ROLE OF ETHOMEEN® T/25 AND SILWET® L-77 ADJUVANTS ON RAINFASTNESS OF HERBICIDE FORMULATION OF VISION® FOR THE CONTROL OF TREMBLING ASPEN (*Populus tremuloides* Michx.)

A Thesis Presented to

The Faculty of Graduate Studies

(Department of Soil Science)

of

The University of Manitoba

by

John Wing Leung

In Partial Fulfillment of The Requirements for

The Degree of

Master of Science

April, 1992

ISBN 0-315-77771-0

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ABSTRACT

ROLE OF ETHOMEEN® T/25 AND SILWET® L-77 ADJUVANTS ON RAINFASTNESS OF HERBICIDE FORMULATION OF VISION® FOR THE CONTROL OF TREMBLING ASPEN (*Populus tremuloides* Michx.)

J.W.Leung, M.Sc.
University of Manitoba, 1992

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G.R.B. Webster, Ph.D.
A. Sundaram, Ph.D.

Preliminary studies found that the surfactants Ethomeen® T/25 [polyoxyethylene (POE 15) tallowamine] and Silwet® L-77 (polyalkylene oxide modified dimethyl polysiloxane) show promise in protecting spray deposits on trees from being washed off by rain. In this thesis, five studies - studies 1-4 were done in the lab and study 5 was carried out in the field - were conducted to determine the possibility of using these adjuvants to enhance rainfastness of the herbicide formulation Vision® [i.e., active ingredient (AI) is glyphosate] on trembling aspen (*Populus tremuloides* Michx.).

The effect of various concentrations of Ethomeen T/25 and Silwet L-77 on the compatibility of glyphosate with adjuvants in the end-use mixtures (EUMs) was investigated. The EUMs, stored at 25°C for 16 h and 4°C for 8 h, were examined for [^{14}C]glyphosate degradation by thin layer chromatography (TLC) and liquid scintillation counting (LSC). Ethomeen T/25 could be used at concentrations < 1.35% and Silwet L-77 at concentrations ≤ 0.15%. The maximum translocation of the [^{14}C]glyphosate to the untreated parts of the trembling aspen branch tips was quantified by LSC (on the combusted samples). The result was used to determine the optimum adjuvant concentration of Ethomeen T/25 (0.45%) and Silwet L-77 (0.15%) in Vision EUM for the remaining rainfastness studies.

An investigation of the physical properties of EUMs of Vision with and without Ethomeen T/25 or silwet L-77 in relation to droplet spreading and drying rates on aspen foliage was also explored. Ethomeen T/25 increased the $T_{1/2}$ (i.e., the time required for the volatile components to evaporate down to 50% of its initial value) of the EUM. Silwet L-77 significantly reduced the surface tension of the EUM, which increased droplet spreading ability. Droplets with a greater spreading ability tend to spread more quickly and to dry faster.

Rainfastness of glyphosate with and without adjuvants under medium rain at various rain free period was evaluated by examining samples of treated leaf washings, treated leaf residue, and untreated parts using [^{14}C]glyphosate. At 36 h after treatment, Ethomeen T/25 and Silwet L-77 significantly reduced glyphosate washed off (13% and 23% respectively), compared to the amount washed off with Vision alone. However, glyphosate wash-off of the EUM with Silwet L-77 was markedly lower than that of the EUM with Ethomeen T/25.

A replicated single tree application field trial was carried out in northern Ontario. At 36 h after treatment, half of the treated sample trees received no rain; the other half received a simulated rainfall of 5 mm. Observations on phytotoxic development on sampling trees indicated that trees that did not receive any rain showed no significant difference between EUMs with or without the adjuvants. However, in the simulated rainfall trial, the EUMs that contained either Ethomeen T/25 or Silwet L-77 showed herbicidal activity substantially faster than Vision alone. The results of foliar analysis were consistent with those from the lab study, and showed that Ethomeen T/25 and Silwet L-77 respectively reduce spray washoff by 15% and 19%. Neither group of EUM treated-trees showed bud regeneration the following spring.

ACKNOWLEDGMENTS

I would like to thank my supervisor, Dr. A. Sundaram, for her continual encouragement to me to pursue this degree. I have greatly appreciated the freedom which she has given me to carry out my M.Sc. research program. This thesis would not have been possible without her unlimited support, advice and guidance.

I would also like to thank Dr. G.R.B. Webster who is the supervisor of my M.Sc. program at The University of Manitoba, has accepted me as his graduate student and has provided me direction, advice and guidance.

Thanks also go to Dr. J. M. Stewart for being a member in the advisory committee for my M.Sc. program and for his advice and guidance on this thesis.

Drs. K.M.S. Sundaram and S.Y. Szeto gave me much advice, endless support and encouragement. I owe them more than words can say.

Thanks must be extended to the management of the Forest Pest Management Institute for giving me such an opportunity to fulfill my dream to pursue my M.Sc.

Above all, I would like to express my sincere thanks to Mrs. J. Curry, and Messrs. J. Hatherley and S. Iuliano for their assistance in carrying out intensive work on the lab studies and on the field spray application.

I am grateful to Dr. R. Fleming and Mr. T. Burn for their assistances on the statistics aspects of this thesis, and Drs. R. Campbell, G. Choudhry, N. Payne, D. Pitt, R. Prasad, and P. Reynolds for their valuable comments on the proposal of the M.Sc. research project.

I especially would like to thank Mrs. K. Jamieson who assisted me on editing this thesis and Ms. J. Novick for her assistance in converting this thesis from the format of Vax-WPS to Wordperfect.

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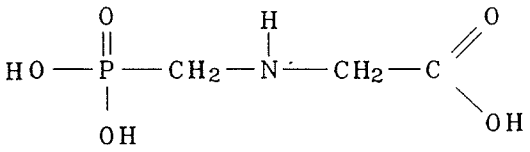
INTRODUCTION

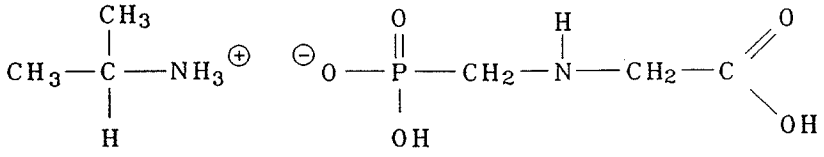
In forestry spray applications, once droplets have been accurately delivered onto the target matrices, it is essential that the active material remain on the target site at optimum concentration levels long enough to produce acceptable pest control activity. A common problem is the wash-off of pesticide deposits by rain (Bailey et al., 1974; Barnett et al., 1967; Laflen et al., 1978; Trichell et al., 1968; Willis et al., 1975). Rainfall that occurs shortly after pesticide spray application causes reduction in pesticidal activity (Baker et al., 1978; Leistera, 1975; Upchurch and Price, 1957), and repeated spray applications may be required for pest control. The repeated use of pesticides not only increases the cost of forest management but may also cause serious environmental concerns. Reduction of pesticide deposits from target surfaces as a result of rain-wash can cause possible adverse side-effects on non-target species. Furthermore, if the pesticide is mobile, it may contaminate the soil or ground- or surface-water (Sheets et al., 1972). If the pesticide is non-mobile and persistent, it may remain and if biologically available, induce resistance in the target pest (Adkisson, 1968; Nemec and Adkisson, 1969; Whitten and Bull, 1970).

Vision®, a commercial formulation concentrate that contains the isopropylamine salt of glyphosate [N-(phosphonomethyl) glycine] (Figure 1.1), is currently registered in Canada for conifer release and site preparation by aerial application. Glyphosate is a broad spectrum, post-emergence herbicide. It inhibits the shikimic acid pathway of plants, and as a result prevents secondary compound (aromatic amino acid) formation and reduces protein synthesis. Animals do not have such a pathway, thus glyphosate has low toxicity to animals and people. Because of

Figure 1.1 Chemical structures of Glyphosate.

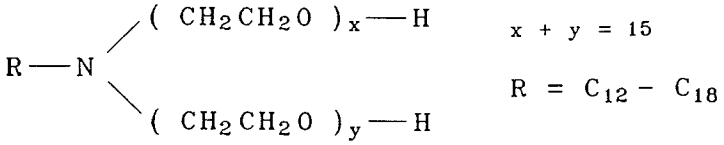


Acid Form

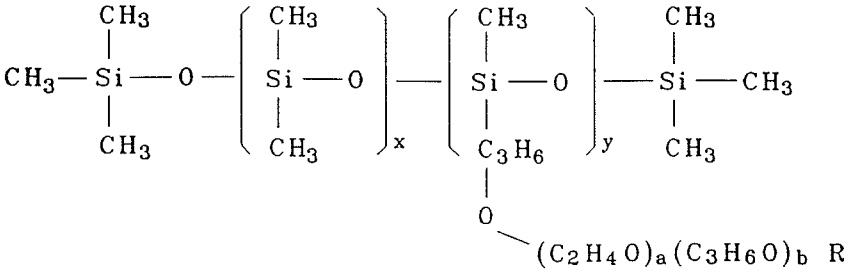


Isopropyl Amine Salt Form

Figure 1.2 Chemical structures of Ethomeen T/25 and Silwet L-77.



Ethomeen® T/25



Silwet® L-77 *

* " R " can be either hydrogen or a lower alkyl radical and the values of a, b, x and y are not provided here because they are proprietary information.

its safety characteristics (Cole, 1985; Atkinson, 1985) and its speed and pattern of degradation (Sprankle et al., 1975b; Tate and Alexander, 1974), glyphosate accounted for more than 80% (176,536 ha) of the total herbicide used for forest management in Canada in 1988 (Campbell, 1990). It may become a successor to the chlorophenoxy acids (e.g., 2,4-D).

The technical glyphosate is only moderately soluble in water (i.e., ca. 1.2 % at 25°C). For the purpose of easy mixing or preparing the end-use mixture (EUM), the commercial formulation concentrate (such as Vision® or Roundup®) contains the isopropylamine salt of glyphosate (Figure 1.1), which is highly soluble in water. Because of its high water solubility, glyphosate deposits on foliage are particularly vulnerable to removal by rainfall until a lethal quantity of active ingredient (AI) has penetrated the foliage (Bryson, 1987, 1988; Caseley et al., 1975, 1976; Caseley and Coupland, 1985). Behrens and Elakkad (1976) found that 1.0 mm/h of rain severely reduced the herbicidal activity of glyphosate, and 12.5 mm 2 h after herbicide application resulted in complete loss of activity. Several researchers have reported that a 6-8 h rainfree period is required for penetration of sufficient active ingredient to give acceptable performance (Baird and Upchurch, 1972; Behrens and Elakkad, 1972; Coupland and Caseley, 1981). Even though other factors (e.g., dose, concentration and formulation of AI, physiological condition, and stage of growth of species) are also important in the penetration of the herbicides, the environmental conditions during and between application and the onset of rainfall are critical (Caseley et al., 1976). For example, a rainfree period of 2 h is sufficient for a lethal dose of herbicide to enter the plant at 95% relative humidity (RH), but if plants are kept at 48% RH after spraying, a substantial amount of herbicide will be washed off

by a simulated rainfall (Caseley et al., 1975).

It is likely then, that any plant or environmental factor that slows the entry of glyphosate into the plant will increase the vulnerability of the glyphosate treatment to rainfall in the post application period. Manipulation of the formulation may enhance the rate of glyphosate penetration into the plant and may thus reduce the risk of diminished glyphosate activity. For example, Turner (1981) showed that adding 0.5% of a surfactant (Ethomeen®) to Roundup spray mixtures increased the performance about fourfold when 5 mm of rain was applied to *A. repens* one hour after herbicide application. The conclusion was that the surfactant enhanced the rate of uptake and was thus a major factor in reducing the vulnerability of the glyphosate deposits on foliage to rainfall.

Recently, Sundaram (1991) and Thonke et al. (1989) reported the possibility of improving the rainfastness of glyphosate by introducing an adjuvant into the EUM of the commercial formulation. With this objective in mind, we studied six adjuvants and found that surfactant adjuvants, in general, do not enhance the rate of uptake enough to provide rain-protection for the foliar deposits. The results of these studies also indicate that the surfactant (which is already in Vision), is the most powerful in enhancing the rate of uptake, and the addition of any other surfactant does not significantly further enhance the rate of uptake. An alternative option to improve the rainfastness of Vision is to look for a product that will protect foliar deposits from rain. Fortunately, two surfactant compounds have shown promise in such a study. The first is an ethoxylated aliphatic amine, Ethomeen® T/25 [polyoxyethylene (POE 15) tallowamine]; and the second is a copolymer, Silwet® L-77 (polyalkylene oxide modified dimethyl polysiloxane) (Figure 1.2); In our laboratory studies, Ethomeen

T/25 and Silwet L-77 acted both as a surfactant and a rain protectant because they show a combined mechanism involving enhanced rate of uptake and actual protection of the foliar deposit against rain. However, Silwet L-77 appeared to be more effective in protecting foliar deposits than in enhancing the rate of uptake (Sundaram, A. 1990a; Sundaram, A. 1990b).

Currently the only method used to retain pesticide deposits on target is the introduction of an adhesive or sticking agent (e.g., Rhoplex, Chervron sticker, and Bond). However, some of these adhesives are very specific and they are suitable only for a particular formulation. They are intended mainly to protect pesticide formulations from being worn off or blown off by the wind (Morris et al., 1977). Conducting spray applications without the concern of rainfastness in the EUMs, is neither logical, scientific nor economical.

In this thesis, five studies were conducted to determine the possibilities of using the adjuvants Ethomeen T/25 and Silwet L-77 to enhance the rainfastness of Vision formulation on trembling aspen as a preliminary model to investigate the rainfastness of various pesticides. The first four studies were done in the laboratory and the last one was conducted in the field. Studies I and II were designed to determine respectively the compatibility of Ethomeen T/25 and Silwet L-77 with Vision EUM and to select the optimum adjuvant concentration level of these adjuvants for the remaining rainfastness studies; Study III looks into the possible explanation for the behaviour of Ethomeen T/25 and Silwet L-77, which are considered rain protection agents; Study IV investigated the minimum rain free period for the Vision EUMs with and without the adjuvants of Ethomeen T/25 or Silwet L-77 and the rainfastness of these EUMs for the control of trembling aspen

(*Populus tremuloides* Michx.); and Study V was a single tree field trial that was executed to produce more realistic data, in an attempt to support and verify the laboratory studies.

CHAPTER 1.

Effect of Ethomeen T/25 and Silwet L-77 on Compatibility of Glyphosate with Adjuvants in The Eund-Use Mixtures.

ABSTRACT

Three end-use mixtures (EUMs), such as Vision with Ethomeen (VE) and Vision with Silwet (VS) [with incorporated concentration levels of 0.05, 0.15, 0.45, 0.90, and 1.35% (v/v) for each adjuvant], plus Vision (V) alone as a control, were prepared and kept for 4 d under a daily temperature regime of 25°C for 16 h and at 4°C for 8 h. The compatibility of AI (active ingredient, i.e., glyphosate) with the adjuvant was determined by observing the EUMs for any physical changes (e.g., phase separation, crystallization, or formation of a suspension), and by evaluating EUMs for any AI degradation (e.g., using TLC Rf values for identification and LSC for quantification) at sampling periods of 0, 1, 2, and 4 d. Results of these studies indicated that no physical changes occurred in any EUM at any sampling period. Furthermore, Rf-values from TLC analysis and percentage recovery of [^{14}C]glyphosate by LSC supported the observation that Ethomeen is compatible with Vision at most of the concentration levels studied, but that Silwet can be used only at concentration levels $\leq$ 0.15%.

1 INTRODUCTION

In pesticide formulation research, additives of polymeric surfactant adjuvants are often used in the end-use mixture (EUM) to reduce off-target drift (Goering and Butler, 1975; McNulty et al., 1977; Richardson, 1974; Yates et al., 1974), and to alter droplet size spectra, thus increasing spray deposits on target sites (Sundaram et al., 1987; Sparks et al., 1987). Nevertheless, Doub et al.(1988) found that certain polymers could have adverse side effects, such as the reduction of herbicidal activity *via* entrapment of the herbicide molecules in the polymeric structure. In addition, certain adjuvants also altered the physicochemical properties of the EUM resulting in phase separation (McWhorter, 1982; Turner and Loader, 1978; Turner, 1985; Zamora and Thill, 1988).

Preliminary studies (Sundaram, 1990a, 1990b) in the Pesticide Formulations Project of Forest Pest Management Institute, Forestry Canada, showed that the adjuvants Ethomeen T/25 [polyoxyethylene (POE 15) tallowamine], a cationic surfactant which ionizes in water (Akzo Chemical Ltd., Toronto, ON) and Silwet L-77 (polyalkylene oxide modified dimethyl polysiloxane), which is an organosilicone surface active copolymer (Union Carbide Corporation, Specialty Chemicals Division, Danbury, CT, USA.), have the potential of improving rainfastness of glyphosate [N-(phosphonomethyl) glycine, Monsanto Agricultural Products Company, St. Louis, MO, USA] in Vision formulation. However, little is available in the literature on the compatibility between glyphosate and adjuvants. The purpose of this study was to investigate the effect of Ethomeen and Silwet on compatibility aspects of glyphosate in EUMs of Vision formulation prior to pursuing any extensive rainfastness studies

with these two adjuvants.

