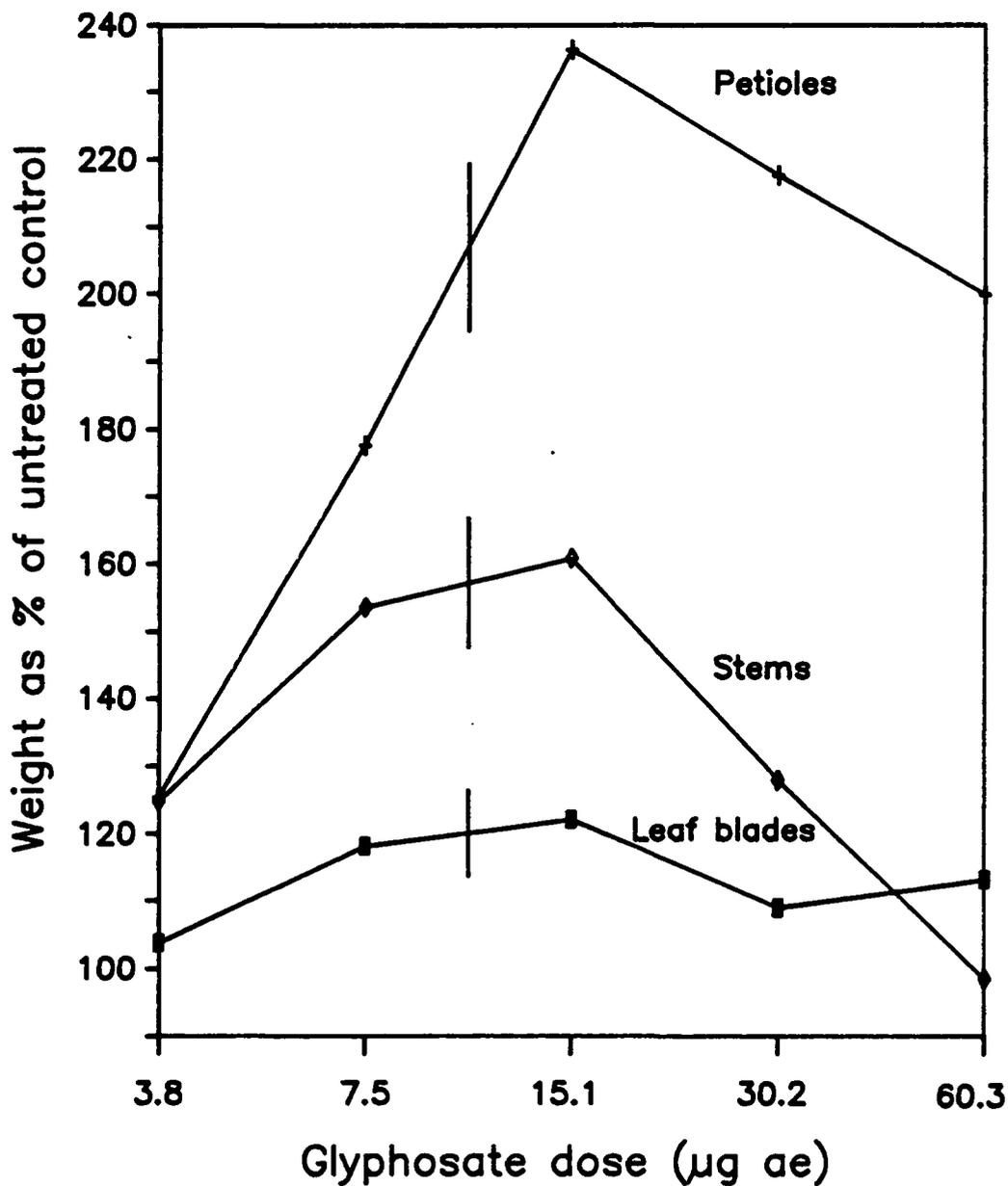


Figure 2. Dry weights of lower bean shoot parts as percent of untreated control 12 to 14 days after glyphosate treatment. Mean weights of corresponding parts from untreated controls were stem 180 mg; petioles 34 mg; leaves 328 mg. Vertical bars represent LSD .05.

Response of individual upper shoot parts were also consistent with each other and were essentially linear over the log dose range from 3.8 to 30.2 ug glyphosate, showing reduced growth with increased dose (Figure 3). The  $r^2$  values over that range for trifoliolate leaf blades, upper stems, and trifoliolate leaf petioles were all greater than .99.

Simple leaves were almost fully expanded when treated and showed no significant area response to glyphosate except at the highest dose, which inhibited leaf expansion at least 13.5 cm<sup>2</sup> per plant (Figure 4A). Trifoliolate leaf area expansion was suppressed with increasing dose up to 30.2 ug ( $r^2$  of .98). Total leaf area paralleled trifoliolate leaf area, giving an  $r^2$  of .99 over the same log dose range.

Leaf weights per area were the same in both simple and trifoliolate leaves and increased to a maximum of 146% of the control at the 15.1 ug dose (Figure 4B). From this, as well as the weight gains in lower shoot parts, it may be inferred that low doses of glyphosate are more effective at reducing growth by preventing synthesis of new tissue than in slowing weight gain by reduced photosynthetic rates. It appears that lessened sink strength in new tissue causes photosynthates to accumulate in older parts.

It can be concluded from this study that some parts of bean seedling shoots reflect sublethal glyphosate activity very well, making them useful bioassays to study factors affecting glyphosate activity. When plotted on a log dose scale over the dose range of 3.8 to 30.2 ug, growth of all upper shoot parts, either individually or together as a whole, and leaf area of all foliage or only new