2 MATERIALS AND METHODS

2.1 The End-use Mixtures

Glyphosate EUMs and adjuvants used in the study, are listed in Table 1.1, along with the percentage compositions of the ingredients used. All EUMs were prepared to obtain a dosage rate of 1 kg AI in 35 L. The EUMs with [^{14}C]labeled glyphosate (at the N-phosphonomethyl carbon position, purchased from Amersham Corp. Oakville, ON) were used only for the study of AI degradation; another set of non-radiolabelled EUMs was used for observation of aspects of physical changes.

2.2 Methods

2.2.1 Phase Separation, Crystallization and Suspension Formation

According to the percent composition (as shown in Table 1.1), the individual non-radiolabelled EUM was separately prepared in a 20 mL LSC vial with a volume of 10 mL each. These EUMs were kept in an environmental chamber for 4 d under a daily temperature scheme of 25°C for 16 h and 4°C for 8 h. Observations of any physical changes (e.g., phase separation, crystallization, or formation of suspension etc.) were recorded at sampling periods of 0, 1, 2 and 4 d.

2.2.2 AI Degradation Investigation

EUMs with radiolabelled glyphosate were used for the AI degradation study. They were prepared and stored in the same manner as those in section 2.2.1, but with a final volume of 0.5 mL in a 2 mL HPLC vial. They were also collected at the same

Table 1.1 Percentage composition of ingredients used in glyphosate EUMs for compatibility study

End-use mixtures[1]	Percentage composition (v/v)				
	Vision[2]	[^{14}C]gly.[3]	Ethomeen	Silwet	Water
V	8.03	9.40	--	--	82.57
$VE_{0.05}$	8.03	9.40	0.05	--	82.52
$VE_{0.15}$	8.03	9.40	0.15	--	82.42
$VE_{0.45}$	8.03	9.40	0.45	--	82.12
$VE_{0.90}$	8.03	9.40	0.90	--	81.67
$VE_{1.35}$	8.03	9.40	1.35	--	81.22
$VS_{0.05}$	8.03	9.40	--	0.05	82.52
$VS_{0.15}$	8.03	9.40	--	0.15	82.42
$VS_{0.45}$	8.03	9.40	--	0.45	82.12
$VS_{0.90}$	8.03	9.40	--	0.90	81.67
$VS_{1.35}$	8.03	9.40	--	1.35	81.22

[1]V = Vision; VE = Vision with added Ethomeen; VS = Vision with added Silwet.

[2]Vision contains 356 g of glyphosate A.E. (acid equivalent) per L.

[3][^{14}C]glyphosate with N-phosphonomethyl carbon labeled, had a spec. act. of 0.049 µCi per µg of AI.

sample periods (i.e., 0, 1, 2 and 4 d) to determine the AI content for degradation determination. The techniques used in this study were thin-layer chromatography (TLC) and liquid-scintillation counting (LSC), which are described below:

For TLC quantification of the AI, 20 individual bands *ca.* 1 cm wide, were made on a 20 cm x 20 cm silica gel plate (Whatman 4855820 LK5, Chromatographic Specialties, Inc., Brockville, ON) using a TLC plate scriber (The Chemical Rubber CO., 18901 Cranwood Parkway, Cleveland, OH, USA). Two TLC plates were required to accommodate the eleven EUMs of this study. On an individual band of a TLC plate, a 10-μL Hamilton syringe was used, and a 2-μL aliquot (containing *ca.* 25 Bq ^{14}C) of an EUM was spotted in volume of 0.5 μL with oven-drying at 70°C between each spotting. This procedure was carried out simultaneously on alternating bands (i.e., with a buffer band between two sample bands) for another EUM until the EUMs of V and VEs were loaded on one plate while the EUMs of V and VSs were on the other. Following this, 3 μL of 500 μg/mL aminomethylphosponic acid (AMPA) standard solution was also spotted on top of each sample spot using the same spotting technique. Each of two control spots, spotted with a 5-μL mixture of standard glyphosate (1000 μg/mL) and AMPA (500 μg/mL), was placed on the edge bands of each TLC plate to locate glyphosate and AMPA. The TLC plates were developed for 55-60 min (until the solvent front reached the 10-cm mark) in a multi-plate developing tank, containing 100 mL of 60 : 55 : 10 MeOH / H_2O / 0.5M $MgCl_2$. When development was complete, the plates were dried in an oven at 70°C, and were allowed to cool. The plates were then sprayed with a solution of ninhydrin in ethanol (0.3 % w/v) with a twin fluid nozzle sprayer (Desaga spray gun, Desaga, Heidelberg, Germany) and dried at 105°C for 5 min. At that time the spots were

apparent, and the Rf-values of the spots were recorded [orange-red : glyphosate (Rf-value = 0.720-0.760), lavender-pink: AMPA (Rf-value = 0.602-0.623)].

Each individual band on the TLC plate was divided into three sections (initial spotting point, AMPA and glyphosate regions) which were scraped into three separate LSC vials, containing 20 mL scintillation cocktail (Scintil Verse II, Fisher Scientific Company, Unionville, ON). The vials were agitated and left overnight before the ^{14}C-assay was conducted with a Beckman LS6000 LSC. Counting efficiency was 95 to 98%; all counts were corrected.

The experiment was replicated four times for all EUMs at each sampling period. The means ± SD values were calculated. Under the conditions of the TLC method, each sample spot contained the equivalent of 57 μg of glyphosate and 1.5 μg of AMPA. In the control spots, these values were 5 μg and 2.5 μg respectively.

3 RESULTS AND DISCUSSION

The compatibility of Ethomeen and Silwet with EUMs of Vision formulation was determined by i) observing the VEs- and VSs-EUMs for any physical changes such as phase separation, crystallization, and suspension formation, phenomena which would indicate alteration of the physicochemical properties of the EUMs; ii) analyzing for the presence of AMPA, the major metabolite of glyphosate in EUMs, by TLC and LSC as an indication of AI degradation; and iii) evaluating the Rf-values of the components of the EUMs for possible formation of an AI-adjuvant complex which might change the herbicidal activity of the Vision formulation.

3.1 Phase Separation, Crystallization and Suspension Formation

Phase separation, crystallization, or formation of suspensions was not observed at any sampling period for any EUM under the storage conditions of this study. These results imply that the additions of Ethomeen and Silwet appear not to result in the formation of any complex with compounds which may alter the physico-chemical properties of the Vision EUM, or to induce precipitate of these compounds.

3.2 AI Degradation

Using the statistical analysis of Student-Newman-Keuls' test (S-N-Ks' test, α=0.05) (Steel and Torrie, 1980), the EUM TLC data indicated that glyphosate had an Rf-value of between 0.720 and 0.760. If the chemical nature of the AI did not change after preparation, glyphosate should remain and it should have the same Rf-value as before. If AI degradation occurred, the percentage radioactivity recovery from the glyphosate and AMPA spots would determine the extent of AI loss. The data on percentages of [^{14}C]glyphosate recovery and Rf-values are given in Tables 1.2, 1.3, 1.4 and 1.5.

The results from Tables 1.2 and 1.3 both indicate that the amount of [^{14}C]glyphosate applied on the TLC plate was recovered completely (range 93 to 104%). Three samples in the VEs showed slightly lower recovery (84 to 90%) than most of the samples. These lower recoveries can be explained by static electricity causing sample loss during transfer of the scraped TLC plate material into the polyethylene LSC vials. This problem was overcome by using glass LSC vials. However, the results also showed relatively little or no recovery of [^{14}C]AMPA (i.e., < 5% for all EUMs) demonstrating that the possibility of AI degradation due to the

Table 1.2 Percent recovery (mean±s.d.) of [^{14}C]glyphosate in EUMs that contained Ethomeen T/25.

Time		Vision + Ethomeen T/25[1]				
(d)	V	$VE_{0.05}$	$VE_{0.15}$	$VE_{0.45}$	$VE_{0.90}$	$VE_{1.35}$
0	--	100	100	100	100	100
1	--	96 ± 1	94 ± 2	95 ± 4	98 ± 4	93 ± 1
2	--	97 ± 1	95 ± 2	89 ± 5*	97 ± 2	98 ± 8
4	--	96 ± 2	96 ± 10	84 ± 4*	90 ± 2*	95 ± 3

Table 1.3 Percent recovery (mean±s.d.) of [^{14}C]glyphosate in EUMs that contained Silwet L-77.

Time		Vision + Silwet L-77[1]				
(d)	V	$VS_{0.05}$	$VS_{0.15}$	$VS_{0.45}$	$VS_{0.90}$	$VS_{1.35}$
0	--	100	100	100	100	100
1	--	97 ± 1	95 ± 1	97 ± 2	96 ± 2	104 ± 1*
2	--	96 ± 1	95 ± 2	99 ± 3	99 ± 4	102 ± 4*
4	--	96 ± 2	97 ± 3	100 ± 4	98 ± 9	97 ± 5

[1] Student-Newman-Keuls' test, for $\alpha = 0.05$, comparison was made between values of percent recovery of [^{14}C]glyphosate at all conc. levels and sampling periods. Only those without '*' are not significantly different from one another in the entire sample population.

existence of both adjuvants is not likely during the 4 day study period. Since EUM of V did not contain any additional adjuvant, the [^{14}C]glyphosate recovery for V between sampling periods was not investigated.

3.3 Rf-values Evaluation

For EUMs of VEs, the results (Table 1.4) show that the glyphosate Rf-values of most of the EUMs were not significantly different (S-N-Ks' test, with $\alpha = 0.05$) from one another when the comparisons were made with respect to adjuvant concentration or sampling time. Although, at the concentration level of 1.35% (i.e., $VE_{1.35}$), which the Rf-values of glyphosate at 1 d (0.755) and 2 d (0.751) showed statistically higher than that of 0 d (0.718), but they were within the glyphosate Rf-value range (i.e., 0.720 to 0.760). For EUMs of VSs, when comparing the Rf-values within the same sampling period (Table 1.5), no significant difference was found for those with the Silwet concentration levels of 0.15% or less. However, within the same concentration level, no significant difference was detected between the Rf-values of VSs at different sampling period. The results also show that an increase in the concentration level of adjuvant in EUMs seems to retard the migration of the AI and this suggests two possibilities: i) interaction between the AI (glyphosate) and adjuvant [e.g., forming a complex or coagulating into a bigger compound, which would be expected to migrate to a lesser extent (smaller Rf) than glyphosate alone] might occur or ii) the adjuvant might increase the chemical affinity of the TLC plate by coating the TLC plate material, and as a result, increasing the chemical affinity for the AI (glyphosate) thus reducing the AI migration and lowering the Rf-value of glyphosate. To verify these two possibilities requires a study of the

Table 1.4 Rf-values of glyphosate in EUMs that contained Ethomeen T/25.

Time	Vision + Ethomeen T/25[1]					
(d)	V	$VE_{0.05}$	$VE_{0.15}$	$VE_{0.45}$	$VE_{0.90}$	$VE_{1.35}$[2]
0[3]	0.727 (0.017)	0.726 (0.020)	0.724 (0.014)	0.733 (0.013)	0.732 (0.002)	0.718 a (0.015)
1	0.741 (0.005)	0.743 (0.014)	0.734 (0.022)	0.734 (0.033)	0.746 (0.010)	0.755 b (0.015)
2	0.755 (0.019)	0.732 (0.007)	0.737 (0.028)	0.759 (0.015)	0.753 (0.019)	0.751 b (0.023)
4	0.752 (0.021)	0.741 (0.008)	0.734 (0.025)	0.739 (0.023)	0.746 (0.026)	0.748 ab (0.021)

[1] Values represent the mean (± s.d.) of four sets of data obtained from four replications of the study.

[2] Student-Newman-Keuls' test, for $\alpha = 0.05$. All Rf-values within the same column are not significantly different from one another, except column $VE_{1.35}$, where Rf-values with the same letters are not significantly different from one another.

[3] Student-Newman-Keuls' test, for $\alpha = 0.05$. All Rf-values within the same row are not significantly different from one another.

Table 1.5 Rf-values of glyphosate in EUMs that contained Silwet L-77.

Time	Vision + Silwet L-77[1]					
(d)	V^2	$VS_{0.05}$	$VS_{0.15}$	$VS_{0.45}$	$VS_{0.90}$	$VS_{1.35}$
0[3]	0.727 b (0.017)	0.721 b (0.014)	0.729 b (0.009)	0.706 ab (0.016)	0.688 a (0.009)	0.684 a (0.006)
1	0.741 b (0.005)	0.732 b (0.011)	0.730 b (0.005)	0.704 a (0.012)	0.699 a (0.012)	0.698 a (0.003)
2	0.755 b (0.019)	0.731 b (0.013)	0.726 ab (0.025)	0.718 a (0.025)	0.706 a (0.018)	0.698 a (0.014)
4	0.752 b (0.021)	0.728 b (0.024)	0.720 ab (0.010)	0.712 a (0.029)	0.709 a (0.011)	0.709 a (0.013)

[1] Values represent the mean (± s.d.) of four sets of data obtained from four replications of the study.

[2] Student-Newman-Keuls' test, for $\alpha = 0.05$. All Rf-values within the same column are not significantly different from one another.

[3] Student-Newman-Keuls' test, for $\alpha = 0.05$. All Rf-values with the same letters within the same row are not significantly different from one another.

EUMs using techniques such as the X-ray crystallography or complexometric titrations of the EUM, which is not the objective of this study. Therefore compatibility of Silwet with Vision formulation at concentration level above 0.15% remains uncertain.

Because of the uncertainty associated with the EUMs containing high adjuvant concentrations, it is reasonable to surmise that the chemical characteristics of glyphosate in Vision were not affected by Ethomeen at concentration levels $\leq$ 0.90%; whereas, Silwet mixtures were affected at concentration levels > 0.15%.

4 CONCLUSION

Because phase separation, crystallization and suspension formation were not observed, no physical changes appear to have occurred for any of the EUMs throughout the study. This demonstrates that within the adjuvant concentration level sand the time period of the study, Ethomeen and Silwet appear not to be chemically incompatible with Vision formulation. The results of the percent recovery of [^{14}C]glyphosate (i.e., range from 93 to 104%) reflected that relatively little or no recovery of [^{14}C]AMPA (data are not showed here), and the statistical analysis of these values (Tables 1.2 and 1.3) indicated that no AI degradation occurred for all EUMs during the four days study period. Because of the uncertainty in AI entrapment associated with EUMs containing high adjuvant concentrations, evaluation of the Rf-values of glyphosate suggested that Ethomeen is compatible with Vision at concentration levels of 0.90% or less; whereas, Silwet is limited at levels equal to or lesser than 0.15%.

CHAPTER 2.

Optimizing Adjuvant Concentration in End-use Mixtures to Maximize Rainfastness of Vision Without Reducing Uptake and Translocation for Bioavailability.

ABSTRACT

The influence of Ethomeen and Silwet on glyphosate washoff from trembling aspen foliage was studied. Vision EUMs containing one of these two adjuvants were used to determine optimum adjuvant concentration (OAC) level for maximum foliar uptake and minimum washoff without causing reduction in bioavailability or translocation of the AI. The study was conducted using uniform-sized droplets (0.5 μL in volume, or about 1.0 mm in diameter) generated using a microapplicator. The droplets were applied to the adaxial surface of foliage of trembling aspen branch tips at an equivalent rate of 1.0 kg of glyphosate (A.E.) in 35 L/ha of foliar surface. Forty-eight hours after treatment (HAT), the treated leaves were washed with distilled water and [^{14}C]glyphosate residues quantified by LSC in the treated-leaf-wash (TLW), in the post-oxidized treated-leaf- residue (TLR), and the untreated parts (UTP) of the branch tips. The maximum translocation of radiolabelled glyphosate to the UTP for EUMs that contained Ethomeen T/25 and Silwet L-77 was at adjuvant concentration level of 0.45% and 0.15%, respectively.

1 INTRODUCTION

Polymeric adjuvants and related materials used in pesticide formulations sometimes have enhanced residual activity (Cadogan, 1986; Dahl and Lowell, 1984; Gorski et al., 1985; Ivy, 1972; Smith and Verna, 1977; White and Schreiber, 1984), but at other times have resulted in little difference (Schreiber et al, 1978). In a recent study (Sundaram, 1990c), some adjuvants were shown to provide beneficial effects in herbicide applications. However, there appears to be an optimum adjuvant concentration (OAC) for maximum benefit. Beyond this level, the adjuvants may have adsorbed or bound AI, hence, reducing translocation of AI for biochemical interaction with plants.