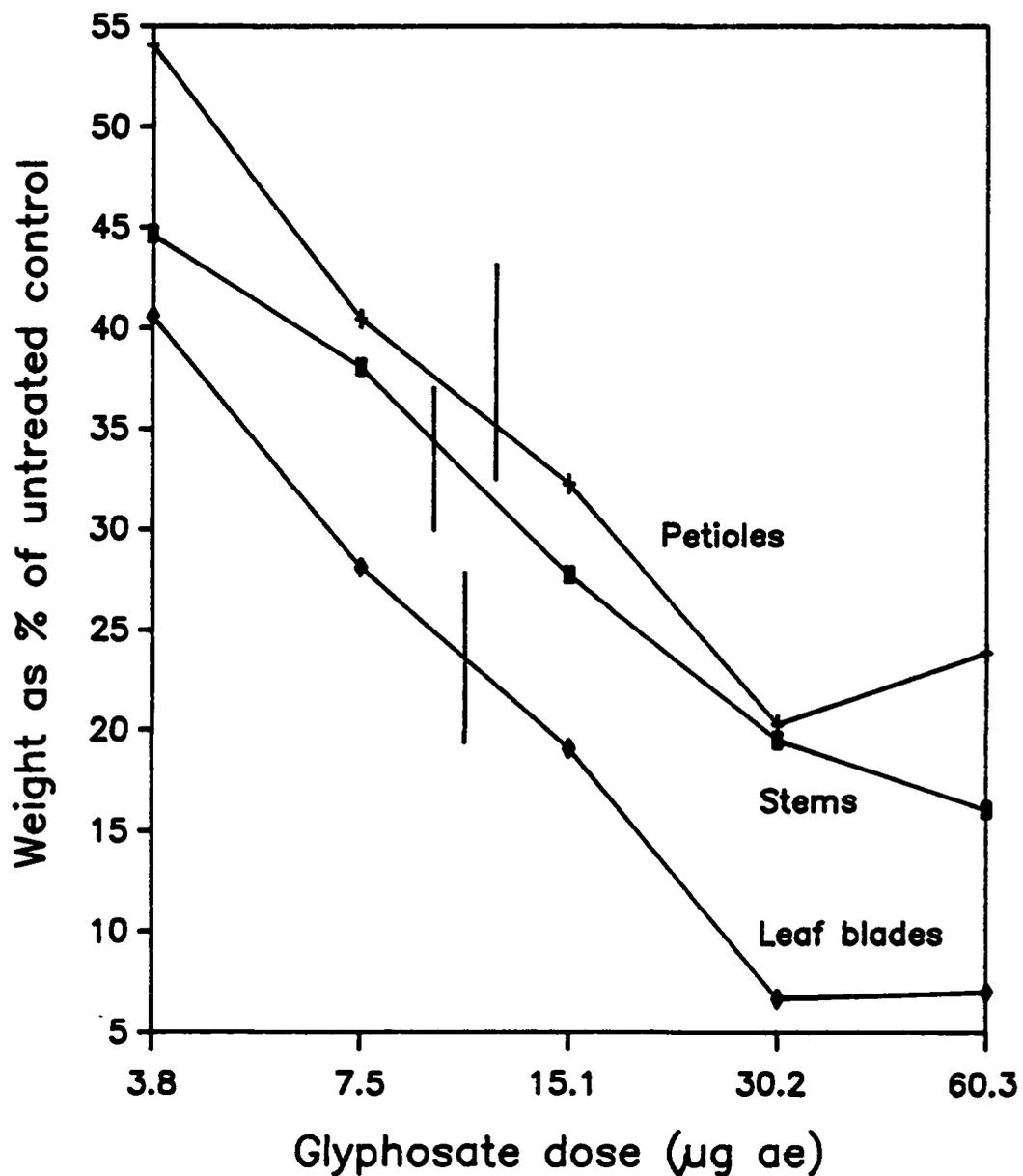


Figure 3. Dry weights of upper bean shoot parts as percent of untreated control 12 to 14 days after glyphosate treatment. Mean weights of corresponding parts from untreated controls were stem 248 mg; petioles 167 mg; leaves 1,237 mg. Vertical bars represent LSD.05.

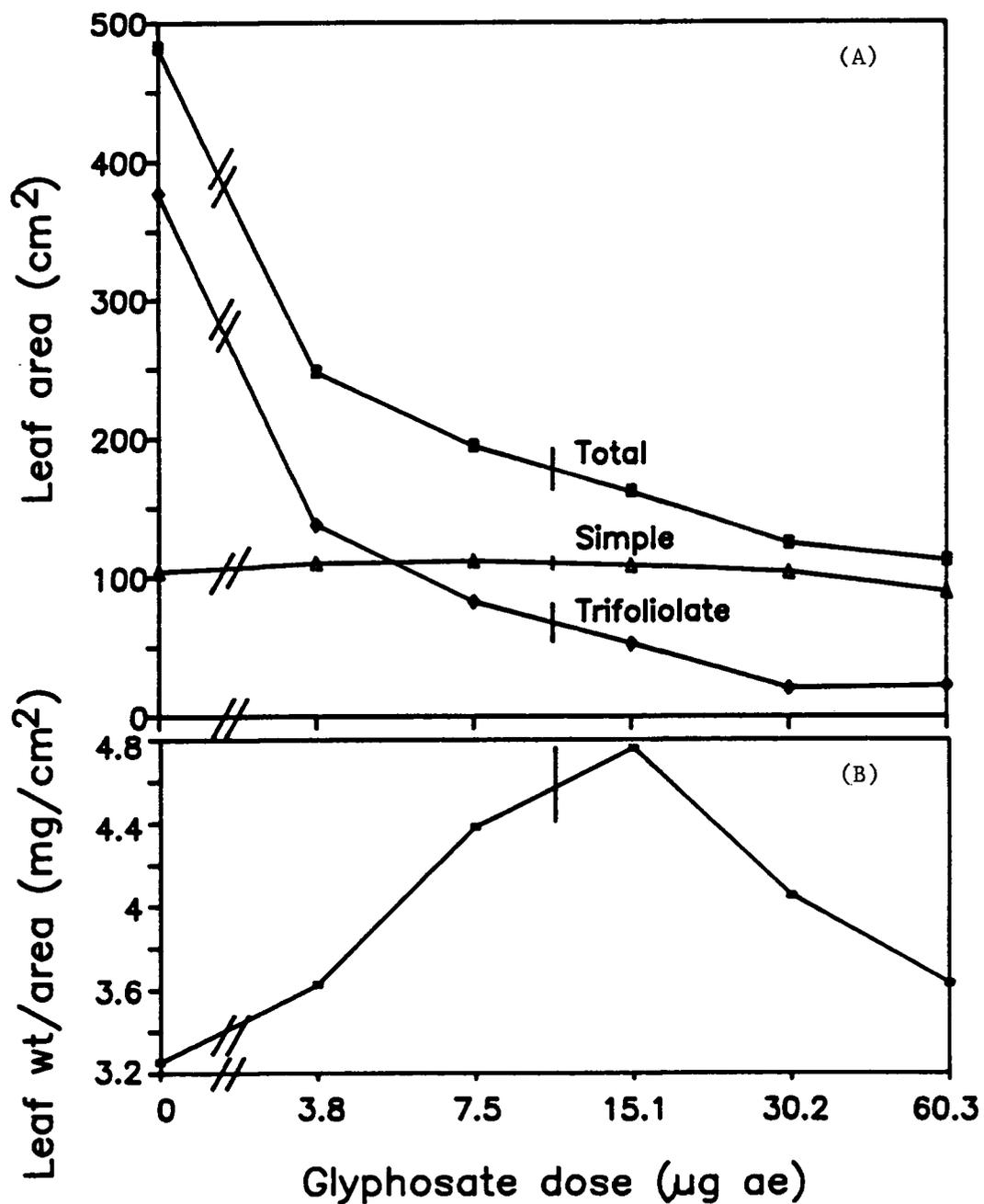


Figure 4. Leaf areas (A) and leaf weights per area (B) as affected by glyphosate dose 12 to 14 days after treatment. There were no differences between the simple and trifoliolate leaf weights per area. Vertical bars represent LSD .05.

growth, decreased linearly with  $r^2$  values of .98 or more. Because these measurements correlate so well with glyphosate dose, the choice of which to use in a particular situation can be based on convenience.