Preliminary studies have shown that Ethomeen T/25 and Silwet L-77 have the potential of improving rainfastness of glyphosate in the EUM of Vision formulation. However, little is known about the concentration levels required for these adjuvants to provide the maximum beneficial effect (i.e., maximum foliar uptake with minimum washoff) with little reduction in translocation of glyphosate into untreated parts of plants. The purpose of this study was to determine the relative OAC of Ethomeen T/25 and Silwet L-77 in EUMs of Vision formulation used for the rainfastness studies of glyphosate.

2 MATERIALS AND METHODS

2.1 Materials

2.1.1 Treatment of End-use Mixtures

Eleven EUMs were prepared using radiolabelled glyphosate as described in Table 1.1 of Chapter 1, and were used for the three treatments (e.g., Vision alone, Vision with Ethomeen and Vision with Silwet) in this study.

2.1.2 Trembling Aspen Branch Tips

One-year-old seedlings were transferred just before bud-flush from the outdoor nursery into a greenhouse under constant temperature (15 ± 1°C), photoperiod (16 h light, 8 h darkness) and relative humidity (75 ± 7%), for four weeks of acclimatization. Sixty-eight (68) branch tips (ca. three weeks after bud-flush, each 18-cm long with leaf stage of four fully developed and two underdeveloped leaves) were clipped from the mid-crown area of these seedlings. The stem of each branch was placed at once in a 50 mL capacity plastic vial containing tap water. The branch was supported upright by tubing and a lid with a hole (Figure 2.1). Similar branch clippings were tested for their survival rate and growth patterns for a period up to 7 d in a preliminary investigation prior to the actual study. It was observed that the branches remained healthy but showed small reductions in weight during the first two days. However, weight gain was noted from the third day on, and the growth of plants was able to be sustained for more than a week. The clipped branch tips were kept in the greenhouse for three days' acclimatization prior to their use in the experiment. The average surface area of the fully developed leaves was 12 ± 1 cm^2 (Figure 2.2).

Figure 2.1 Experimental set-up for branch tips used for the OAC study

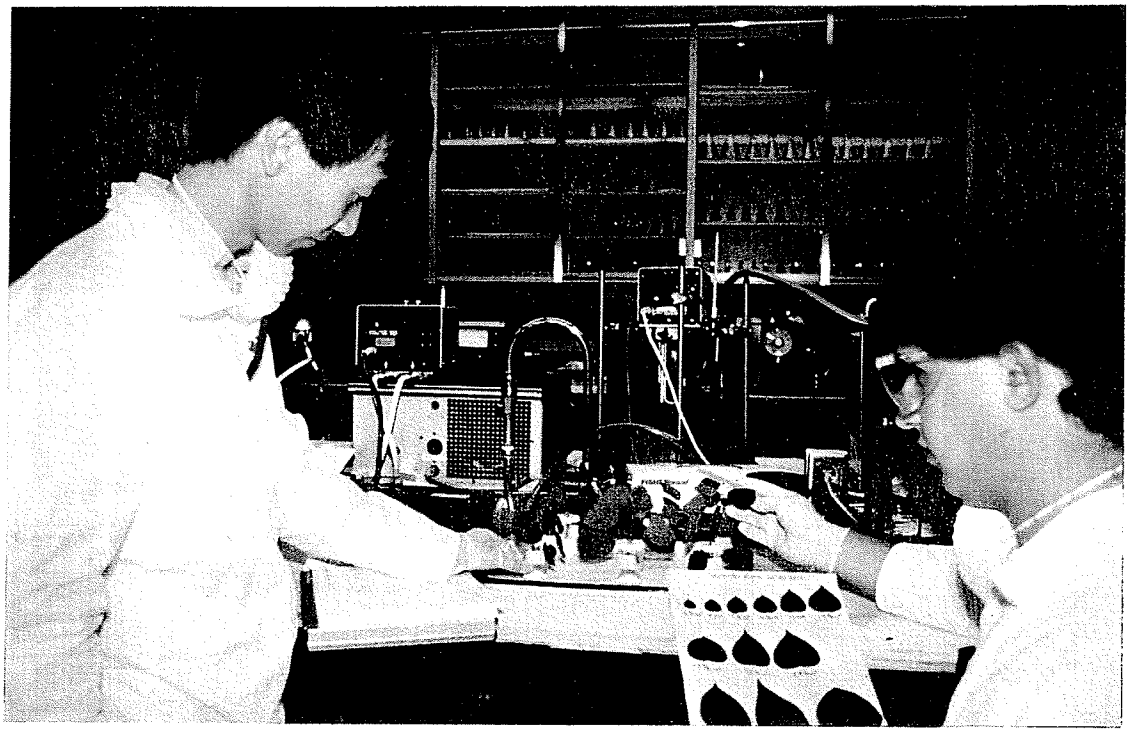


Figure 2.2 Estimation of the average foliar surface area for the OAC study

2.2 Methods

Sixty-six (66) branches were divided into 11 groups corresponding to the 11 EUMs, each consisting of six branches. The remaining two branches served as the control group to measure the background radioactivity in the branches. Table 2.1 shows the assigning of branch tips and the illustration of the experimental design of the study.

The experiment was conducted so that uniform-sized droplets (0.5 μL in volume, or about 1.0 mm in diameter) of the radiolabelled EUM were generated using a microapplicator (Instrumentation Specialties Company, 4700 Superior, Lincoln, NB, USA). The droplets were then applied to the adaxial surface of the four fully developed leaves of trembling aspen branch tips at the rate of 9 drops per leaf to obtain a dosage rate equivalent to 1.0 kg of glyphosate in 35 L/ha area of foliar surface. Each branch tip received the amount of radioactivity approximately equal to 222 Bq. Forty-eight (48) hours after treatment (HAT), each branch tip was divided into three segments, viz., treated leaf (TL), untreated leaf (UTL) and the stem and petioles (SP). The tap water in the vial (TWV) was also collected for radioassay to examine glyphosate movement via stem into water. The treated leaves were removed from the plant, placed in a glass funnel over a graduated cylinder (100 mL capacity), washed twice with 20 mL of distilled water for 30 second each. The wash-liquid (called 'treated leaf wash', TLW) was assayed for ^{14}C-activity by analyzing by LSC duplicate samples of 2 ML plus 18 mL of a scintillaiton cocktail (ScintiVerse II, Fisher Scientific Company, Unionville, ON) using a Beckman LS6000 LSC system. All plant segments, including the treated leaf residue (TLR), were then oven-dried for 48 h at 60°C, weighed, and then broken into small pieces using

Table 2.1 Experimental design for the Optimum Adjuvant Concentration (OAC) study.

Conc. of Adjuvant % (v/v)	No. of branch tips in treatments			
	Control	Vision alone	Vision Ethomeen	Vision Silwet
0	2	6	---	---
0.05	---	---	6	6
0.15	---	---	6	6
0.45	---	---	6	6
0.90	---	---	6	6
1.35	---	---	6	6

scissors and a mortar and pestle. Subsamples of these dried plant parts (e.g., SP, UTL, and TLR), were combusted in a biological sample oxidizer (Packard Oxidizer, Model 306, United Technologies Packard, Packard Instrument Company, Ill, USA). The evolved $^{14}CO_2$ activity was absorbed in a LSC vial containing Carbosorb (scintillation cocktail for CO_2 absorption from United Technologies Packard, Ill, USA). The data are given in Tables 2.2 and 2.3.

Table 2.2 Percentage distribution of radioactivity in the different samples analyzed (mean ± s.d.) - for EUMs containing Ethomeen T/25

Sample[1]	Percentage distribution[2]					
Abbrev.	V	$VE_{0.05}$	$VE_{0.15}$	$VE_{0.45}$	$VE_{0.90}$	$VE_{1.35}$
TLW	86 ± 2 d	84 ± 1 c	82 ± 2 c	83 ± 1 c	78 ± 2 b	73 ± 3 a
TLR	12 ± 2 a	14 ± 2 a	14 ± 3 a	13 ± 2 a	19 ± 2 b	24 ± 2 c
UTP[3]	1.6 ± 0.6 ab	2.1 ± 0.1 ab	2.4 ± 0.6 b	3.5 ± 1.0 c	2.1 ± 0.5 ab	1.6 ± 0.6 a
UTL	0.24 ± 0.3 a	0.33 ± 0.3 a	0.49 ± 0.5 a	0.26 ± 0.1 a	0.41 ± 0.3 a	0.49 ± 0.3 a
SP	1.4 ± 0.4 ab	1.8 ± 0.3 ab	1.9 ± 0.6 b	3.2 ± 0.9 c	1.7 ± 0.5 ab	1.1 ± 0.5 a
% TOTAL[4]	99.6 ± 0.4	100.1 ± 3	98.4 ± 3	99.5 ± 2	99.1 ± 1	98.6 ± 3

[1]TLW: treated leaf wash; TLR: treated leaf residue; UTP: untreated parts; UTL: untreated leaves; SP: stem and petioles.

[2]Values with the same letters within row are not significantly different from one another (S-N-Ks' test, α=0.05).

[3]Mean and s.d. of six sets of data obtained by calculating the sum of UTL and SP.

[4]Mean and s.d. of six sets of data obtained by calculating the sum of TLW, TLR, and UTP.

Table 2.3 Percentage distribution of radioactivity in the different samples analyzed (mean ± s.d.) - for EUMs containing Silwet L-77

Sample[1]	Percentage distribution[2]					
Abbrev.	V	$VS_{0.05}$	$VS_{0.15}$	$VS_{0.45}$	$VS_{0.90}$	$VS_{1.35}$
TLW	86 ± 2 c	85 ± 2 c	82 ± 2 b	82 ± 2 b	80 ± 1 b	75 ± 3 a
TLR	12 ± 2 a	13 ± 2 a	16 ± 4 ab	16 ± 4 ab	18 ± 3 b	23 ± 5 c
UTP[3]	1.6 ± 0.6 ab	1.5 ± 0.6 ab	2.1 ± 0.4 b	1.8 ± 0.7 ab	1.4 ± 0.4 a	1.4 ± 0.2 a
UTL	0.24 ± 0.3 a	0.42 ± 0.4 ab	0.62 ± 0.2 b	0.32 ± 0.3 ab	0.26 ± 0.1 ab	0.39 ± 0.2 ab
SP	1.4 ± 0.4 a	1.1 ± 0.9 a	1.5 ± 0.4 a	1.5 ± 0.6 a	1.1 ± 0.4 a	0.97 ± 0.4 a
% TOTAL[4]	99.6 ± 0.4	99.5 ± 3	100.1 ± 5	99.8 ± 5	99.4 ± 3	99.4 ± 5

[1 2 3 4 *]: See footnotes of Table 2.2.

3 RESULTS AND DISCUSSION

A large number of seedlings would be required to investigate foliar uptake and translocation of glyphosate with several concentrations of the two adjuvants. However, the use of a large number of seedlings would involve extensive labor, time, and materials. Since the objective of this study was to determine the relative OAC of Ethomeen and Silwet in EUMs of Vision formulation being used for the rainfastness study of glyphosate formulations, small branch tips were used in this study to overcome the problems mentioned above. Because $^{14}CO_2$ was the radioactive compound for LSC ^{14}C-assay and few glyphosate metabolites have been reported in plants within 48 HAT (Devine and Bandeen, 1983; Gottrup et al, 1976; Zandstra and Nishimoto, 1977) the 48 h duration of the study was to ensure that the radioactivity recovered could be referred to as [^{14}C]glyphosate. Also, the technique used to wash the treated leaves had been shown in a previous study (Sundaram, 1990d) to completely remove the washable glyphosate from the foliage.

From the data on the distribution of [^{14}C]glyphosate in different parts of the plant (Tables 2.2 and 2.3), it was evident that the majority of the applied amount (*ca.* 73 to 86%) was extracted into the TLW. With the addition of Ethomeen and Silwet to the EUMs, TLW values decreased with increasing adjuvant concentration. The TLW values of VEs and VSs (except that of $VS_{0.05}$) showed significant reduction compared to the TLW value of V. This reduction implies increased rain protection potential of these two adjuvants for the EUM of Vision. For TLR, the applied [^{14}C]glyphosate remaining on the treated leaves after washing was *ca.* 12 to 24%. Increasing the adjuvant concentration increased the amount of recovery in TLR.

However, significant differences between VEs and V, and VSs and V occurred when the adjuvant concentration levels reached 0.90% or higher. For the translocation of [^{14}C]glyphosate to the UTP of plants, the relationship between the amount of recovery and adjuvant concentration level was a bell shape for both VEs and VSs. This means that the increase of adjuvant concentration will increase translocation up to a maximum level, and decrease the translocation afterwards. With EUMs containing Ethomeen, only $VE_{0.45}$ showed significantly higher recovery (S-N-K's test, $\alpha = 0.05$) than that of V. With EUMs containing Silwet, no significant difference was detected between VSs and V. In a further expansion of the analysis of results of UTP to its components such as UTL and SP, we found that no significant difference between VEs and V was detected in UTL. The only SP sample of VE which showed significantly higher recovery than that of V was found to be the samples of $VE_{0.45}$. On the other hand, the [^{14}C]glyphosate recovery in samples of UTL for the VSs indicated that only $VS_{0.15}$ was significantly higher than that of V and no significant difference was noted between VSs and V for samples of SP. An almost complete recovery of radioactive material was reached (i.e., the total recovery of [^{14}C]glyphosate was from 98.4 to 100.1%) thus the amount of glyphosate that might have moved via the stem into the TWV is $\leq$ 1.6%.

4 CONCLUSION

As adjuvant concentration levels increased, the amount of glyphosate being washed off was reduced; whereas, the 'apparent' foliar uptake of glyphosate increased (see data of TLW and TLR in Tables of 2.2 and 2.3). However, the significant

increase in this 'apparent' foliar uptake of the glyphosate (i.e., in TLR) does not necessarily indicate an increase in penetration of glyphosate through the leaf cuticle, since these polymeric adjuvants could simply have provided a protective layer over the droplets, thus reducing the amount being washed off during rinsing (Sundaram, 1990e). Without detailed investigations using extracted plant cuticle (Baker et al., 1983), it would not be possible to conclude that the higher adjuvant concentration level was the OAC level for rain protection of glyphosate in Vision formulation. On the other hand, the data of Tables 2.2 and 2.3 show that with the increase of adjuvant concentration level, the amount of glyphosate in UTP increased slightly, and then passed through a maximum. Because the glyphosate that is present in the UTP was considered to be the amount of glyphosate which would not be vulnerable for rain wash, the results shown in Chapter 1 and the maximum amount of radiolabelled glyphosate present in UTP were used as the parameters for selecting the OAC of the EUM. As a result, 0.45% and 0.15% were respectively considered to be the OAC levels for Ethomeen T/25 and Silwet L-77 used in Vision EUM to maximize rainfastness of glyphosate for the remaining studies of this thesis.

CHAPTER 3.

Effect of Two Adjuvants on Physical Properties, Droplet Spreading and Drying Rates of Glyphosate in Vision Formulation.

ABSTRACT

The physical properties of three EUMs (e.g., V, $VE_{0.45}$ and $VS_{0.15}$), were measured to examine the role of adjuvants on droplet spreading and drying rates. The presence of adjuvants did not markedly alter the viscosities or volatilities of EUMs for this study. The surface tensions of V and $VE_{0.45}$ were similar; whereas, the surface tension of $VS_{0.15}$ was substantially lower. This implied that Silwet L-77 wetted the foliar surface better than Ethomeen T/25. To demonstrate the wetting ability of Silwet L-77, a spreading study was conducted on the surface of trembling aspen leaves (TAL) and glass slides coated with trembling aspen wax (WCGS). The results showed that droplets of $VS_{0.15}$ have more spreading ability than $VE_{0.45}$ and V, while the spreading abilities of $VE_{0.45}$ and V were similar. Correspondingly, on TAL, droplets of $VS_{0.15}$ took a shorter time than those of $VE_{0.45}$ and V to complete spreading, but on WCGS, drops of all EUMs completed spreading within 20 sec. From the drying study, droplets of $VS_{0.15}$ required shorter droplet drying time than either V or $VE_{0.45}$, whereas no significant difference was detected between V and $VE_{0.45}$ for the same event on both surfaces.

1 INTRODUCTION

Once the pesticide spray droplets are deposited on the target surfaces, the retention of the active ingredient on the surface layer, and its rate of uptake or penetration into the sub-surface layer of the target species, are the most important factors for protecting the AI against rain wash. However, these factors are highly dependent upon the association between the chemical components of the EUM used (i.e., the active ingredient, the adjuvants, and the carrier etc.), the interfacial area (i.e., area of contact on which interaction take place), and the surface of target matrices (which is referred to as the epicuticular wax surface of the trembling aspen leaf for this study).