## GLYPHOSATE ACTIVITY AS INFLUENCED BY SPRAY DROP SIZE

### Introduction

As equipment is developed to allow greater flexibility and control in spray application, it is important that we understand how changing application factors affect herbicide activity so that maximum application efficiency can be achieved. Greater efficiency would reduce costs to growers and lessen the environmental impact of chemicals used. Spray drop size is one important application factor. The experiments reported in this section were to determine spray drop size effects on glyphosate activity.

### Materials and Methods

A rotary atomizer (a 'Herbi,' from Micron Corp.) was used to obtain uniform spray drops of 140, 240, and 430  $\mu\text{m}$  diameter. Drops generated from a horizontal rotary atomizer, uniform in density and initial velocity, travel horizontally in still air for a distance that is a function of their diameter, if their fall is not interrupted before horizontal movement diminishes. This makes it possible to treat small plants with spray drops of very similar size if the plants are placed an appropriate distance below and to the side of the stationary, spinning disk.

Commercial rotary atomizers for pesticide application are designed to produce a narrow drop size spectrum when used with flow rates recommended by the manufacturer. However, a much wider spectrum can be achieved by increasing the flow rate so that ligament or sheet formation of drops occurs (Gebhardt, 1988). Feed

rates greater than 70 mls per minute produced sufficient quantities of each of the three drop sizes used in these experiments.

The motor driving the rotary atomizer disk was powered from a 9 volt DC power supply that maintained rotation speed more consistently than a 12 or 13 volt DC power supply, or a 12 volt battery, when rotation speed under a load (when spraying) was compared to no load. Speed varied during treatment from 2003 to 2009 rpm's as monitored with a phototachometer. The atomizer was mounted horizontally at the top and one end of a box-like, rectangular chamber measuring 142 cm long by 40 cm wide by 100 cm high. The chamber was framed with 2.4 by 4.8 cm pieces of wood which were nailed together and then enclosed with 2 layers of transparent plastic film.

A vaseline and light paraffin oil matrix (1:4, w/w) in 5.3 cm petri dishes was used to collect drops which were measured under a microscope equipped with a calibrated ocular micrometer. When collected in this way, aqueous drops remain suspended within the matrix as near perfect spheres and are prevented from rapid drying by careful placement of a few drops of light paraffin oil over the surface immediately after drop collection (Matthews, 1979, page 69).

Drops were collected in a plane 67 cm below and from 11 to 110 cm to the side of the disk center. Drop diameter was found to increase almost linearly with horizontal distance at 3.4  $\mu\text{m}$  per cm. Drop measurements of a distilled water spray compared to distilled water plus 1% X-77 surfactant at constant disk rotation speed showed that drops of a given size and density always traveled the same distance from the disk regardless of flow rate or surface tension,

although surfactant greatly altered the relative numbers of drops of a given size compared to water alone.

For a given solution and feed rate at constant disk rotation speed, the volume of liquid applied over the horizontal distance was uneven, with bands of heavy and light application. Moreover, the flux of drops at a given distance was somewhat variable for undetermined reasons. This problem was greatest with the smallest drop size used and necessitated a method for determining the volume of spray applied to each plant, the variability preventing a single calibration from being accurate for more than one treatment.

Three 24 by 38 cm acrylic sheets, 0.8 mm in thickness, and each with a triangular,  $19 \text{ cm}^2$  hole near the middle, were affixed horizontally in the chamber 67 cm under the plane of the atomizer so that the holes were on a line from the center of the disk at distances of 13, 47, and 99 cm. Positions of the holes corresponded to drop sizes of  $138 \pm 6 \text{ }\mu\text{m}$ ,  $240 \pm 8 \text{ }\mu\text{m}$ , and  $430 \pm 10 \text{ }\mu\text{m}$  diameter, respectively. A plant to be treated was placed on the bottom of the spray chamber under an acrylic sheet so as to be shielded from all spray, except for one simple leaf which was directly underneath the  $19 \text{ cm}^2$  hole. A cover was positioned over the hole. Between the hole and the leaf an 8.7 cm petri dish, lined with commercial plastic food wrap and containing approximately 10 mls of 4:1 v/v kerosene:n-hexane solution, was placed on a movable support. Treatment consisted of removing the cover (with a handle that extended to the outside of the chamber) while the sprayer was operating, collecting in the hydrocarbon solution for 2 minutes the drops that fell through the hole, moving the petri dish to allow the

leaf to be treated for a previously estimated time period, replacing the petri dish to collect for 2 more minutes, then replacing the cover.

After spraying, the dish was removed from the chamber and the plastic food wrap removed from the petri dish by lifting all 4 corners together so that the hydrocarbon collection mixture plus the captured spray was suspended in the center of the sheet of plastic. The sheet plus suspended liquid were set in a funnel to allow coalescence of all the spray drops at the lowest point. The amount of spray solution collected was determined by carefully drawing it into a graduated 250 ul syringe and directly measuring the volume. The accuracy of this method was checked spectrophotometrically with a dyed spray solution and the two methods always varied by less than 3%. The quantity of spray received by the plant was estimated by multiplying the volume of spray collected in the dish in 4 minutes by the fraction of 4 minutes the leaf was sprayed. The length of time a leaf was sprayed depended on the intended dose and the previous measurements of volume per time for that drop size.

Nine seeds of Oregon 91G snap beans were planted in a moist peat/vermiculite potting mix in 10.5 cm square pots. Seeds were pushed just into the media surface and covered with dry media. Pots were placed in a growth chamber with a 13 hr 28 C light period with a PPF of  $320 \text{ } \mu\text{m}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$  and an 11 hr dark period of 20 C. Relative humidity was not controlled or monitored. Pots were watered as needed and, as seedlings emerged and leaves began to expand six and seven days after planting, plants were thinned for uniformity to one per pot. Sixteen days after planting, when the simple leaves

appeared fully expanded and the first trifoliolate leaves were just open, plants were sorted for uniformity by size and development into four replications, treated with glyphosate, then allowed to grow in the growth chamber for an additional 13 days until harvest.

There were nine herbicide treatments consisting of three doses at each of three drop sizes. The largest size drops, 1230  $\mu\text{m}$  diameter, were applied in doses of 2.9, 5.8, and 11.6  $\mu\text{g ae}$  glyphosate per plant to a 19  $\text{cm}^2$  area of one simple leaf using a microsyringe equipped with a repeating dispenser. The two smaller drop sizes, 138 and 430  $\mu\text{m}$  diameters, were applied with the rotary atomizer. Doses of the smaller drop sizes could not be precisely controlled but were measured, as described above, and ranged from 1.4 to 17.8  $\mu\text{g ae}$  per plant. All treatments were with aqueous 3.0 mM glyphosate solution prepared from the commercial formulation of the isopropylamine salt, containing 360 grams per liter with a surfactant. There were also two control treatments which received no herbicide, one was harvested at treatment time and the other was harvested at the same time as the herbicide treated plants.

Plant parts harvested were shoot growth beyond the first trifoliolate leaf node plus axillary buds that had begun to grow from the first (simple) leaf nodes. At harvest there appeared to be little difference in size of first trifoliolate leaves regardless of treatment, so they were not included in the harvested portion from each plant. Harvested material was placed in a forced air oven and dried for at least 72 hr at 52 C before being weighed.