Literature evidence on physical properties, spreading and drying rates of spray EUMs in relation to foliar uptake and translocation is controversial. Some studies have indicated a positive relationship and some have shown none. For example, Sundaram (1984) found that formulation viscosity influenced the retention, spreading, and rate of dissipation of spray droplets, and that the rate of evaporation appeared to have a direct influence on the rate of cuticular absorption (Figure 3.1). Sundaram and Sundaram (1987) and Zabkiewicz et al. (1985) used polymeric adjuvants in pesticide formulations to increase spreading or wetting ability of spray droplet on foliar surfaces (i.e., to increase the interfacial area, and thus improve the droplet retention as well as increase the rate of uptake), resulting in enhanced pesticidal activity. In contrast with these findings, the loss of liquid phase from foliar surfaces reduces cuticular penetration, implying that polymers increase droplet spreading, accelerate drying rate, and reduce uptake (Hess, 1984; Zabkiewicz et al., 1988).

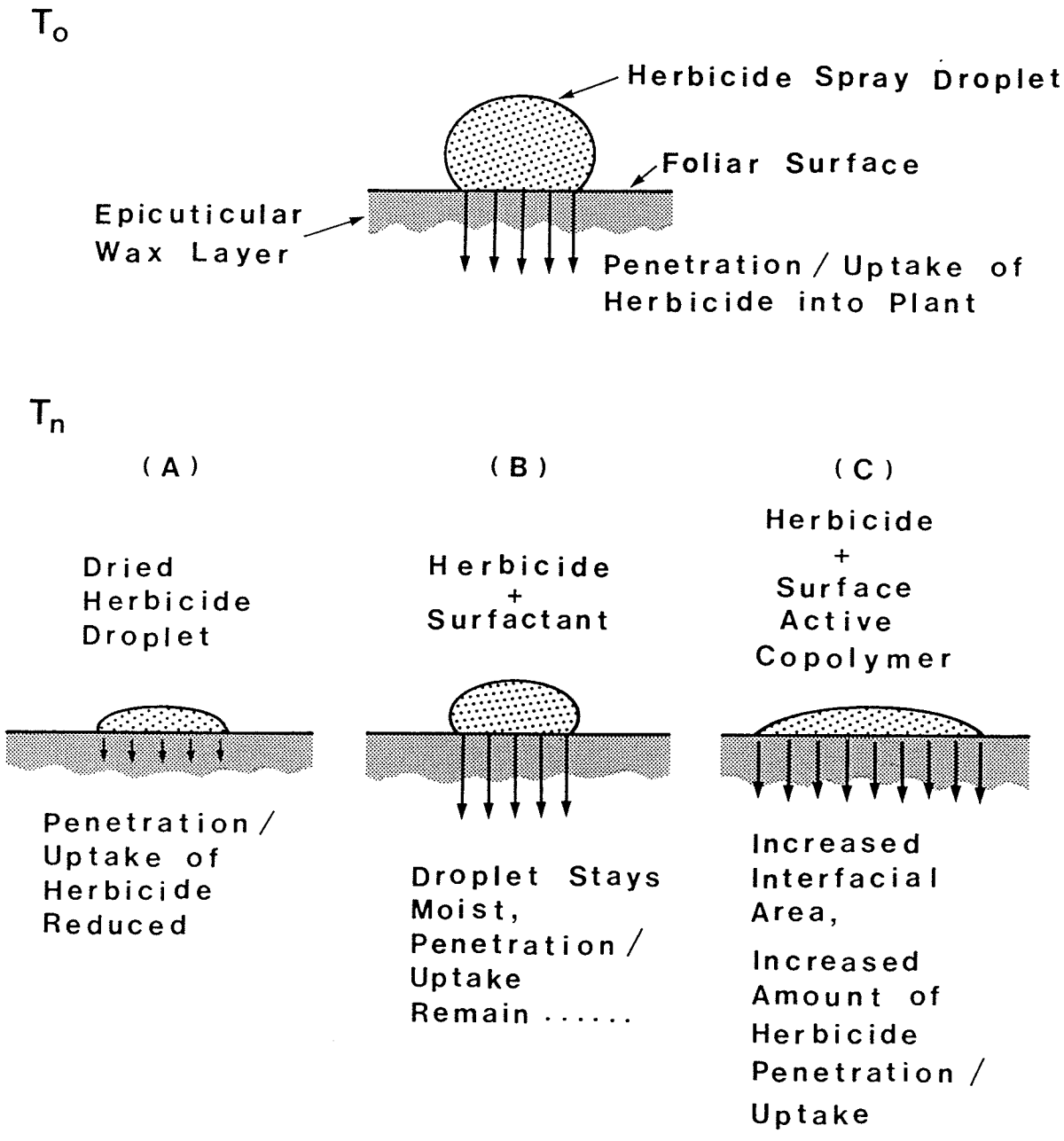


Figure 3.1 Diagrammatic representation of the interaction between droplet deposits and leaf epicuticular wax surfaces.

Furthermore, there are few quantitative data in the literature using trembling aspen foliage. In view of these, it is necessary to know if there is a relationship between physical properties, spreading, and drying rates of Vision with and without adjuvants in order to understand the role of adjuvants in the rainfastness of glyphosate.

2 MATERIALS AND METHODS

2.1 Materials

2.1.1 Treatment of End-Use Mixtures

With their concentration levels predetermined by the previous studies, three EUMs (e.g., V, $VE_{0.45}$ and $VS_{0.15}$) were used in this study. The EUMs were prepared as described in Chapter 1.

2.1.2 Trembling Aspen Leaf (TAL) Disc Slides

Four trembling aspen leaf discs with diam. *ca.* 15 mm were removed from the fully developed leaf of trembling aspen seedlings (see 2.1.2 of study 2) with a No. 11 cork borer (Fisher Scientific Co., Cat. No. 07-845E, Unionville, ON). To provide a uniform surface structure for droplet spreading and drying processes, they were then mounted flat with a piece of double sided adhesive tape (Loma Coll, Lohmann GMBH & Co., Germany) on a microscope slide. Measurements were carried out immediately after mounting the leaf discs to maintain the freshness of the leaf surface during the study.

2.1.3 Wax Coated Glass Slides (WCGS)

The combined epicuticular wax of 50 fully developed trembling aspen leaves was extracted by dipping them one after another into a bath of 100 mL chloroform for 30 sec. The extracted solution was then flash-evaporated to a final volume of *ca.* 40 mL. Using an Eppendorf micro-pipette, four 40-μL aliquots of the extract were separately delivered onto the four quadrants of each of 60 precleaned microscope slides (size 75x50 mm, Fisher Scientific Co., Cat. No. 12-550C, Unionville, ON). Each slide was then placed in a fume hood and the extracts allowed to evaporate overnight. At the end of this procedure, each microscope slide contained four evenly coated trembling aspen wax spots (diam. *ca.* 15 mm).

2.2 Methods

2.2.1 Physical Properties

Viscosity, surface tension and volatility were the physical properties of the EUMs under investigation in this study. Viscosity (cP) was measured using a size 100 Ostwald viscometer (Glasstone, 1955). Surface tension (dynes/cm) was measured using a Surface Tensiomat Model 21 (Fisher Scientific Co., Cat. No. 14-814, Unionville, ON) and volatility was measured by the method of Sundaram and Leung (1986). Volatility parameters are expressed as rate of evaporation, R(Evap), half-life (i.e., $t_{1/2}$, the time required for the volatile components to reach 50% of their initial concentrations), and the percent non-volatile components (NVC%, the residual amounts that were left unevaporated until at least 24 h after). All measurements were carried out at room temperature (20 ± 2 °C) and 75 ± 5% relative humidity.

2.2.2 Droplet Spreading and Drying Rates

Erio Acid Red XB400 (EAR), a water-soluble fluorescent dye, (St. Lawrence Aniline Company, Brockville, ON) was added to the EUM to facilitate the visualization of the droplets under microscope for the investigation of the spreading and drying characteristics. The EAR dye was used at a concentration level of 0.2% (w/v). In a previous experiment (not published), this quantity did not alter the physical properties of the EUM.

2.2.2.1 Equilibrium Spread Areas and The Associated Rates

Uniform-sized droplets (0.5 μL, *ca.* 1.0 mm dia.) were generated using a microapplicator as described in Chapter 2. At t_o (Figure 3.2), they were collected on the surface of the trembling aspen leaf (TAL) disc. The droplets were observed under a stereoscopic microscope (AO Stereostar / Zoom, American Optical Scientific Instruments, Div. of Warner-Lambert Technologies, Inc., Buffalo, N.Y., USA) with a cool light source (Reichert fiber optic illuminator, Fisher Scientific Co., Cat. No. AO 1177) to avoid undue heating of the droplet (which would increase the rate of evaporation). When the diameter of the spread area reached a constant value (when $D_i = D_{i+1} = D_{i+2} .... = D_{i+n}$, the equilibrium state of spreading), it was measured. The time (t_i or t_{i+1} or..... t_{i+n}) it took to reach equilibrium was also recorded. The same procedure was replicated 20 times for each EUM, on TAL discs and on the wax coated glass slides (WCGS). The values of the spread areas were used to calculate the value (Mean ± s.d.) of spread factors (SF) for the three EUMs. The resultant equation was

$$SF = \frac{\text{Dia. of the spread area on a surface}}{\text{Dia. of the spherical droplet}}$$

In some instances, especially on the surface of the TAL disc, the spread area of the droplet failed to form a circular stain. In such cases, another circular stain was selected until the results of twenty replications were obtained for each EUM.

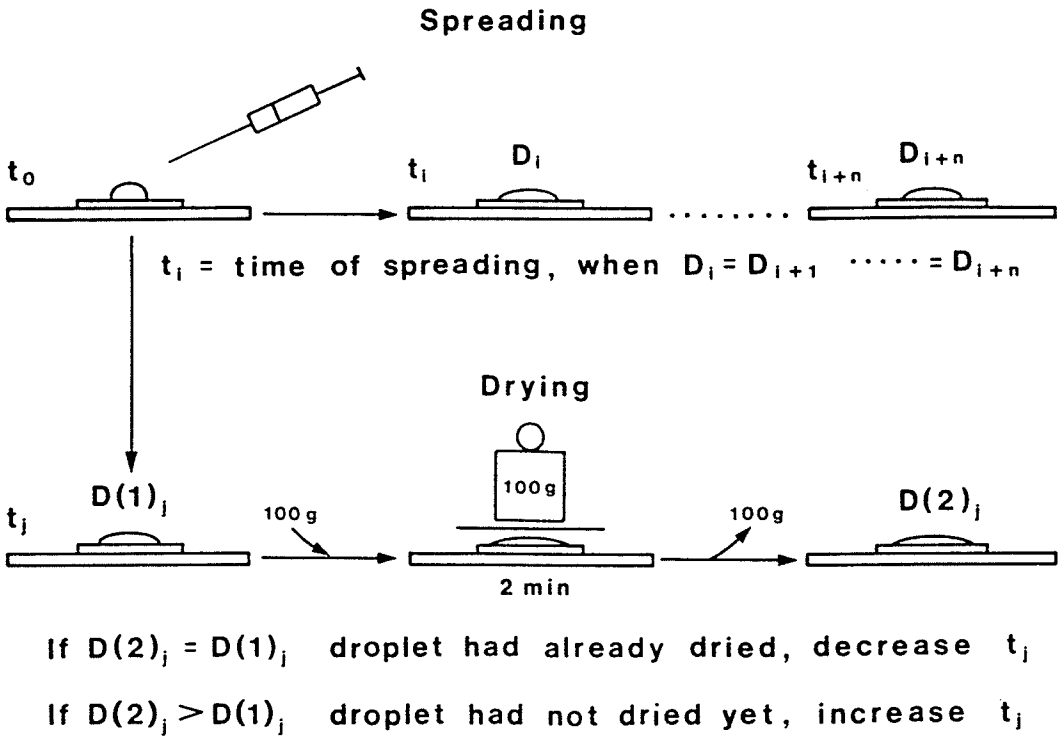
2.2.2.2 Droplet Drying Rates

The procedure used to determine droplet drying rates was based on the methods developed by Sundaram (1990e). Droplets (0.5 μL) were collected on surfaces of TAL disc and WCGS in the same manner as those for the spreading study. Observations were made every two minutes under a microscope until the appearance of the droplet stop changed as an endpoint [approx. 10 min, the solution changed (when red micelles appeared in the droplet) due to the removal of the volatile components (e.g., water) of the droplet via evaporation process, which implies the offset of the favorable solubility relationship (i.e., the favorable hydrophilic and lypophilic balance in solubility) of the EUM (Van Valkenburg, 1969)]. At this stage, the diameter was measured and recorded as $D(1)_j$ at time lapse of t_j. Since the droplet may or may not have undergone complete evaporation, a further test of droplet dryness, was carried out by placing a clean microscope cover glass (18 mm x 18 mm in size, Fisher Scientific Co., Cat. No. 12-540A, Unionville, ON) over the droplet and gently applying a force of 100-g weight onto the top of the cover glass without causing any lateral movement of the assembly. The weight was removed after two min. To measure the droplet spread area, the glass-covered-droplet was observed under a microscope and recorded as diameter $D(2)_j$. If $D(2)_j$ was the same as $D(1)_j$, we considered that the droplet had dried completely. The experiment was repeated with the reduction of t_j until the point of t_{j-r}, when $D(n-1)_{j-r}$ was less than

$D(n)_{j-r}$. Therefore, the time t_{j-r+1} was considered the time that was required for the droplet to dry completely. However, if $D(2)_j$ was greater than $D(1)_j$, it implied that the droplet had not dried at the time the 100-g weight was applied. The experiment was repeated with increasing t_j until the point of t_{j+r}, when either $D(n-1)_{j+r}$ was the same as $D(n)_{j+r}$ or constant values of D(n) [i.e., $D(n)_{j+r+1}$, $D(n+1)_{j+r+2}$, ...$D(n+q+1)_{j+r+q}$] were obtained from the time of t_{j+r} onward. Thus t_{j+r} was taken as the time required for the droplet to become completely dried.

The time diagrams of the procedures for droplet spreading and drying rate studies are illustrated in Figure 3.2.

Figure 3.2 Time diagrams of the droplet spreading and drying rate studies



3 RESULTS AND DISCUSSION

3.1 Physical Properties

Data on the physical properties of the three EUMs used in this study are shown on Table 3.1. The evaporation rates are plotted in Figure 3.3. These results indicate that the viscosities of EUMs, i.e., V (1.17 cP), $VE_{0.45}$ (1.25 cP) and $VS_{0.15}$ (1.18 cP) were similar and suggest the adjuvant in $VE_{0.45}$ and $VS_{0.15}$ had little effect on V to alter viscosity. The surface tension values of the EUMs of V (43.59 dynes/cm) and $VE_{0.45}$ (43.55 dynes/cm) also showed little difference; perhaps because the commercially formulated Vision already contained Ethomeen T/25. Therefore the additional 0.45% of the same adjuvant did not significantly alter the surface tension. The surface tension of $VS_{0.15}$ was significantly lower (27.96 dynes/cm) than that of the other two, which implies that Silwet L-77 would probably be a better wetting agent than Ethomeen T/25. A derivative-free non-linear regression program (BMDP Statistical Software, Inc., Los Angeles, CA, USA) was used to test the best fit equations of the evaporation rate curve of the EUMs (Figure 3.3) and found no significant difference between them. However, the $T_{1/2}$ values of $VE_{0.45}$ (19.8 min) was somewhat greater than V (15.7 min) and $VS_{0.15}$ (15.9 min). Such a difference may be attributed to a relatively higher concentration level of adjuvant in the EUM of VE (0.45%) compared to VS (0.15%); this slightly higher amount of Ethomeen may have been sufficient to retard the process of evaporation.

Table 3.1 Physical properties (mean ± s.d.)[1] of EUMs used in the study at temperature 20 ± 2°C and 75 ± 5% relative humidity.

Physical properties	EUMs of the study		
	V	$VE_{0.45}$	$VS_{0.15}$
Viscosity (cP)	1.17 a (0.01)	1.25 c (0.00)	1.18 b (0.00)
Surface tension (dynes per cm)	43.59 b (0.15)	43.55 b (0.00)	27.96 a (0.09)
Volatility data:			
a. R(Evap)[2]	7.60 a (0.17)	7.91 a (0.33)	7.50 a (0.05)
b. NVC%[3]	5.36 a (0.14)	6.15 b (0.07)	5.28 a (0.07)
c. $t_{1/2}$ (min)[4]	15.7	19.8	15.9

[1]Values with the same letters within row are not signifi- cantly different from one another (S-N-K's test, $\alpha=0.05$).

[2]Percentage weight decrease of the liquid film per min, as calculated during the initial 10 min of evaporation.

[3]The residual amounts which were left unevaporated until at least 24 h after the start of the experiment.

[4]Half-life, $t_{1/2}$, refers to the time required for the volatile components of the mixtures to evaporate down to 50% of their initial values. It was calculated by using the equation

$$t_{1/2} = \frac{2.303 \times \text{Log } 2}{C}$$

where C is the rate constant of evaporation from the evaporation curves of Figure 3.3.