Another, similar, experiment was conducted with bean plants grown outdoors. Five drop sizes were tested, with four doses of

each drop size. A 12.2 mM solution of aqueous glyphosate was used. Doses for the 740 and 1230  $\mu\text{m}$  diameter drops were 10, 20, 40, and 80  $\mu\text{g ae}$  and were applied with microsyringes. Doses of the 138, 240, and 430  $\mu\text{m}$  drops were intended to be the same as used with the larger drops, but because doses could not be predicted accurately (but were measured accurately) with the rotary atomizer, the four doses for each of the three smaller drop sizes ranged between 4.0 and 112  $\mu\text{g ae}$  per plant. All drops were applied to a 19  $\text{cm}^2$  area of one simple leaf.

Oregon 91G bean seeds were germinated in moist perlite then transplanted, 4 per 10.5 cm square pot containing a moist peat/vermiculite medium, in a greenhouse with 27:21 C day:night temperatures. At or shortly after emergence pots were moved outdoors onto a sawdust covered area. Pots were watered as needed, and plants were thinned for uniformity to one per pot as the simple leaves began to expand.

When the simple leaves appeared fully expanded and the first trifoliolate leaf was unfolded and beginning to expand, plants were sorted by size and development into 4 replications and treated as described above. After treatment, plants were returned outdoors and, after 14 additional days of growth, were harvested, dried, and weighed as previously described.

Data from each of the two experiments were analyzed using multiple linear regression. Log dry weight, log dose, drop radius, and replication factors gave acceptable linear response and homogeneous variance.

Bean leaves from plants grown outdoors were used to determine

the extent of spread of each of the five drop sizes used with the 12.2 mM glyphosate solution. Uniform sized drops were applied to leaves which had just been detached from bean plants and placed on moist paper in square petri dishes. Covers were then placed on the dishes to prevent rapid drying of drops while diameters of areas covered by the drops were measured under a dissecting microscope with a calibrated ocular micrometer. Reflected light was used to discern drop margins on the leaves. The spread diameters of at least three drops were measured on each of four different leaves, with a total of at least 25 drop spread diameters measured per drop size. Spread factors were calculated by dividing the diameter of leaf surface covered by a drop by its spherical (in flight) diameter.

#### Results and Discussion

Statistical analysis showed significant effects of, but no interaction between, the three independent variables (glyphosate dose, spray drop size, and replication) on shoot growth of beans grown in the controlled environment. The model that best describes this response is

$$Y=1.21422+(-.3851068*d)+(-.000549884*r)+(.000001277*r^2)+\xi$$

(Equation 1)

where Y=log dry weight, d=log of glyphosate dose, r=spray drop radius, and  $\xi$  =error.

This model predicts a spray drop diameter of 429  $\mu$ m to give maximum herbicidal activity (Figure 5). It also predicts that glyphosate applied in 1230  $\mu$ m diameter drops is only 59% as

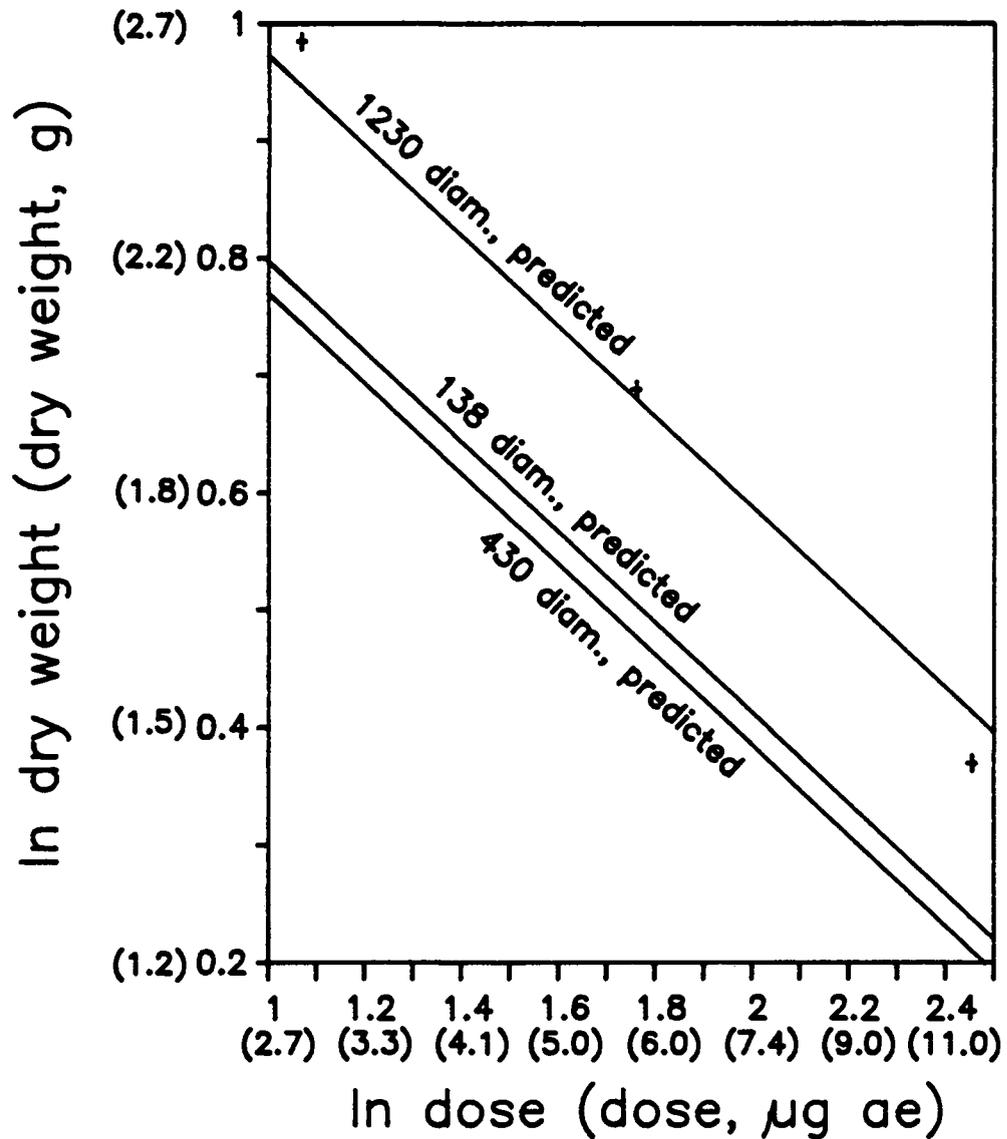


Figure 5. Dose-response of bean plants, grown in a controlled environment, to glyphosate applied in three spray drop sizes. Curves are predicted from Equation 1. Means are presented only for the 1230  $\mu\text{m}$  diameter drop size because dose was repeatable between replications within a treatment only for that size. Untreated plants grew to a log weight of .843 (2.32 g) and were log weight -1.69 (.184 g) when treated.

effective as when applied in drops 430  $\mu\text{m}$  diameter, i.e., it took 1.70 times as much glyphosate applied in 1230 as in 430  $\mu\text{m}$  diameter drops for equal growth suppression. The 138  $\mu\text{m}$  diameter size is predicted to be 93% as effective (1.07 times as much glyphosate needed) as the 430  $\mu\text{m}$  diameter drops for equal activity. However, a means comparison test (Tukey's LSD at the .05 level) revealed no significant activity difference between the 138 and 430  $\mu\text{m}$  diameter drop sizes, but the 1230  $\mu\text{m}$  size was significantly less effective than either smaller size.

Bean plants grown outdoors responded to drop size differences much the same as when grown in the controlled environment. Dose, spray drop size, and replication each had a significant effect on growth of glyphosate treated plants, with no significant interactions. The regression model describing growth response is similar to Equation 1:

$$Y=1.38975+(.89007227*d)+(-.00171716*r)+(.000004215*r^2)+\epsilon$$

(Equation 2).

Symbols are as described for Equation 1.

This model predicts a drop diameter of 407  $\mu\text{m}$  to give greatest glyphosate activity and, from the 5 sizes tested, predicts the 430  $\mu\text{m}$  size to be most effective (Figure 6). For herbicidal activity equivalent to the 430  $\mu\text{m}$  drop size it took, as estimated by Equation 2, 1.09, 1.03, 1.14, and 2.22 times as much glyphosate when applied in drops of 138, 240, 740, and 1230  $\mu\text{m}$  diameter, respectively. As in the controlled environment experiment, Tukey's LSD test showed that none of the sizes produced significantly different herbicidal

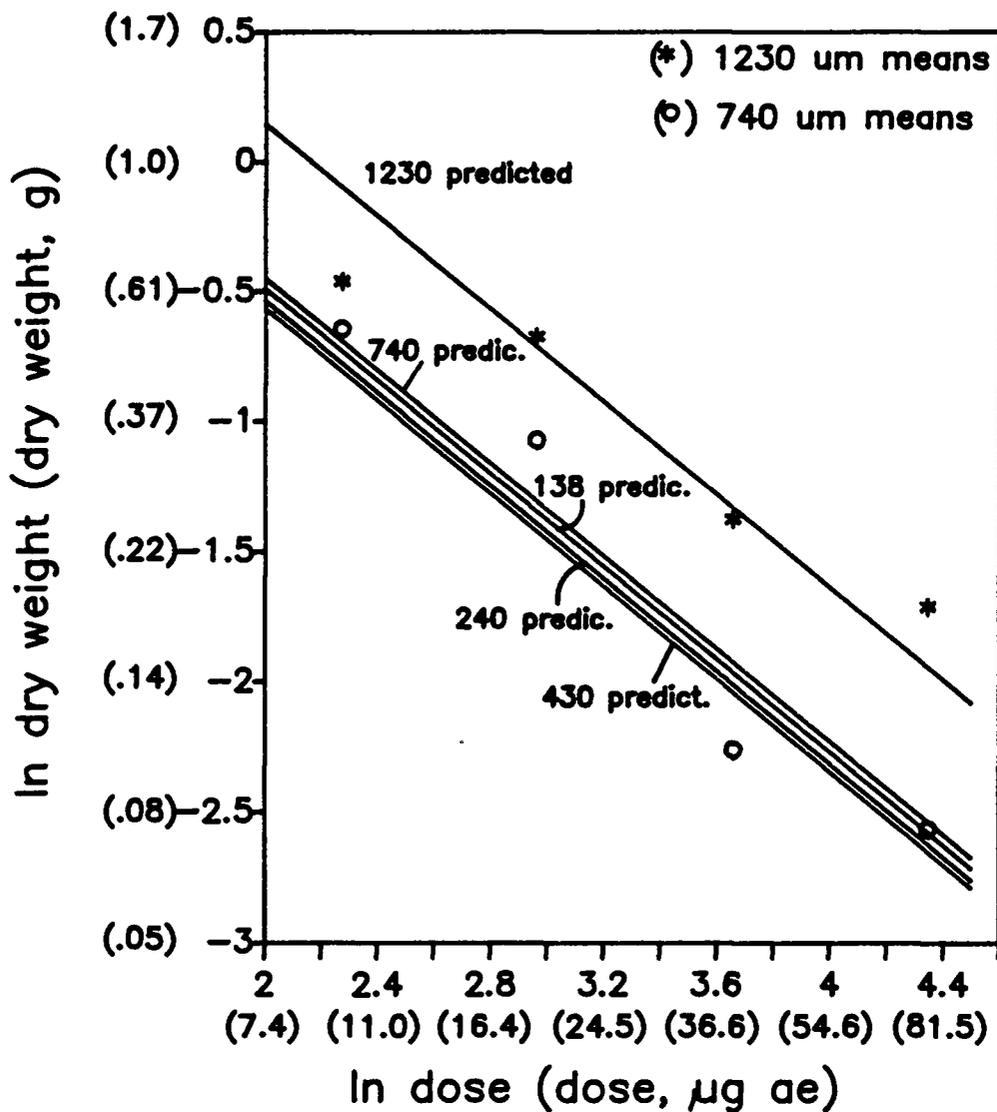


Figure 6. Dose-response of bean plants grown outdoors to glyphosate applied in five spray drop sizes. Curves are predicted from Equation 2. Means are presented only for the 740 and 1230  $\mu\text{m}$  drop sizes because dose was repeatable between replications within a treatment only for those sizes. Untreated plants grew to log weight of .392 (1.48 g) and plants were log weight -3.47 (.03 g) when treated.

activity except the largest size which was significantly less effective than the other four sizes tested.

Though both regression models predict maximum glyphosate activity from spray drops of similar size, 429 and 407  $\mu\text{m}$  diameter, it cannot be justifiably concluded from these two experiments that such a size is optimum for greatest glyphosate activity. The possibility that these predictions are artifacts of the quadratic nature of the regression models is supported by the lack of finding significant activity differences between sizes smaller than 1230  $\mu\text{m}$  diameter in each experiment.

Spread factors (Table 1) were similar for all drop sizes

Table 1. Coverage of bean leaves by five drop sizes with 12.2 mM glyphosate solution.

Drop diameter	Bean leaf spread factor	Bean leaf area ( $\text{mm}^2$ ) covered by one drop	Number of drops from 20 $\mu\text{l}$	Theoretical max. leaf area ( $\text{cm}^2$ ) covered by 20 $\mu\text{l}$
138	1.8 $\pm$ .2	.048 $\pm$ .010	14,534	7.0 $\pm$ 1.5
240	1.4 $\pm$ .1	.09 $\pm$ .02	2,763	2.5 $\pm$ .6
430	1.6 $\pm$ .2	.39 $\pm$ .07	480	1.9 $\pm$ .3
740	1.4 $\pm$ .1	.88 $\pm$ .16	94	.83 $\pm$ .15
1230	1.5 $\pm$ .1	2.6 $\pm$ .5	21	.55 $\pm$ .11

measured (1.4 to 1.6) except the smallest size, which had a slightly larger spread factor of 1.8. When the leaf areas covered by a single drop of a particular size are multiplied by the number of drops of that size from a given volume, a theoretical maximum area covered by that volume is calculated. The area is a theoretical one

because drops which coalesce together, and therefore cover less area than if separate, are not accounted for. It can be seen from Table 1 that leaf area covered decreases with increase in drop size, with drops 138  $\mu\text{m}$  diameter occupying more than 12 times the area of the same volume in drops of 1230  $\mu\text{m}$  diameter.