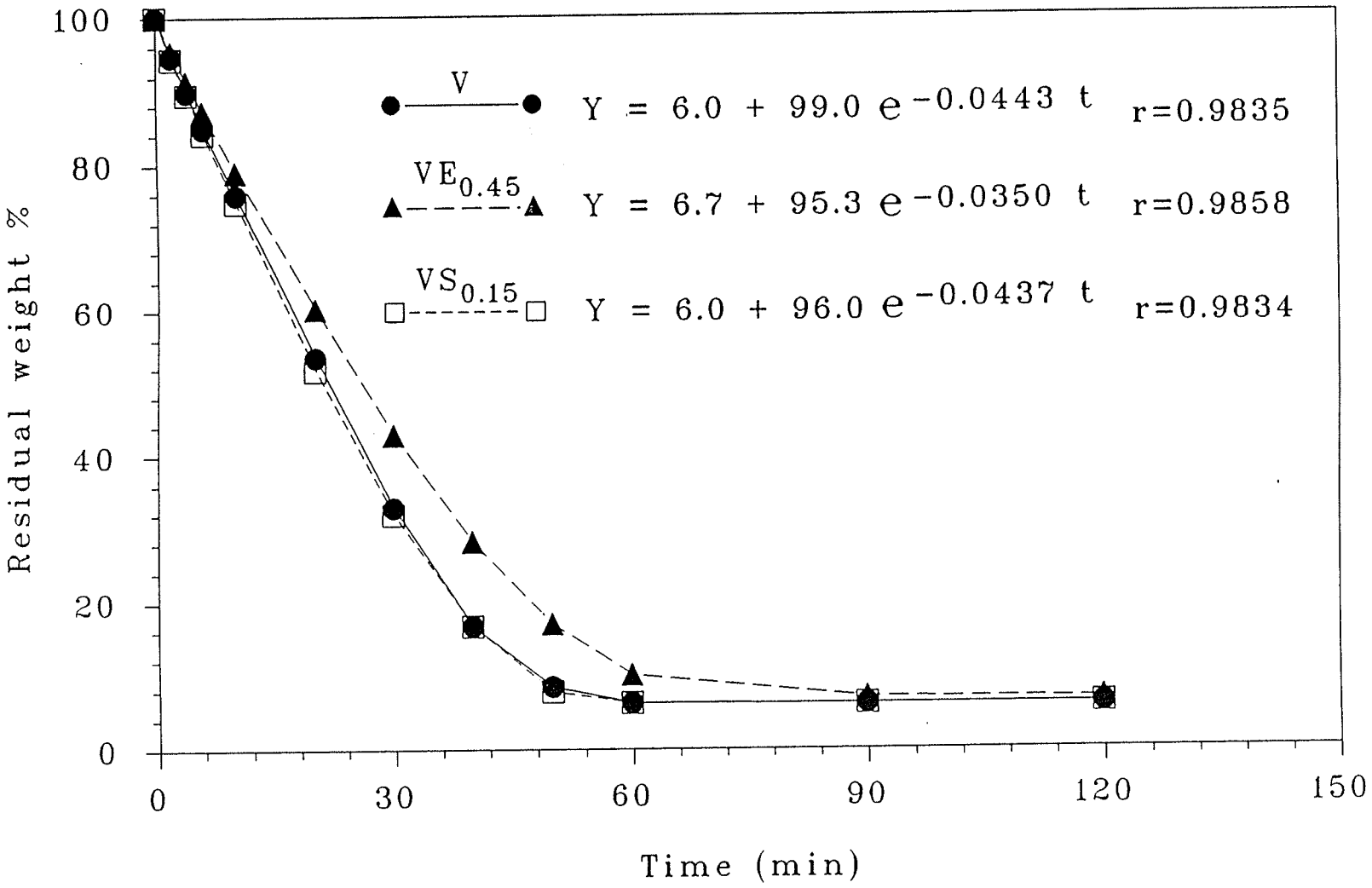


Figure 3.3 Evaporation curves of the EUMs of the study

3.2 Influence of Physical Properties on Droplet Spreading and The Time Required for Complete Spreading

The spread factor data and the time required for EUMs to spread completely on trembling aspen leaves (TAL) and wax coated glass slide (WCGS) are given in Table 3.2. Generally, all three EUMs were significantly different (S-N-K's test, α =0.05) from one another in terms of their spreading characteristics on both surfaces; however, the difference between V and $VE_{0.45}$ was small compared to the difference between V and $VS_{0.15}$ or the difference between $VE_{0.45}$ and $VS_{0.15}$. In fact, droplets of all EUMs spread more on the surface of TAL than on the surface of WCGS. Furthermore, on TAL, V and $VE_{0.45}$ took a similar amount of time (i.e., 420 sec and 413 sec, respectively) for their droplets to be spread completely, while $VS_{0.15}$ achieved the same in *ca.* 65% (i.e., 270 sec) of that time. Nevertheless, the difference between these values is statistically insignificant due to the large s.d. that existed in V and $VE_{0.45}$. Besides, all droplets of EUMs were spread completely within 20 sec on surface of WCGS.

In general, the relationships between physical properties and droplet spreading characteristics were as follows: some slight difference in terms of viscosity and volatility was found between $VE_{0.45}$ and the others, but this difference did not appear to contribute to a difference in the droplet spreading characteristics between EUMs. This agrees with the conclusion made by Sundaram (1989). But the influence of surface tension on droplet spreading characteristics was more pronounced. Surface tension and droplet spreading were inversely associated (i.e., decreased surface tension increased droplet spreading). In fact, the effect shown was less influence on the surface of WCGS than on the surface of TAL. The results therefore indicate

Table 3.2 Spread factor data for the EUMs on different surfaces and the time required for complete spreading of droplets[1] at temperature 20 ± 2°C and 75 ± 5% relative humidity.

Description of Surface type	EUMs of the study		
	V	$VE_{0.45}$	$VS_{0.15}$
	Mean spread factor values[2] - (± s.d.)		
1. Trembling aspe leaf (TAL)	1.63 a (0.03)	1.72 b (0.06)	2.11 c (0.12)
Relative spread area (RSA)[3]	1	1.10	1.68
2. Wax coated glass slide (WCGS)	1.22 a (0.08)	1.28 b (0.05)	1.38 c (0.04)
Relative spread area (RSA)	1	1.11	1.28
	Time (sec) required for complete spreading - mean (± s.d.)[2]		
1. Trembling aspen leaf (TAL)	420 a (154)	413 a (163)	270 a (35)
2. Wax coated glass slide (WCGS)	< 20	< 20	< 20

[1]Droplet size used equal 0.5 μL in volume or 1.0 mm in diameter.

[2]Mean and s.d. of 20 replicate measurements carried out in the study; values with the same letters within row are not significantly different from one another (S-N-K's test, $\alpha = 0.05$).

[3]Calculated as

$$RSA = \frac{\pi / 4 \text{ (diam. of area for either V, VE or VS)}^2}{\pi / 4 \text{ (diam. of area for V)}^2}$$

that the chemical nature of the adjuvants appears to have played a more important role on droplet spreading than the physical properties. Other researchers (Zabkiewicz et al. 1985, 1988; and Sands and Bachelard, 1973) have also found that the chemical nature of adjuvants played an important role in droplet contact angle, wettability and equilibrium spread areas. However, further investigations with different concentrations of the same adjuvant are necessary before any final conclusions can be made concerning the role of surface tension on droplet spreading. In addition, the adverse effect of the interactions between Silwet L-77 and other adjuvants known to be present in Vision cannot be totally ignored (Neustadter, 1984).

3.3 Influence of Physical Properties on Droplet Drying Rates

A study of the physical properties data in Table 3.1 and the droplet drying data in Table 3.3, indicate that viscosities and volatilities would not contribute to marked differences in the droplet drying process. A positive relationship was found between the surface tensions and the droplet drying time (i.e., decreased surface tension reduced the time for droplet drying). This finding implies that there is an inverse relationship between droplet spreading and droplet drying (i.e., increasing the spreading ability, reduces the time for droplet drying). Since the volatility study (Table 3.1) demonstrated that no significant difference in terms of evaporation was detected between the three EUMs (e.g., V, $VE_{0.45}$ and $VS_{0.15}$), one might expect their drying to be the same as well. In fact, droplets of $VS_{0.15}$ (10.5 min on TAL, 14.0 min on WCGS) required shorter droplet drying time than either V (15.0 min on TAL, 22.8 min on WCGS) or $VE_{0.45}$ (15.4 min on TAL, 22.3 min on WCGS); whereas, no significant difference was detected between V and $VE_{0.45}$. Theoretically,

Table 3.3 Time (min) required for droplet[1] drying on TAL and WCGS for the EUMs used in the study at 20 ± 2°C and relative humidity 75 ± 5%.

Description of Surface type	EUMs of the study		
	V	$VE_{0.45}$	$VS_{0.15}$
Trembling aspen leaf (TAL)	15.0[2] b (2.2)	15.4 b (2.3)	10.5 a (0.9)
Wax coated glass slide (WCGS)	22.8 b (2.7)	22.3 b (1.8)	14.0 a (1.5)

[1] 2: See footnote of Table 3.2 for details

drying involves the evaporation of the volatile component of liquid (largely water), and the evaporation process is highly dependent upon the interfacial area between the liquid and air (e.g., for the same volume of liquid, increasing the interfacial area increased the rate of evaporation). For this reason, as the amount of the volatile component (water) in the EUMs of V, $VE_{0.45}$ and $VS_{0.15}$ was about the same, and a uniform volume of 0.5 μL droplet was used, $VS_{0.15}$ increased the spreading area (Table 3.2), thereby hastening the complete drying of the droplets (Table 3.3). These drying characteristics have also been demonstrated on both TAL and WCGS surfaces.

3.4 Inter-relationship between Spreading Characteristics and Drying Process of A Spray Deposit Droplet

It has been demonstrated that, by the reduction of surface tension of EUMs, adjuvants can alter droplet spreading characteristics of the spray deposit. This results in an increase of the interfacial area between the droplet liquid and the foliar surface which is expected to increase the rate of uptake. An increase of the interfacial area also increases the rate of drying (i.e., via evaporation) or hastens the droplet drying process and results in a decrease in the rate of uptake. Therefore, the possibility of using Silwet L-77 in Vision as a rainfastness agent will partly depend upon the rates of these two processes. However, hastening the drying rate may not be completely negative from a rainfastness point of view. As long as the droplet does not become completely dried out (i.e., when uptake ceases), the uptake of pesticide continues. Once the droplet solvent has completely evaporated, the concentration ratio of the pesticides with respect to the leaf cuticle will increase, and the rate of uptake may increase due to the diffusion effect. Ethomeen T/25 showed no signs of improving the spreading ability of Vision; thus, its rainfastness ability for Vision in the preliminary study may have been due to other factors, which is essential for maintaining AI uptake. Such assumptions require further investigation.

When droplets become truly dried, uptake may cease, although the moisture content of live foliage may still continue to dissolve the herbicide at a much slower rate possibly causing a continuous but slow uptake. The complete drying of droplets may be part of the reason that the results of some studies (Caseley et al., 1975; Gottrup et al., 1976) showed that, over a constant time period, plants kept under conditions of high humidity received a lethal dose of herbicide, while plants kept at

low RH failed to do so. Therefore, to decide whether an adjuvant could be used as a rainfastness agent for pesticide spray, it is important to know not only the physical properties of EUMs with respect to droplet spreading to the rate of uptake, but is also important to to know the probable RH at the time of application and to understand the drying process.

4 CONCLUSION

In summary, this study indicated that the addition of the adjuvant Ethomeen T/25 did not change the viscosity and surface tension characteristics of the Vision EUM, but it did slightly slow the process of volatility. This may enable the spray deposit to be maintained under moist conditions, so that the uptake of glyphosate would continue. Silwet L-77 did not change the viscosity and volatility but it reduced the surface tension of the Vision EUM significantly. With low surface tension, Silwet L-77 also enhanced the spreading ability of the EUM. This increase of spreading ability may improve the rate of uptake of glyphosate because the interfacial area has been increased. However, an increase of spreading ability also may result in hastening the drying process, which forces the uptake to cease. These suggest that the spreading and drying processes appear to have played a more important role in AI uptake than the physical properties. Nevertheless, the time available for the pesticide to perform what is required of it before it rains, and the effects of plant physiology and biochemistry (individual or combination) on increasing the rates of uptake and translocation of the AI to the target site, thus providing rain protection are also important (Sprankle, et al., 1975a, 1975b, 1975c; Wyrill and Burnside, 1976; WSSA-Herbicide Handbook, 1983).

CHAPTER 4.

Influence of Two Adjuvants on Rainfastness of Glyphosate in EUM of Vision Formulation Applied on Trembling Aspen Seedlings under Simulated Medium Rainfall Activity : Laboratory Study

ABSTRACT

Under laboratory conditions, uniform-sized droplets of EUMs containing radiolabelled glyphosate (as described in Chapter 3) were applied to the adaxial surface of foliage of trembling aspen seedlings at 1.0 kg of glyphosate in 35 L/ha of foliar surface. Simulated rainfall of 5 mm with intensity of 10 mm/h was applied at time intervals of 0.5, 8, 24, 36, 48, 72, and 96 h after treatment (HAT). Rainfastness of glyphosate in Vision with and without adjuvants (e.g., V, $VE_{0.45}$, and $VS_{0.15}$) was evaluated by examining the samples of treated leaf rainwash run-off, treated leaf residue, and the untreated parts using the radiotracer technique. At 36 HAT, both $VE_{0.45}$ (69.6%) and $VS_{0.15}$ (59.7%) significantly reduced the amount of glyphosate washed off from the treated foliage, compared to the amount washed off when Vision was applied alone (82.6%). However, glyphosate wash-off from the $VS_{0.15}$ treated foliage was markedly lower than the amount from the $VE_{0.45}$ treated foliage. Plant bioassays indicated that with a rainfree period of 8 h or more, the growth of most seedlings was stunted within 1 or 2 d. The percentage of foliage browning 20 d after treatment with RFP of 8 to 48 h was 8% to 80% for V, 95% to 100% for $VE_{0.45}$ and 100% for $VS_{0.15}$.

1 INTRODUCTION

Many pesticides have a short life on foliage, which in part is the result of weathering. In general, rainfall activity is considered as the most typical weathering factor affecting the efficacy of herbicides on foliar surfaces, especially those rainfall events which occur soon after spray applications. Several studies (Anderson and Arnold, 1985; Ashton and Crafts, 1973; Behrens and Elakkad, 1981; Bovey and Diaz-Colon, 1969; Doran and Andersen, 1975; Upchurch et al., 1969; Weaver et al., 1946) have shown that rainfall causes reduction of herbicidal activity because herbicides are washed off foliage before they can be absorbed by the plant. Detailed studies on the effect of rainfall on foliar-applied herbicides concluded that for a given plant species, formulation ingredients, rain-free period and rainfall amount (i.e., the total volume of rainfall) had greater influence than rainfall intensity (i.e., volume of rainfall per time period) on the amount of wash-off from the plant (McDowell et al. 1984, 1985, 1987; Pick et al. 1984; Willis et al. 1982, 1986, 1988).

Vision spray deposits on foliage is also expected to be vulnerable to rainfall. Numerous studies have been conducted to develop methodology for glyphosate quantification (Cowell et al., 1986; Deyrup et al., 1985; Lundgren, 1986), environmental fate (Newton et al., 1984; Sprankle et al., 1975b, 1975c; Torstensson and Stark, 1981), mode of action (Duke, 1985; Gougler and Geiger, 1984; Smart et al., 1985) of glyphosate and its major metabolite, AMPA (aminomethyl- phosphonic acid). However, information is sparse on rain- fastness of glyphosate. Bryson (1988) stated that at 0.99 kg glyphosate (AE)/ha, under a simulated rainfall of 1.27 cm in 10 min., glyphosate required a rain-free period $\geq$ 240 min to control johnsongrass

seedlings effectively. Winton et al. (1985) reported that with dosage rates of 1.1, 2.2, and 3.3 kg AE/ha, followed by 1.9 cm of simulated rainfall at 1, 2, 4, and 6 HAT, the control of johnsongrass with 2, 4, and 6 HAT was approximately 80%. The product label from Monsanto states that the effectiveness of glyphosate may be reduced if rainfall occurs within 6 h after treatment, and a repeat treatment may be required if heavy rainfall occurs within 2 h. Thonke et al. (1989) stated that a minimum 24 h rain-free period is required for acceptable control of quackgrass [*Elymus repens* (L.)], after treatment with Roundup (another commercial formulation of glyphosate) without rainfastening adjuvants; whereas, a noticeable amount of rain-protection was evident even at 45 min after treatment when the EUMs contained some rainfastening adjuvants.

It is known that herbicide spray deposit penetration, surface retention, and rain wash-off are dependent on many factors, such as, the type of herbicide, carriers, diluents, volatilization, and environmental conditions as well as the type of adjuvants and weed species. Little information is available on these aspects in relation to trembling aspen (*Populus tremuloides* Michx.), the major competitive weed species in forestry for conifer release in Canada. The objectives of the present study were to determine (i) the rain free period required to control trembling aspen seed- lings using EUMs of Vision with or without the two adjuvants, and (ii) the influence of the adjuvants Ethomeen T/25 and Silwet L-77 on rainfastness of glyphosate in Vision formulation applied on trembling aspen foliar surfaces.

The study was divided into two parts. Part one was conducted in such a way that [^{14}C]glyphosate EUMs (i.e., V, $VE_{0.45}$ and $VS_{0.15}$) were used to determine the rainfastness of Vision with and without adjuvants by examining the samples of treated

leaf rainwash run-off (RW), treated leaf residue (TLR), and the untreated parts (UTP) with the radiotracer technique. Part two was a bioassay experiment with non-radiolabelled EUMs, which was conducted to see the effect of the adjuvants on the rainfastness of glyphosate in Vision as used for controlling trembling aspen seedlings.

2 MATERIALS AND METHODS

2.1 Materials

2.1.1 Treatment of End-Use Mixtures

Two sets of EUMs of V, $VE_{0.45}$ and $VS_{0.15}$ were prepared, one with radiolabelled and the other with non-radiolabelled glyphosate, equivalent to a dosage rate of 1 kg AE in 35 L/ha. The radiolabelled set was used to study rainfastness, and was prepared by adding [^{14}C]glyphosate to the non-labeled EUMs (see Chapter 1, Table 1.1 for details of the specific activity) to obtain a final radioactivity of 27836 Bq per mL, while the non-radiolabelled set was used for the bioassay study and was prepared in the same way as described in Chapter 3.

2.1.2 Trembling Aspen Seedlings

In early spring, seedlings of newly emerged aspen were collected from the field. Before transplanting into plastic pots (15 cm diam.) with commercial potting soil (Pro-Mix, Shaw Milling Ltd., Sault Ste. Marie, ON), the seedlings were cleaned by dipping the entire plant into a tray of water (to remove all field soil), then into a 5% solution of Benlate [E.I. du Pont de Nemours & Co., Wilmington, DE, USA] for

sterilization. They were kept in a greenhouse at a temperature of 20 ± 2°C, 75 ± 5% relative humidity, and a photoperiod of 16 h light and 8 h darkness for at least four weeks of acclimatization. At the time of the experiment, the seedlings were developed to a stage with average heights of 20 ± 4 cm, the number of leaves per seedling was 10 ± 2, and the surface area of the leaves at the mid-crown level was 14 ± 3 cm^2 per leaf. The total number of trembling aspen seedlings used for this study was 170. Ninety eight (98) of them were used for the rainfastness study, the remaining 72 were used for the bioassay study. A split-plot experimental design was used as shown in Tables 4.1 and 4.2.

2.2 Methods

2.2.1 Rainfastness Experiment

The ninety-eight seedlings of this study were divided into seven groups $G_{0.5}$, G_8, G_{24}, G_{36}, G_{48}, G_{72} and G_{96} (i.e., fourteen seedling per group). These seven groups were used to investigate the seven rainfree periods by exposing them to a 5 mm rain at 0.5 HAT for $G_{0.5}$, at 8 HAT for G_8, ... and 96 HAT for G_{96}. Each group was further divided into four subgroups. Three of the four sub-groups, each contain- ing four seedlings, were treated with V, $VE_{0.45}$ and $VS_{0.15}$. The remaining sub-group contained two control seedlings.

Uniform-sized droplets (0.5 μL in volume, or about 1.0 mm in diameter) of radiolabelled glyphosate EUMs were generated using a microapplicator as described in Chapter 2. They were applied to the adaxial surface of four fully developed leaves of each seedling at the rate of 10 droplets per leaf [i.e., total 5 μL of EUM, containing 143 μg of glyphosate (AE) per 14 cm^2 of leaf or equivalnent to a dosage

Table 4.1 Experimental design for the rainfastness of glyphosate

RFP and Sampling Period (h)	No. of seedlings per treatment			
	Background	V	$VE_{0.45}$	$VS_{0.15}$
0.5	2	4	4	4
8	2	4	4	4
24	2	4	4	4
36	2	4	4	4
48	2	4	4	4
72	2	4	4	4
96	2	4	4	4

Table 4.2 Experimental design for the bioassay study of glyphosate

Rain free Period (h)	No. of seedlings per treatment					
	C1[1]	C2	C3	V	$VE_{0.45}$	$VS_{0.15}$
0.5	3	---	---	3	3	3
8	---	---	---	3	3	3
24	---	---	---	3	3	3
36	---	---	---	3	3	3
48	---	---	---	3	3	3
72	---	---	---	3	3	3
96	---	3	3	3	3	3

[1]C1 = Control 1, no treatment and no rainfall were applied; C2 = Control 2, and C3 = Control 3, respectively they were treated with 0.45% Ethomeen and 0.15% Silwet in water at 35L/ha and exposed to 5 mm rainfall at 96 HAT.

rate of 1.0 kg of AE in 35 L/ha area of foliar surface]. Such a treatment provided a total of 20 μL of EUM per seedling, containing a radioactivity level of 557 Bq. As soon as all treatments were completed (i.e., a total of 84 treated seedlings), a V-treated, a $VE_{0.45}$-treated, a $VS_{0.15}$-treated, and an untreated seedling were selected for rainfall exposure. The soil in the pots of these seedlings were covered with a 30 x 30 cm^2 Teflon sheet to prevent loss of radioactive glyphosate to soil as a result of rain-wash. Each of these potted seedlings was then placed on a 14 cm diameter petri dish and the entire assembly was put in a thermostatic tank (40 cm in height and 30 cm in diameter), which was in a simulated rainfall system (SRS) - equipped laboratory spray chamber (Sundaram and Sundaram, 1987). The SRS was calibrated to obtain the medium rainfall of 10 mm/h. The detailed information is listed in Table 4.3. Beside each thermostatic tank, a rain gauge (Taylor, Cat. No. 89068, Forestry Suppliers, Inc., Jackson, Mississippi, 39284-8397, USA.), was used to monitor the collected amount of rainfall. Also two petri dishes with 40 mL Castor oil (Regal Pharmaceutical and Surgical Supply Co. Ltd., Burlington, ON), were placed between the first two and second two tanks to verify the rain drop spectrum, as shown in Figure 4.1 (Sundaram, 1990a). The sliding doors of the spray chamber were immediately closed and rainfall was introduced to the seedlings. When 5 mm rain had been recorded in the rain gauge (30 min were required to reach this stage), the nozzle was closed, and the seedling was carefully removed from the tank.

The washed treated leaves (hereafter called treated leaf residue 'TLR'), the untreated leaves (UTL), the stem and petioles (SP) were removed from the plant with care. The roots (R) were also removed from the pot and the soil was washed away with water. The Teflon sheet, UTL and SP were washed with 2 x 20 mL of

Table 4.3 Details on rainfall simulation[1] using a laboratory spray chamber equipped with a SRS

Nozzle type :	Vejet 8003
Nozzle speed :	33 cm/sec
Flow rate (L/min) :	0.44
Spray pressure :	180 kPa
Intensity of rain :	10 mm/h
Duration of rain :	30 min
Cumulative rain :	5 mm

Rain drop spectra:[2]

D_{max}	(μm)	:	2083
D_{min}	(μm)	:	40
NMD	(μm)	:	524
VMD	(μm)	:	1129

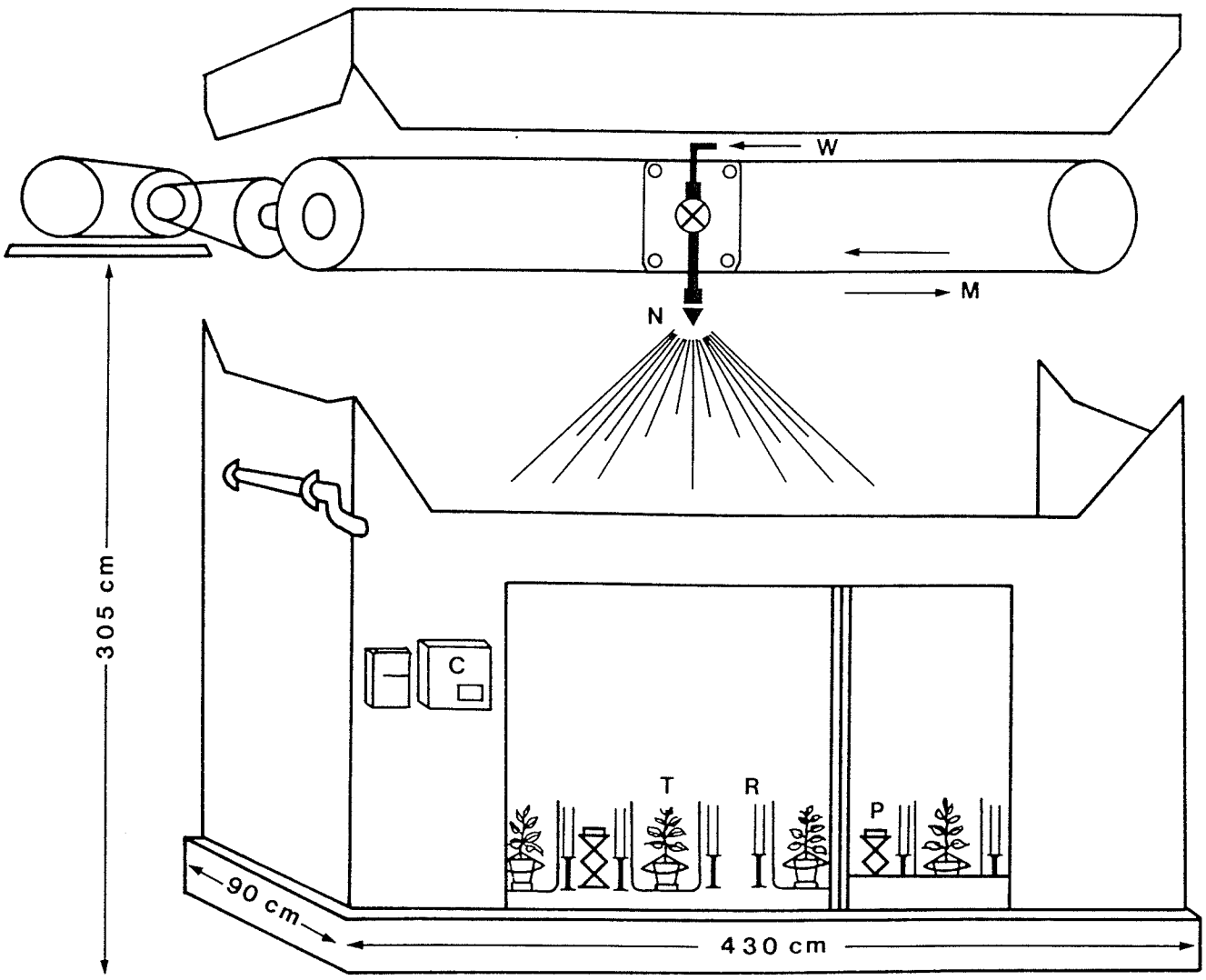
[1]Determination of rain drop spectra was carried out by collecting rain drops in a 40 mL volume of castor oil (using a petri dish as shown in Figure 4.1) for 20 sec in the spray chamber during rainfall. Droplet sizes were assessed by microscopy, during sedimentation of the drops towards the bottom of the petri dish. The data listed here represent mean ± s.d. of 5 sets (with at least 100 drops per set) of values obtained from the 5 replicates.

[2]D_{max} : Maximum diameter of rain drop

D_{min} : Minimum diameter

NMD : Number median diameter (diameter that contains 50% of numbers in drops of smaller size).

VMD : Volume median diameter (diameter that contains 50% of volume in drops of smaller size).



C : Control panel
M : To and fro nozzle movement
N : Veejet nozzle
P : Petri dish of Castor oil
R : Rain gauge
W : Water supply for SRS
T : Thermostatic tank

Figure 4.1 Diagramatic illustration of the exposure of the tremblifng aspen seedlings to 5 mm of similated rainfall activity.

distilled water. This water was then added to the rain water (RW), i.e., the water which washed off glyphosate deposits from treated leaves and fell into the tank. The combined water solution was then transferred into Teflon bottles and stored at -20°C until analysis. The procedure was repeated for another three sets of seedlings (the two untreated plant were presented only with the first and the last set of samples) to complete all four replications. These samples were taken as the samples with rainfree period of 0.5 HAT (a time period of 0.5 h had elapsed between glyphosate droplet treatment and rainfall application), except the sample of RW, which needed to be flash evaporated to a volume of 25 mL prior to analysis. All samples of RW, TLR, UTL, SP, and R were processed and were analyzed as those described in Chapter 2. The entire procedure was then repeated 8, 24, 36, 48, 72 and 96 HAT for the samples of G_8, G_{24}, G_{36}, G_{48}, G_{72} and G_{96}, respectively.

2.2.2 Bioassay Experiment

The bioassay experiment had a similar experimental design as the rainfastness study. However, three seedlings rather than four were used for each treatment, and instead of having two seedlings for background measurement, three control groups each containing three seedlings were used to monitor the entire study (see Table 4.2). The measurements of growth (plant height changes) and percentage of foliar phytotoxicity (plant necrosis) of the seedlings were the parameters used for bioassay. Only the developing part of the stem (the green shoot of the main stem) was used to determine plant growth. The initial height was taken by measuring from the base (using a copper wire ring put around the lowest position of the green part of stem to mark the origin point) to the tip of the green shoot. Phytotoxicity was determined

by evaluating the percentage of necrotic foliage (i.e., the foliar surface that turned brown) of the entire seedling. Using the method of determining the leaves surface area in Chapter 2, the necrotic area and the total area of the leaves surfaces of each seedling were measured and the percentage of phytotoxicity was calculated (Table II of Appendix I). Because EUMs were not radiolabeled, a spinning disc atomizer (Herbi, Micron Corporation, Houston, TX, USA) was used as the spray application system. It was calibrated to deliver a volume rate of 35 L of the EUMs per ha of surface area prior to spray application. The droplet size spectra were characterized at the same site where the sample seedlings received the spray of the EUMs, using the castor oil method similar to that of determining the rain drop size spectra. Details on the application system and spray droplet characteristics are given in Table 4.4. After the seedlings had been exposed to their corresponding treatments, they were subjected to 5 mm of cumulative simulated rainfall at the same time intervals as in the rainfastness study described above. The seedlings were kept in a greenhouse (except when they were receiving the simulated rainfall) under the same environmental conditions that were used for acclimatization.

3 RESULTS AND DISCUSSION

3.1 Rain Free Period (RFP) and Rainfastness Investigations

In general, as the rainfree period (RFP) extended, the amount of [^{14}C]glyphosate washed off from the TL foliar surface was reduced and the translocation of [^{14}C]glyphosate into the UTP of the plant increased. The amount of [^{14}C]glyphosate remaining on the TLR increased to a maximum then decreased

Table 4.4 Details on application system and spray droplet characteristics of the EUMs of the study

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Application system :

Atomizer : Herbi (Micron Corporation)

Spinning disc speed : 1900 ± 20 RPM

Pump system[1] : Harvard Apparatus Compact Infusion Pump Model 975

Flow rate : 0.540 ± 0.008 mL/sec

Time exposure to obtain 35 L/ha[2] : 18 ± 2 sec

Spray Droplet Characteristics :[3]

	V	$VE_{0.45}$	$VS_{0.15}$
D_{max} (μm) :	280	280	330
D_{min} (μm) :	120	120	170
NMD (μm) :	235	236	257
VMD (μm) :	245	247	265

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[1]Harvard Apparatus, South Natick, Mass, 01760

[2]The data represent mean ± s.d. of 18 replications of calibration

[3]By using the method similar to that of determining the rain drops spectra (see footnote of Table 4.3), with minimum of 20 drops per each of the 18 calibrations (i.e., no lesser than 160 drops per EUM), spray droplet spectra were obtained.

D_{max} : Maximum diameter of spray drop

D_{min} : Minimum diameter

NMD and VMD : Number and volume median diameters.

thereafter (this may due to the increase of AI translocation to the UTP) for all three EUMs.

3.1.1 Rain Free Period:

The minimum RFP for each EUM, was determined by evaluating a minimum amount of [^{14}C]glyphosate (*ca.* 20% of the dosage amount applied), which partially had been retained on the TLR and translocated to the UTP (after a time interval of a RFP and after 5 mm rainfall activity) to maintain sufficient effectiveness of the herbicidal activity on the target (Curtis, 1974). This technique suggested that 80% of the EUM deposit is the maximum permissible amount in sample RW. For example, data on the distribution of percent [^{14}C]glyphosate of EUM of V (Table 4.5) showed these amounts of glyphosate occurred between the RFP of 36 and 48 h. For the EUM of $VE_{0.45}$ (Table 4.6), the same event occurred shortly after 24 h, but between 8 and 24 h for the EUM of $VS_{0.15}$ (Table 4.7), thus, suggesting that the minimum RFP for the EUM of V is 36 h, while for EUMs of $VE_{0.45}$ and $VS_{0.15}$, they are 24 h. The multiple comparison S-N-Ks test was used to analyze the values of % [^{14}C]glyphosate in samples of UTP, TLR and RW for all RFPs and was used to determine the significant difference between the seven RFPs within same kind of samples.