Results from these experiments do not preclude three different explanations of drop size effect on glyphosate activity. One is that 1230  $\mu\text{m}$  drops are less effective than smaller sizes, and that among smaller sizes there is no difference in influence on activity. A second possible explanation is that glyphosate activity increases as drop size decreases, but the experimental techniques were not sensitive enough to discern differences between sizes less than 1230  $\mu\text{m}$  diameter. The other explanation is that an optimum drop size or size range for maximum activity exists, but these experiments were not sensitive enough to show differences between drops 740  $\mu\text{m}$  or smaller, or else did not include treatments with drops small enough to reduce activity, which would be less than 138  $\mu\text{m}$ .

There are several possible reasons for drop size effects. As seen in Table 1, large drops concentrate the nonvolatile portion of spray in much smaller areas than an equal volume of small drops. Such concentrated active ingredient or surfactant may damage cells and prevent maximum uptake and/or movement of the glyphosate. Even if tissues are not damaged, and if glyphosate cellular uptake and translocation is dependent on a process higher than first order, the large quantity of glyphosate in a small area may exceed the maximum uptake and/or translocation capacity of the tissue, resulting in sub-optimal herbicide performance.

Low carrier volumes have been widely shown to result in better glyphosate performance than high volumes. If the carrier water does nothing but deliver glyphosate plus adjuvant to the plant surface, leaving a dried deposit, the assumption could be made that low volumes are more effective than high volumes because of the difference in surface area covered by the dried deposits, being less with low volumes. If this is the case, large drops would be expected to give greater glyphosate activity than small drops. It may be that a certain minimum amount of coverage is necessary for maximum activity, and the largest drop size failed to provide that amount in these experiments.

If an optimum drop size or size range that facilitates maximum glyphosate activity exists, at least two factors might be expected to reduce activity, one for drops smaller and one for drops larger than that size. If this is the case, then conditions that affect those factors would change the optimum drop size. Such an interaction may exist between concentration and drop size, with small drops becoming less effective as glyphosate concentration is decreased, provided that the commonly observed concentration (carrier volume) effect is related to the degree of coverage and not some other factor. The concentration used to treat outdoor grown plants was over four times as strong as was used to treat growth chamber grown plants (12.2 verses 2.96 mM), and relative effects from drop size were similar. However, it should not be concluded that 1230  $\mu\text{m}$  drops are less effective at all glyphosate concentrations. This is because the plants used were grown in very

different environments, and environment can greatly affect a plant's response to herbicides.

## GLYPHOSATE ACTIVITY AS INFLUENCED BY DROP SPREADING

## Introduction

If glyphosate phytotoxicity differences due to drop size result from differences in the leaf surface area covered by the different drop sizes (when a given volume of solution is applied), then changing the area covered in a way other than changing drop size should give the same result. The objective of these experiments was to determine if differences in leaf coverage affect glyphosate activity when drop size is not a factor.

## Materials and Methods

Oregon 91G bean seeds were germinated in moist perlite in a growth chamber set for a 13 hr light period at an irradiance of  $320 \mu\text{m}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$  (PPF) and 28:20 C light:dark temperatures. Relative humidity was not controlled or monitored. After three days, when roots were 0.8 to 2.0 cm long, four seedlings were transplanted into a moist peat/vermiculite potting mix in 10.5 cm square pots. Plants were thinned to one per pot as they emerged and leaves expanded. Eleven days after transplanting, when the simple leaves were expanded and leaflets of the first trifoliolate leaf were unfolded and less than 1.5 cm long, plants were sorted for uniformity of size and development into six replications of eleven plants each, nine for glyphosate treatment and two for controls. One control plant was harvested at treatment time, the other with the glyphosate treated plants.

Each glyphosate treated plant received 8 ul of one of three aqueous glyphosate solutions (3 mM, 6 mM, and 12 mM) with a 50 ul syringe equipped with a repeating dispenser. Two 1.0-ul drops were placed on each side of the midrib on the adaxial surface of each simple leaf. Of the three plants within a replication treated with each glyphosate solution, one was left with no additional spreading of the applied drops (each drop covered a circular area of 2 mm diameter), one had each drop spread to cover a circular area of 8 mm diameter, and one had each drop spread to cover a circular area of 14 mm diameter. These correspond to areas covered of 0.25, 4, and 12 cm<sup>2</sup> per plant.

A 1.5-mm diameter glass rod (made by melting the tip of a Pasteur pipette in a flame) was used to spread drops by gently moving the rod tip in contact with the drop in a circular motion on the surface of the leaf. Previous attempts to spread drops in this way failed because drops fragmented rather than spread. It was found that exhaling onto the leaf a few times would condense enough moisture on the leaf surface to facilitate reasonably uniform drop spreading by this method. To aid in condensation and prevent rapid drying of condensed moisture, plants were treated in a room at 15 C under low light and 75% relative humidity. The decrease in glyphosate concentration within a drop from the additional, condensed moisture was not estimated and was assumed negligible.

To minimize the quantity of solution removed by the glass rod, all replications of a treatment were treated sequentially without rinsing the rod. Just prior to spreading drops in one sequence the glass rod was used to spread two drops, identical to those in the

sequence, on bean leaves that were not part of the experiment, and then was not rinsed before spreading the treatment drops.

Plants were returned to the growth chamber following treatment, but after seven days three of the replications were removed and placed in a greenhouse because of crowding. After six additional days, all shoot material from each plant distal to the simple leaf node was removed and dried in a forced air oven at 55 C for at least 72 hours and weighed.

A second, similar experiment was conducted with bean plants grown outdoors. Nine Oregon 91G bean seeds were sown directly in a moist peat/vermiculite potting mix in 10.5 cm square pots that were placed outdoors on a gravel and sawdust covered area July 16, 1987, at Corvallis, Oregon. Plants were thinned for uniformity to one per pot as they emerged and grew. Treatments were as previously described except with four replications and the addition of another glyphosate concentration, 24 mM, making 12 herbicide treatments (three areas covered with each of four glyphosate concentrations). Plants were allowed to grow for 12 days after treatment, and on August 14, 1987, were harvested and placed in a drier as described above.

Dry weight data from only glyphosate treatments from each experiment were statistically analyzed. Analysis for each experiment was as a completely randomized block design and a protected LSD value was obtained for each.

Leaf disks of 12 mm diameter were removed from the simple leaves of untreated, outdoor grown, control plants that were harvested at treatment time. Cuticles were isolated from the disks

by the method of Holloway and Baker (1968), which involved soaking the disks in a solution of  $ZnCl_2$  in concentrated HCl (1.0:1.7 w/v). After 20 hours at room temperature, the isolated adaxial cuticles were removed from the solution, rinsed, and stored in distilled water at 3 C for 13 days, at which time they were rinsed three times in a 10% aqueous solution of HCl then three more times with distilled water and air dried. The cuticles were delicate, and many fragmented during isolation or rinsing procedures. Those that were undamaged from a pair of replications were dried together in an aggregation on a nonstick surface of a frying pan. This drying method was necessary, as efforts to remove single, whole cuticle pieces from any surface to which they adhered while drying were unsuccessful.

Cuticles were also isolated from bean plants grown in a growth chamber under conditions described above, and leaf disks were removed when the plants were at the treatment growth stage. None of the cuticle disks from growth chamber grown plants remained completely intact during isolation; however, fragments of adaxial cuticles were isolated, rinsed, and dried together in aggregations. Dried cuticle aggregations were weighed on a microbalance.

To determine glyphosate sorption to bean leaf cuticles, a dried cuticle aggregation from each set of growing conditions was shaken at 25 C in one ml of aqueous  $^{14}C$ -glyphosate solution for nearly 81 hours. Fifty  $\mu$ l aliquats were taken from the solutions at times of 0, 13 minutes, 53 minutes, 2 hours 53 minutes, 8 hours 53 minutes, 26 hours 53 minutes, and 80 hours 53 minutes. Glyphosate content of the equilibration solutions was determined by measuring the

radioactivity of the aliquats by standard liquid scintillation counting techniques. One incubation vial was included without cuticle material to confirm that glyphosate did not adsorb to glass. The  $^{14}\text{C}$ -glyphosate was formulated as the isopropylamine salt with the surfactant MON-0818 added in the same ratio as the commercially available herbicide.

#### Results and Discussion

Increased spreading of drops of glyphosate solution on bean leaves reduced activity of the glyphosate at each dose (Figure 7). Results were not significantly different between the 12 and 4  $\text{cm}^2$  coverages at the 4  $\mu\text{g}$  dose and between the .25 and 4  $\text{cm}^2$  coverages at the 16  $\mu\text{g}$  dose, although the means for all groups revealed a consistent pattern. Lack of significant differences occurred because the extremes of plant response were approached. The 8  $\mu\text{g}$  dose that was spread to cover 4  $\text{cm}^2$  was only as effective as 4  $\mu\text{g}$  in drops left unspread (.25  $\text{cm}^2$ ). At the 16  $\mu\text{g}$  dose, spreading drops to 12  $\text{cm}^2$  reduced activity to that of 8  $\mu\text{g}$  in unspread drops.

Reductions in glyphosate activity due to additional drop spread were apparent from plants grown in the growth chamber. However, when the same treatments were applied to plants grown outdoors, no differences were found in glyphosate activity due to increased drop spread (Figure 8).

No explanation for the different response between experiments is apparent. The possibility of differences in cuticular adsorption between the two experiments was investigated with isolated adaxial leaf cuticles from plants grown in the same environments as those in

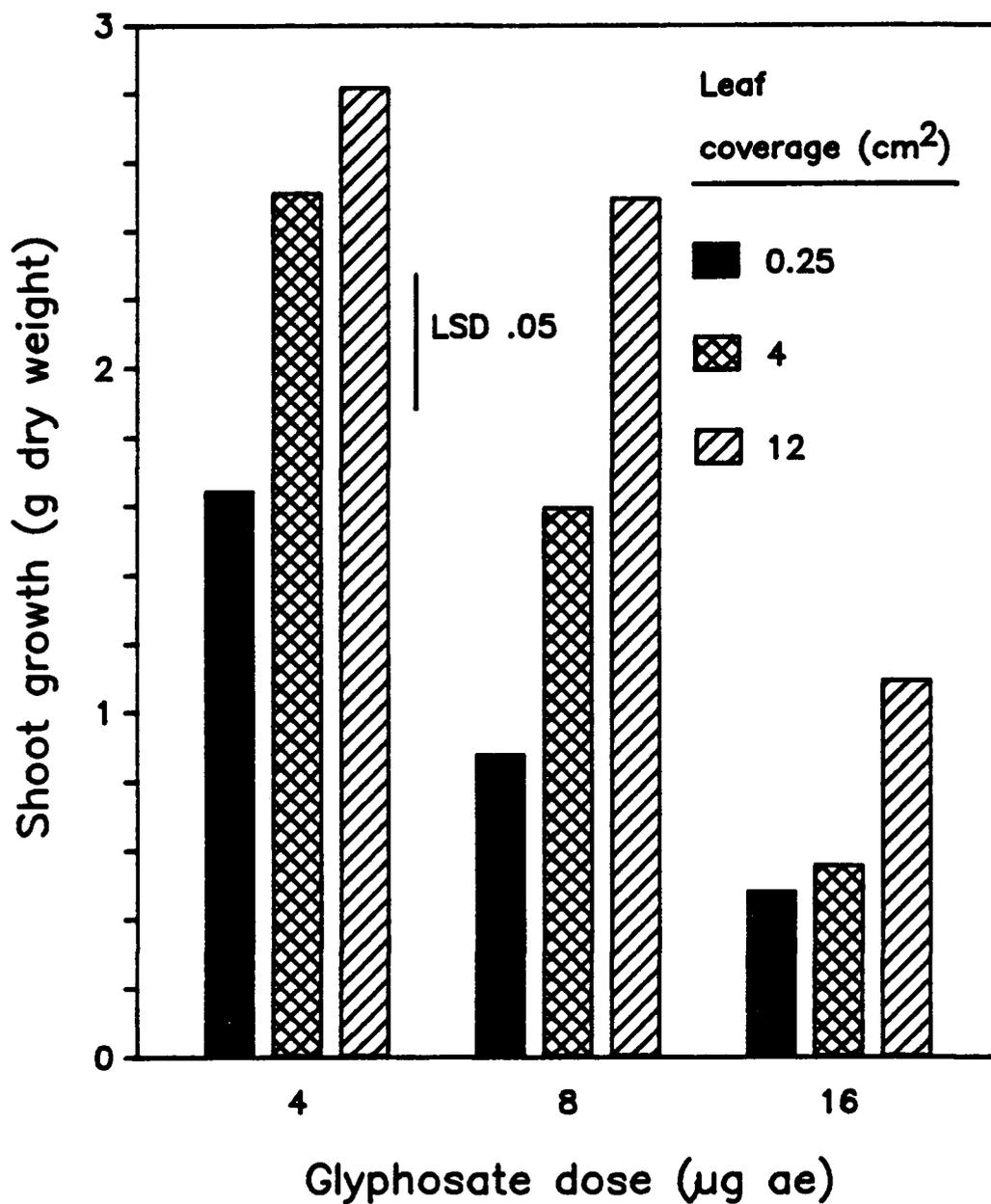


Figure 7. Effect of variable leaf coverage of three glyphosate concentrations, each applied in eight 1.0 ul drops, on shoot growth of bean plants grown in a controlled environment.