In general, there were no significant differences between RFPs of 0.5 and 8 h for all samples of the three EUMs studied (except the TLR of $VE_{0.45}$). This implies that RFP of 8 h is not enough to protect the spray deposit against rain wash for all EUMs. For EUM of V, no significant difference was found among the UTP of RFPs of 8, 24 and 36 h but significant increase was detected at RFP of 48 h and

Table 4.5 Percentage distribution[1] of [^{14}C]-Glyphosate from EUM of V in various parts of trembling aspen seedlings exposed to 5 mm rainfall through various rainfree periods after treatment.

Rain Free Period (hours)	% distribution of applied ^{14}C-Glyphosate (mean ± s.d.)[@]							
	R[1]	SP	UTL	**UTP**	TLR	RW	**TL**	Total
0.5	0.2 ± 0.2a	0 ± 0a	0.03 ± 0.01a	**0.2 ± 0.2a**	3.8 ± 2.9a	96 ± 1d	**99.8 ± 3.5c**	**100**
8	0.03 ± 0.04a	0.6 ± 0.3a	0 ± 0a	**0.6 ± 0.3ab**	7.1 ± 0.2ab	92.3 ± 0.3cd	**99.4 ± 0.5c**	**100**
24	2.3 ± 0.5a	3.1 ± 1.1ab	0.6 ± 1.2ab	**6.0 ± 0.1ab**	10 ± 4ab	84 ± 1c	**94 ± 5c**	**100**
36	2.3 ± 0.4a	4.2 ± 1.8ab	0.6 ± 0.9abc	**7.2 ± 1.1b**	10.2 ± 0.8ab	82.6 ± 0.6c	**92.8 ± 1.4c**	**100**
48	11 ± 3b	9.5 ± 4.6bc	3.0 ± 0.3bc	**23 ± 8c**	27 ± 1c	50 ± 1b	**77 ± 3b**	**100**
72	24 ± 6c	7.7 ± 3.1bc	3.2 ± 2.9c	**35 ± 5d**	13 ± 1b	52 ± 1b	**65 ± 2b**	**100**
96	39 ± 5d	10.5 ± 0.4c	1.6 ± 1.0abc	**51 ± 4e**	13 ± 4b	36.6 ± 0.1a	**49 ± 4a**	**100**

[1] : Comparison was made between rainfree period within samples of the same plant parts, values with the same letters within column are not significantly different from one another (S-N-Ks' test, $\alpha > .05$).

[2] : Values represent the mean ± s.d. of four sets of data obtained from four replications of the study

[3] : Where R : Roots; SP : Stem and Petioles; UTL : Untreated leaves; UTP : Untreated parts (sum of R, SP and UTL); TLR : Treated leaves residue; RW : Rain water; TL : Treated leaves (sum of TLR and RW); Total : Sum of UTP and TL.

Table 4.6 Percentage distribution[1] of [^{14}C]-Glyphosate from EUM of $VE_{0.45}$ in various parts of trembling aspen seedlings exposed to 5 mm rainfall through various rainfree periods after treatment.

Rain Free Period (hours)	% distribution of applied ^{14}C-Glyphosate (mean ± s.d.)[2]							
	R[3]	SP	UTL	**UTP**	TLR	RW	**TL**	**Total**
0.5	0.03 ± 0.07a	0 ± 0a	0.02 ± 0.04a	**0.05 ± 0.11a**	3.9 ± 3.8a	96 ± 2e	**99.9 ± 5.6d**	**100**
8	0 ± 0a	0 ± 0a	0.13 ± 0.25a	**0.13 ± 0.25a**	12 ± 1b	88 ± 4de	**99.9 ± 4.6d**	**100**
24	2.4 ± 0.2a	4 ± 2a	0.4 ± 0.5a	**7 ± 2a**	13 ± 2b	80.3 ± 0.2d	**93 ± 2cd**	**100**
36	7 ± 4b	6 ± 3ab	3.6 ± 0.4b	**17 ± 3b**	13 ± 2b	70 ± 1c	**83 ± 3bc**	**100**
48	11 ± 2bc	13 ± 12b	1.3 ± 0.8ab	**26 ± 13c**	18 ± 9b	57 ± 2ab	**74 ± 11ab**	**100**
72	15 ± 2c	11 ± 5b	1.5 ± 1.4ab	**28 ± 7cd**	12 ± 2b	60 ± 1b	**72 ± 4ab**	**100**
96	22 ± 4d	10 ± 4b	1.5 ± 1.6ab	**34 ± 4d**	18 ± 8b	48 ± 2a	**66 ± 10a**	**100**

[1], [2], [3] : See footnote of Table 4.5 for details.

Table 4.7 Percentage distribution[1] of [^{14}C]-Glyphosate from EUM of $VS_{0.15}$ in various parts of trembling aspen seedlings exposed to 5 mm rainfall through various rainfree periods after treatment.

Rain Free Period (hours)	% distribution of applied ^{14}C-Glyphosate (mean ± s.d.)[2]							
	R[3]	SP	UTL	**UTP**	TLR	RW	**TL**	**Total**
0.5	0.1 ± 0.1a	0 ± 0a	0.1 ± 0.1a	**0.2 ± 0.2a**	2.8 ± 2.3a	97 ± 1d	**99.8 ± 3.5d**	**100**
8	0.02 ± 0.03a	0.7 ± 0.4a	0.05 ± 0.07a	**0.8 ± 0.3a**	5.9 ± 1.0ab	93.3 ± 0.3d	**99.2 ± 1.3d**	**100**
24	2.4 ± 0.6ab	4.6 ± 1.8ab	1.2 ± 1.3ab	**8 ± 2b**	19 ± 3c	72.8 ± 0.9c	**92 ± 4cd**	**100**
36	6 ± 2bc	8 ± 1bc	1.4 ± 2.2ab	**15 ± 2c**	25 ± 5c	60 ± 3b	**85 ± 8bc**	**100**
48	8 ± 2c	12 ± 4cd	6.3 ± 2.1c	**26 ± 3d**	21 ± 5c	53 ± 4ab	**74 ± 8ab**	**100**
72	15 ± 2d	12 ± 2cd	1.0 ± 1.2ab	**28 ± 3d**	10 ± 1b	62.0 ± 0.6b	**72 ± 2a**	**100**
96	14 ± 5d	17 ± 3d	4 ± 5b	**35 ± 5e**	20 ± 8c	45 ± 2a	**65 ± 11a**	**100**

[1], [2], [3] : See footnote of Table 4.5 for details.

thereafter. The same types of result were also found with samples of TLR and RW. For the EUM of $VE_{0.45}$, such a significant increase in % [^{14}C]glyphosate occurred at the RFPs of 36 h for the UTP, 8 h for the TLR and decrease at 36 h for the RW. With the EUM of $VS_{0.15}$, these changes were equal to 24 h for UTP, TLR and RW.

These results indicate that the initial uptake and translocation of glyphosate out of the treated-leaves with the EUM of $VS_{0.15}$ is faster than $VE_{0.45}$, and $VE_{0.45}$ is faster than V. Thus, the analysis verified the minimum RFP for the EUMs mentioned above. The TL data shown in Tables 4.5, 4.6 and 4.7 illustrates the translocation dynamics of the glyphosate, which moved out of the treated-leaves to the UTP of plants when rainfall did not occur. The EUMs of $VE_{0.45}$ (0.47% per h) and $VS_{0.15}$ (0.43% per h) were similar; whereas, V (0.20% per h) showed a slower rate than the former two for the first 36 h, then a faster rate afterward (i.e., from 36 to 96 h, the translocation rate for V was 0.73% per h, whereas for $VE_{0.45}$ and $VS_{0.15}$ were 0.29 and 0.32% per h respectively). Observation during the experiment suggested this phenomenon may be attributed to the initial fast uptake of the AI of $VE_{0.45}$ and $VS_{0.15}$, which results in having local phytotoxic effect occur on the spots where the EUM was deposited. Once the plant tissues of the spots had been damaged, the AI could be trapped and the rate of translocation would slow down as the data showed. Furthermore, the trapped AI could also leach out from the damaged tissues by rainfall activity. This would explain the sudden increase of AI in RW at the RFP of 72 h for all EUMs. The amounts of [^{14}C]glyphosate present in the UTL samples are relatively small (maximum up to 6.34%) compared to the samples of SPs (16.74%) and Rs (38.64%) throughout the entire experiment.

Glyphosate interferes with the biosynthesis of aromatic amino acids, which are

essential for protein synthesis (Boocock and Coggin, 1983; Cerdeira et al., 1985; Geiger et al., 1986), and the accumulation of glyphosate happens in the meristematic areas (Cole, 1985). This implies that UTL is not the prime glyphosate sink or the active site for glyphosate to perform its herbicidal activity, but that it may be R, which constitute much of the meristematic tissues.

3.1.2 Rainfastness:

In an attempt to prove the hypothesis of the study (i.e., addition of either Ethomeen or Silwet will improve the rainfastness of glyphosate in Vision formulation), similar comparisons (S-N-Ks' test) were used to determine parameters such as

(a) **whether there are any significant increases in AI uptake and translocation by plants;**

(b) **whether there was significantly decreased [^{14}C]glyphosate in rain wash-off; and**

(c) **whether the occurrence of minimum RFP corresponds to a significant improvement in (a) and (b).**

The data are given in Table 4.8. The results indicate that at RFPs of 0.5, 8, and 24 h, no significant difference was detected between the three EUMs in samples of RW and in most of the plant parts (except TLR at RFP of 24 h where V and $VS_{0.15}$ are significant). At RFP of 36 h, both $VE_{0.45}$ and $VS_{0.15}$ showed a significant difference compared to V in samples of R, UTP, and RW. Such a significant difference was also detected with $VS_{0.15}$ in samples of SP and TLR as well as with $VE_{0.45}$ in sample of UTL. Only samples of TL showed no significant difference

Table 4.8 Percentage distribution[1] of [^{14}C]-Glyphosate from three EUMs of the study in various parts of trembling aspen seedlings exposed to 5 mm rainfall through various rainfree periods after treatment.

Rain Free Period (hours)	EUMs	% distribution of ^{14}C-Glyphosate[2]							
		R[3]	SP	UTL	**UTP**	TLR	RW	**TL**	**Total**
0.5	V	0.2a	0.0a	0.03a	**0.2a**	3.8a	96a	**99.8a**	**100**
	$VE_{0.45}$	0.03a	0.0a	0.02a	**0.05a**	3.9a	96a	**99.9a**	**100**
	$VS_{0.15}$	0.1a	0.0a	0.1a	**0.2a**	2.8a	97a	**99.8a**	**100**
8	V	0.03a	0.6a	0.0a	**0.6a**	7.1a	92.3a	**99.4a**	**100**
	$VE_{0.45}$	0.0a	0.0a	0.13a	**0.13a**	12.0a	88.0a	**99.9a**	**100**
	$VS_{0.15}$	0.02a	0.7a	0.05a	**0.8a**	5.9a	93.3a	**99.2a**	**100**
24	V	2.3a	3.1a	0.6a	**6.0a**	10a	84.0a	**94.0a**	**100**
	$VE_{0.45}$	2.4a	4.0a	0.4a	**7.0a**	13ab	80.3a	**93.0a**	**100**
	$VS_{0.15}$	2.4a	4.6a	1.2a	**8.0a**	19b	72.8a	**92.0a**	**100**
36	V	2.3a	4.2a	0.6a	**7.2a**	10.2a	82.6b	**92.8a**	**100**
	$VE_{0.45}$	7.0b	6.0a	3.6b	**17.0b**	13.0a	70.0a	**83.0a**	**100**
	$VS_{0.15}$	6.0b	8.0b	1.4ab	**15.0b**	25.0b	60.0a	**85.0a**	**100**
48	V	11a	9.5a	3.0a	**23a**	27b	50a	**77a**	**100**
	$VE_{0.45}$	11a	13.0a	1.3a	**26a**	18a	57a	**74a**	**100**
	$VS_{0.15}$	8a	12.0a	6.3b	**26a**	21a	53a	**74a**	**100**
72	V	24b	7.7a	3.2a	**35b**	13a	52a	**65a**	**100**
	$VE_{0.45}$	15a	11.0a	1.5a	**28a**	12a	60a	**72a**	**100**
	$VS_{0.15}$	15a	12.0a	1.0a	**28a**	10a	62a	**72a**	**100**
96	V	39c	10.5a	1.6a	**51b**	13a	36.6a	**49a**	**100**
	$VE_{0.45}$	22b	10.0a	1.5a	**34a**	18ab	48.0a	**66b**	**100**
	$VS_{0.15}$	14a	17.0b	4.0a	**35a**	20b	45.0a	**65b**	**100**

1 : Comparison was made between the EUMs for samples within each rainfree period, values with the same letters between EUMs are not significantly different from one another (S-N-Ks' test, $\alpha > .05$).

2 : Values represent the mean of four sets of data obtained from four replication of the study

3 : See footnote of Table 4.5 for details.

between the EUMs. At RFP of 48 h, most of the samples (i.e., RW, R, SP, UTP, and TL) showed no significant difference between the three EUMs. However, in TLR, significant differences were found for both $VE_{0.45}$ and $VS_{0.15}$ compared to V; whereas, such a difference was only found between $VS_{0.15}$ and V in sample of UTL. At RFP of 72 h, most of the samples (RW, SP, UTL, TLR, and TL) exhibited no significant difference between the three EUMs, except samples of R and UTP, in which both $VE_{0.45}$ and $VS_{0.15}$ were found significantly different from V. In addition, the percent [^{14}C]glyphosate in RW seems somewhat higher than in RFP of 48h, which implies that some leaching of AI from TL back into RW might occur for all three EUMs. However the amounts of leaching between the EUMs were found to be statistically insignificant. At RFP of 96 h, only RW and UTL showed no significant difference among EUMs; however, samples of R, UTP and TL showed that both $VE_{0.45}$ and $VS_{0.15}$ were significantly different from V; but samples of SP and TLR showed only that $VS_{0.15}$ was significant compared to V. In addition, for samples of TL, no significant difference between the three EUMs were showed at RFP of 0.5, 8,...., and 72 h except the 96 h. This implies that if there is no rain, the performance of the three EUMs of Vision will not be significantly different from one another.

3.2 Bioassay Investigations

3.2.1 Growth Development of The Seedlings:

The data for plant growth is given in Table I. of Appendix I. Because no marked difference was found between the Control groups of C1, C2 and C3 (Table 4.2), their measurements were pooled and represent the 'Control' for the study. In

general, the growth of all treated seedlings was stunted, even in those where the 5 mm rain fall activity occurred immediately after treatment application. Throughout the 20 days of observation, the control seedlings grew more than 6.0 ± 0.5 cm in height. Seedlings with RFP of 0.5 h grew from 0.5 to 2.0 ± 0.5 cm; the growth of those with 8 h of RFPs, or greater, was retarded from 0 to 1.0 ± 0.5 cm. There could be two reasons for the lack of difference in the growth response between the 0.5 h and 8 h RFP. The seed- lings were extremely young and therefore, the use of the dosage rate of 1 kg AE/ha could be so high that similar toxic response was noted at both the 0.5 h and 8 h RFP. Secondly, the age of the seedlings could be such that the 0.5 h RFP was sufficient enought to arrest the growth to a maximum level, so that any further increase in the RFP did not contribute to an increase in the growth response.

Because of the limitation of the growth height measurement, no particular correlations can be drawn between factors of growth height (cm), growth ceased period (i.e., the time when growth height stop increase after treatment applied), and RFP (h); and any interaction of the three factors between EUMs with/without the present of adjuvant.