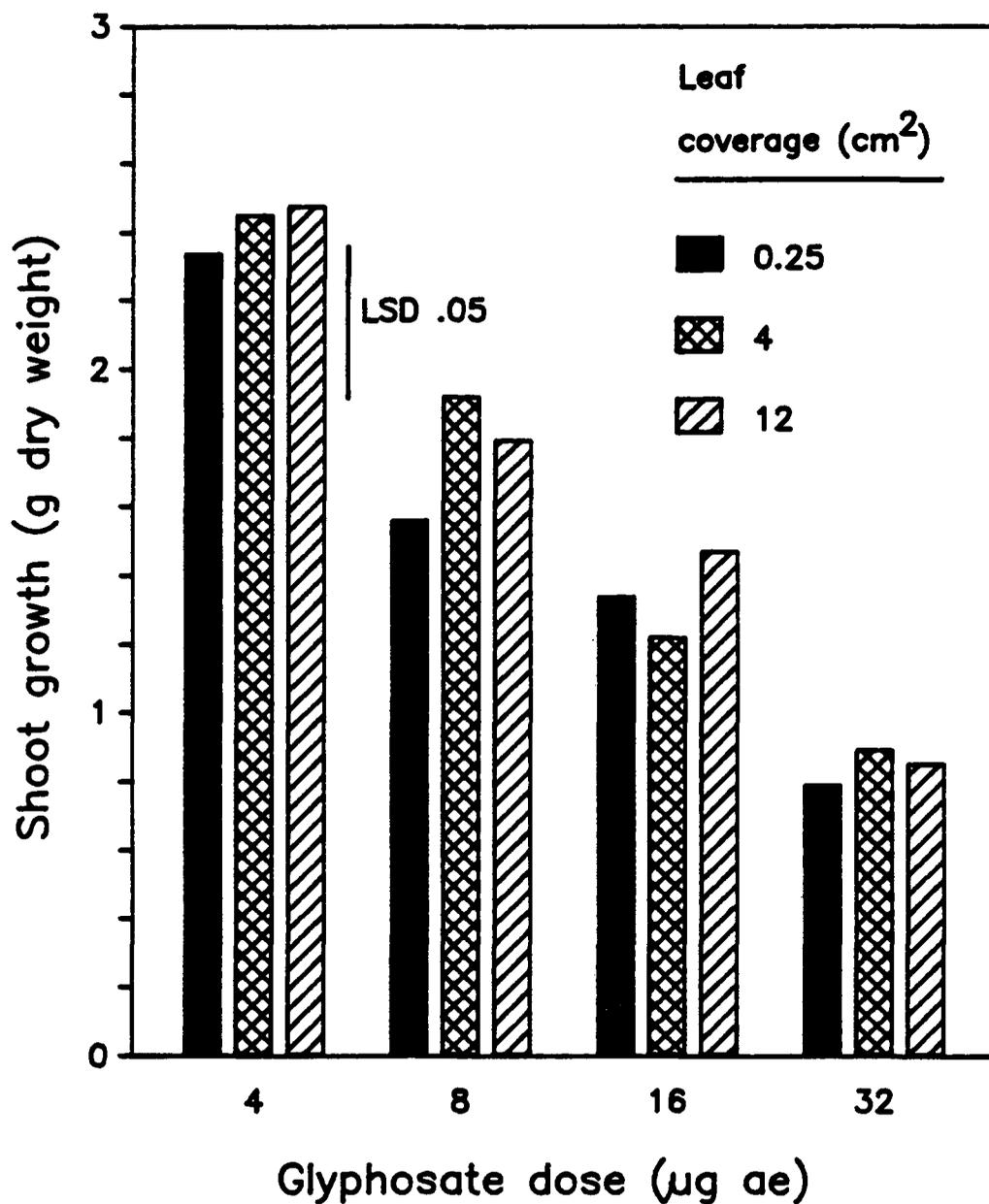


Figure 8. Effect of variable leaf coverage of four glyphosate concentrations, each applied in eight 1.0 ul drops, on shoot growth of bean plants grown outdoors.

the experiments. No  $^{14}\text{C}$ -glyphosate was adsorbed by cuticles from plants grown in either experimental environment when shaken in aqueous glyphosate solution for over 80 hr. In fact, radioactivity in the equilibration solution increased slightly over time, probably due to evaporation of water from the solutions which condensed on the inside of the upper parts of the vials during the equilibration procedure.

Others have shown that 2,4-D partitions from aqueous solution into isolated cuticles of various species (Riederer and Schonherr, 1984; Riederer and Schonherr, 1986). Cuticles from pepper fruits irreversibly bound 2,4-D while tomato fruit cuticle did not. This was attributed to epoxy groups in pepper fruit cuticle that are not found in cuticles of many other species. It was found that acid treatment of pepper cuticles after enzymatic isolation altered the epoxy groups so that irreversible, covalent bonding of the carboxyl groups of 2,4-D to epoxy groups no longer occurred (Shafer and Bukovac, 1987).

Glyphosate contains a carboxyl group, but it is unknown whether it reacts with epoxy groups, if bean leaf cuticles have epoxy groups, or if epoxy groups may develop in bean cuticles in response to certain environmental conditions such as UV radiation or lack thereof, or temperature. The harsh acid treatment used in this study would have eliminated any epoxy groups that may have been present.

Glyphosate chelates some divalent and trivalent metal cations, the reaction causing reduced phytotoxicity. This is usually considered a problem where hard water is used in spraying. Iron,

zinc, aluminum, and calcium have been shown to be inhibitory to glyphosate activity (Stahlman and Phillips, 1979; Sandberg et al., 1980; Buhler and Burnside, 1983B; Hensley et al., 1978) but calcium is usually considered to pose the greatest problem because its quantities are usually much larger in available water supplies, even though its effect is less on an equivalent basis than iron, zinc, or aluminum. Nilsson (1985) provided evidence that polyvalent metal cations inside the plant may be responsible for inactivation of some foliar absorbed glyphosate. Turner and Loader (1978) showed that divalent and trivalent acids enhance glyphosate activity, possibly reflecting the ability of the acids to sequester or immobilize metal cations such as calcium (Turner, 1985). Bean leaves contain 0.8 to 3.0% calcium on a dry weight basis (Walsh and Beaton, 1973), most of which is in the apoplast in pectates or associated with the plasma membrane (Marschner, 1986). In leaves of plants grown under high light intensity a greater proportion of calcium is bound in calcium pectate, perhaps making it less available for interaction with, and inactivation of, glyphosate. Plants grown in a growth chamber under much lower light intensities may have more water soluble calcium available in the apoplast, and distributing glyphosate over a larger surface area of the leaf could increase the amount of calcium available to interact with glyphosate, with a corresponding reduction in phytotoxicity.

Results from only the drop spread experiment with plants grown in the controlled environment support the idea that greater activity of glyphosate from low carrier volumes is due to reduced leaf area contacted. The drop size experiments tended to show the opposite,

with least activity from drops which contacted the least leaf area per volume. It can be concluded that reduced glyphosate activity from large drops is not related to leaf coverage, but increasing coverage in ways independent of drop size can reduce activity under some conditions. Growth environment can affect plant response to variable leaf coverage of glyphosate.

These results suggest that factors other than leaf coverage are important in glyphosate activity. More work needs to be done to elucidate the physical, chemical, or physiological factors influencing glyphosate activity. Possible interactions between drop size and concentration should be explored, and other plant species tested.

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