3.2.2 Phytotoxicity Evaluation of The Seedlings:

The percent phytotoxicity data of the seedlings measured up to 20 d of post-treatment are given in Table II of Appendix I. These data are also put in graphic form and their non-linear regression equations were assessed by a derivative-free non-linear regression program of BMDP. They are illustrated and listed in Fig. 4.2. and Table 4.9. In general, as RFP extends, more spray deposit will

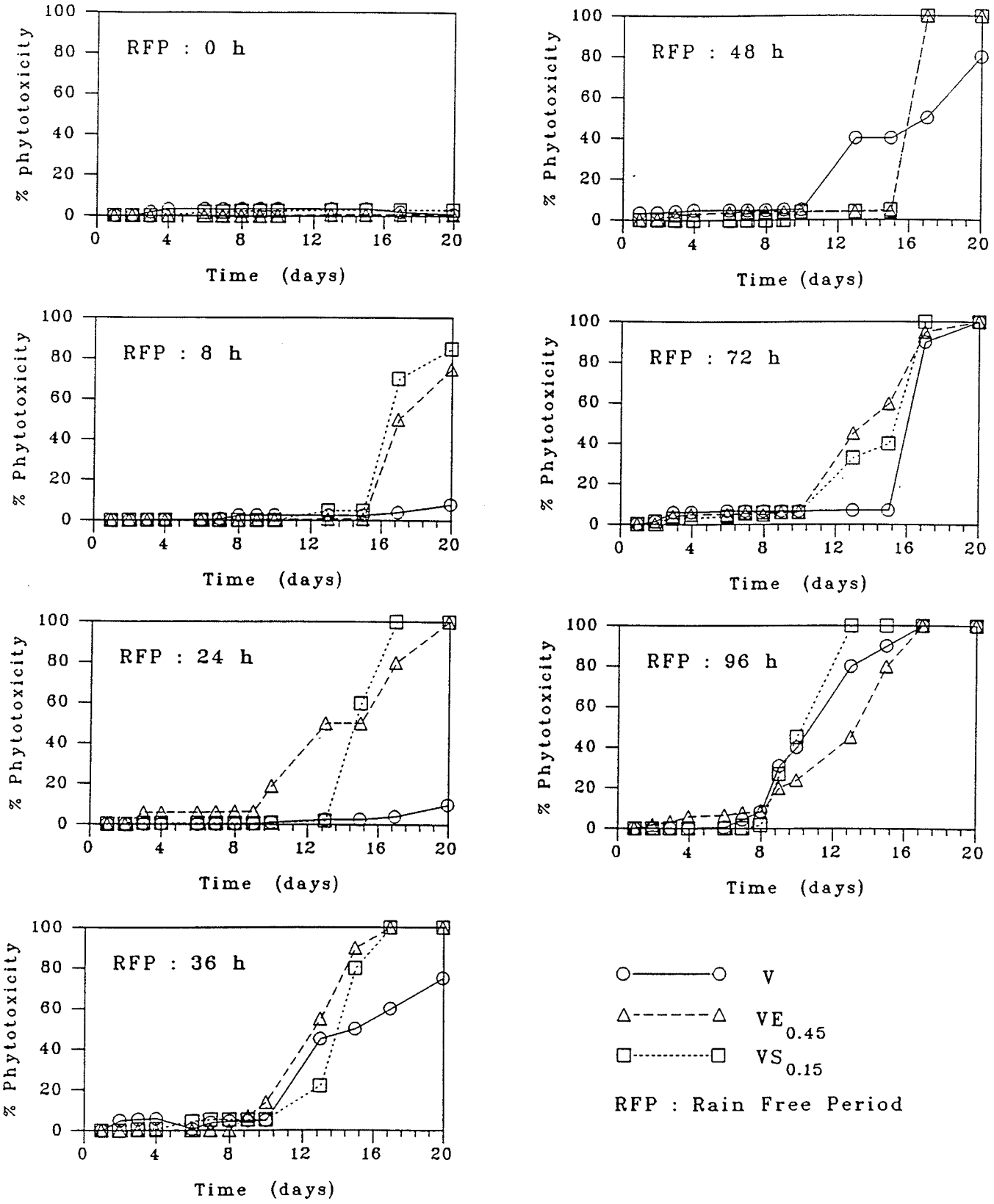

Figure 4.2 Percent phytotoxicity of trembling aspen seedlings which exposure to 5 mm simulated rainfall activity at various RFPs after treatment applications of three EUMs - A bioassay study.

Table 4.9 Non-linear regression equation of the percent phytotoxicity - time curves at various RFPs of the bioassay study.

0.5h	V	:	Y	=	$3.8\ X^{1.03} * \exp[-(X/13.4)^{2.03}]$	S	=	0.8562 a[1]
	$VE_{0.45}$	:	Y	=	$1\ \{1 - \exp[-(X/11.2)^{5.5}]\}$	S	=	0.8983 b
	$VS_{0.15}$	:	Y	=	$3\ \{1 - \exp[-(X/6.5)^{3.2}]\}$	S	=	0.9669 c
8h	V	:	Y	=	$8\ \{1 - \exp[-(X/17.4)^{2.1}]\}$	S	=	0.8217 a
	$VE_{0.45}$	:	Y	=	$75\ \{1 - \exp[-(X/16.9)^{28.5}]\}$	S	=	0.9988 b
	$VS_{0.15}$	:	Y	=	$85\ \{1 - \exp[-(X/14.9)^{28.5}]\}$	S	=	0.9749 b
24h	V	:	Y	=	$10\ \{1 - \exp[-(X/17.8)^{11.0}]\}$	S	=	0.9123 a
	$VE_{0.45}$	:	Y	=	$100\ \{1 - \exp[-(X/15.0)^{4.0}]\}$	S	=	0.9750 b
	$VS_{0.15}$	:	Y	=	$100\ \{1 - \exp[-(X/15.0)^{26.6}]\}$	S	=	0.9990 b
36h	V	:	Y	=	$75\ \{1 - \exp[-(X/14.5)^{4.5}]\}$	S	=	0.9636 a
	$VE_{0.45}$	:	Y	=	$100\ \{1 - \exp[-(X/13.5)^{6.8}]\}$	S	=	0.9990 b
	$VS_{0.15}$	:	Y	=	$100\ \{1 - \exp[-(X/14.5)^{12.8}]\}$	S	=	0.9930 c
48h	V	:	Y	=	$80\ \{1 - \exp[-(X/16.0)^{4.0}]\}$	S	=	0.9487 a
	$VE_{0.45}$	:	Y	=	$100\ \{1 - \exp[-(X/16.2)^{41.5}]\}$	S	=	0.9931 b
	$VS_{0.15}$	:	Y	=	$100\ \{1 - \exp[-(X/16.2)^{38.5}]\}$	S	=	0.9981 b
72h	V	:	Y	=	$100\ \{1 - \exp[-(X/16.5)^{27.5}]\}$	S	=	0.9746 a
	$VE_{0.45}$	:	Y	=	$100\ \{1 - \exp[-(X/14.7)^{5.6}]\}$	S	=	0.9867 b
	$VS_{0.15}$	:	Y	=	$100\ \{1 - \exp[-(X/15.5)^{7.8}]\}$	S	=	0.9616 b
96h	V	:	Y	=	$100\ \{1 - \exp[-(X/11.8)^{4.7}]\}$	S	=	0.9930 a
	$VE_{0.45}$	:	Y	=	$100\ \{1 - \exp[-(X/13.7)^{4.4}]\}$	S	=	0.9845 b
	$VS_{0.15}$	:	Y	=	$100\ \{1 - \exp[-(X/10.5)^{9.3}]\}$	S	=	0.9970 c

[1] : Curves within the same RFP with the same letters are not significantly different from one another (analyzed by BMDP derivative-free non-linear regression).

remain on target for translocation to the active site to perform its herbicidal activity. Therefore the response to the herbicidal effect by the seedlings would have occurred sooner and the rate of such a response would have become higher. Also, the phytotoxic effect of the seedlings at 20 d after treatment will determine the strength of spray deposit which remains after 5 mm rain washing. The results indicate that with 0.5 h RFP, although the differences in phytotoxicity curves between EUMs of V, $VE_{0.45}$ and $VS_{0.15}$ are significant from one another, none of the EUMs would cause the seedlings to have foliar damage > 3.7 %. These results suggest nearly all of the spray deposit had been washed off.

With 8 h RFP, the curves were significantly different between V and $VE_{0.45}$ as well as V and $VS_{0.15}$ but not between $VE_{0.45}$ and $VS_{0.15}$. However, the data showed no significant difference (S-N-Ks test, $\alpha = 0.05$) among any of the EUMS until 17 d after treatment. The phytotoxicity on seedlings that received EUMs of $VE_{0.45}$ and $VS_{0.15}$ were 50 and 70%; and these were significantly higher than V, on which the foliar phytotoxic effects were only 4%. Such differences appeared to be more visible at the 20 d measurement where the effect on V, $VE_{0.45}$ and $VS_{0.15}$ were 8, 75 and 85% respectively.

With 24 h RFP, similar trends are exhibited, in which curve V was significantly different from those of $VE_{0.45}$ and $VS_{0.15}$, but no significant difference was found between curves $VE_{0.45}$ and $VS_{0.15}$. In fact, the differences between EUMs with and without adjuvant were more pronounced when compared to the time when seedlings began to respond to the herbicidal activity. Such a response occurred substantially sooner for $VE_{0.45}$ (at 10 d, 19%) and slightly sooner for $VS_{0.15}$ (at 14 d, 30%) with the intensity of the phytotoxic effect increased dramatically when compared to V

(response at 20 d equal to 10%).

With an RFP of 36 h, the phytotoxicity curves showed that seedlings of all EUMs had responded to their herbicidal effect at 10 d after treatment application, but hereafter the rate of such a response (i.e., the power of the exponen- tial equation in Table 4.9) for $VS_{0.15}$ was significantly greater than for $VE_{0.45}$, and $VE_{0.45}$ was significantly greater than V. This implies that significantly more spray deposit was retained on target by $VS_{0.15}$ than $VE_{0.45}$; and V was retained significantly less than the other two when 5 mm rainfall occurred 36 HAT.

With an RFP of 48 h, the behavior of the phytotoxicity curve of V is similar to that for the 36 h. This indicates that the retention of AI between the two RFPs for V has no marked differences from one another. In fact, significant difference was found between curves of V compared to $VE_{0.45}$ and $VS_{0.15}$. On the other hand, curves of $VE_{0.45}$ and $VS_{0.15}$ are almost identical such that their initial response to AI were very low until 15 d after treatment. This prolonging of response implies a low level of herbicidal activity and suggests that some AI leached out during rainfall activity. However, even with the leach out occurring, the amount of AI remaining on the seedlings after rainfall occur 48 HAT is still high enough to fully control (i.e., 100% phytotoxic effect) the trembling aspen.

With an RFP of 72 h, the curve of V exhibited some AE leach out similar to the $VE_{0.45}$ and $VS_{0.15}$ curves in 48 h RFP; whereas, after 72 h of RFP, the curves of $VE_{0.45}$ and $VS_{0.15}$ showed strength of recovery in herbicidal activity. This result may suggest that AI uptake from V is slower than from the other two EUMs, therefore, requiring longer times to reach the toxic level required for tissue damage to occur. Although statistical analysis (BMDP) showed the curve of V to be significantly

different from those for $VE_{0.45}$ and $VS_{0.15}$, all EUMs showed total control of trembling aspen seedlings at 72 h of RFP.

The result of 96 h RFP showed that, in spite of the fact that significant difference among the curves of the three EUMs occurred, all EUMs exhibit their herbicidal effect on the seedlings substantially sooner than before (i.e., response begin 8 d after treatment, with total control occurring at 12 d for $VS_{0.15}$ and 17 d for both V and $VE_{0.45}$). This implies that, with/without the added adjuvant, 50% of the dosage amount (since approx. 50% of the spray deposit remain after 5 mm rainfall occur at 96 HAT), 0.5 kg AE/ha is effective enough to have a complete control of the one year old trembling aspen when 96 h of RFP is provided.

4 CONCLUSION

In summary, the results of this study indicate that as the RFP was increased, the amount of [^{14}C]glyphosate wash-off from the foliar surface was reduced and the amount that moved into UTP increased, while the amount on TLR increased to a maximum then decreased. If rainfall occurred before 24 HAT or after 48 HAT, differences among the EUMs were minimal. At 36 HAT, both $VE_{0.45}$ (69.69%) and $VS_{0.15}$ (59.7%) significantly reduced the amount of glyphosate washed off from the treated foliage by 13% and 23% respectively, compared to the amount washed off with Vision alone (82.6%). Furthermore, a significant increase in uptake and translocation of the AI in sample of UTP (Table 4.8, the percent distribution of [^{14}C]glyphosate of V, $VE_{0.45}$ and $VS_{0.15}$ was 7.17%, 17.00% and 15.32% respectively)

was also found at 36 HAT. The explanation for the improved rainfastness of the $VS_{0.15}$ EUM may be summed up as follows: (a) the adjuvant acts as a humectant and prevents the spray deposit from completely drying out, which is essential for the processes of glyphosate uptake and translocation; and (b) the adjuvant acts like a spreading agent that regulates the spreading characteristics of the EUM and thus increases the interfacial area of spray deposits and foliar surface for enhanced AE uptake and translocation. A similar trend in response to the spray deposits remaining on the foliar surface of trembling aspen after 5 mm of rainfall activity, was also demonstrated during measurement of percent foliage phytotoxicity in the bioassay study. However, based on the results of this study, the necessary RFP for the three EUMs to receive effective protection on trembling aspen foliar surfaces against 5 mm rainfall activity are considered to be 24 h for $VE_{0.45}$ and $VS_{0.15}$ and a minimum of 36 h for V. These results substantiate the theories that Ethomeen T/25 and Silwet L-77 improve the rainfastness of the EUMs of the commercial Vision formulation significantly.

CHAPTER 5.

Influence of Two Adjuvants on Rain-washing Characteristics of Glyphosate Deposits on Trembling Aspen Following A Field Spray Application

ABSTRACT

A field trial was conducted using the same EUMs that were investigated in the laboratory studies. A "Herbi" spinning disc atomizer was used to deliver a dosage rate of 1 kg of glyphosate in 35 L per ha over each trembling aspen tree (approx. 1 m tall). At 36 h after treatment (HAT), half of the sample trees were subjected to a simulated rainfall of 5 mm rain using a Solo backpack sprayer. A transparent plastic enclosure (each approx. 1 m^3) was used to protect each sample tree from exposure to any undesired natural rain-wash activity for the initial 96 h. During the treatment spray application, water-sensitive paper strips and glass plate units were placed on the forest floor to monitor the ground deposits. Samples of foliage were collected at time intervals prior to treatment, 1 HAT, 36 HAT and 1 h after rain to determine the history of foliar spray deposition patterns and the foliage deposit rain-washing off characteristics. Further, two sets of trembling-aspen-wax-coated glass slides, each spiked with a known amount of [^{14}C]glyphosate EUMs, one covered with Al-foil and another without, were used to determine whether or not these two adjuvants had any effect on the photo-stability of the AI (glyphosate) in Vision EUM (e.g., photo-quencher / photo-sensitizer of the AI).

1 INTRODUCTION

In the previous chapter, the results of laboratory studies demonstrated that the three Vision formulation EUMs (e.g., V, $VE_{0.45}$ and $VS_{0.15}$) provided effective control of trembling aspen seedlings with 5 mm of rainfall activity if a minimum RFP of 36 h was provided after the herbicide application. Some studies (Coupland, 1983, 1987) have shown that environmental factors other than rainfall activity (such as light intensity, soil moisture, relative humidity, and temperature, etc.) also have some effect on the performance of herbicide on weed control practices. In view of these findings, it is worthwhile to find out if the adjuvants Ethomeen T/25 and Silwet L-77 in the Vision EUMs would provide rain-protection for glyphosate under normal field conditions. Thus, a replicated single-tree field trial of appli- cation on a one-year-old cutover site in northern Ontario was conducted to examine the effect of field conditions on the capability of Ethomeen and Silwet to be used as the rain-protection agents in Vision for weed control.

An investigation of the effect of the two adjuvants on the photo-stability of the AI (glyphosate) in the EUMs of Vision formulation on target surface was also conducted to explore any evidence that either Ethomeen or Silwet may have a role of being a photo-quencher or a photo-sensitizer of the AI during the time of treatment spray application.

2 MATERIAL AND METHODS

2.1 Materials

2.1.1 Treatment of End-Use Mixtures

Two sets of EUMs of V, $VE_{0.45}$ and $VS_{0.15}$, one with radiolabelled AI and one without, were prepared in the same way as described in Chapter 4. The radiolabelled set was used for the photo-stability of AI determination of this study while the non-radiolabelled set was used for the field trial spray operation.

2.1.2 Experimental Sites

The study was conducted during the summer of 1989 in a one-year-old trembling aspen cutover site in the Snow Township of Ranger Lake area of Algoma District, Ontario. The site, 83°30.6'W and 46°51.4'N, was approximately five km southwest of the junction of HWY 556 with an access logging trail on Spike Lake (Figure 5.1). Trembling aspen (*Populus tremuloides* Michx.), was the most common tree species and many of them had regenerated from underground root suckers. There were also red maple (*Acer rubrum* L.) and pin cherry (*Prunus pensylvanica* L.f.) species. Six treatment sites, each approximately 10 m x 10 m, were selected in an area at least 100 m from the road. The distance between sampling sites was a minimum 10 m. The six sites were numbered S1, S2, S3, S4, S5 and S6 (Figure 5.1). They all received the treatment spray application of Vision formulation EUMs. At 36 HAT, three of the six sites were chosen to receive simulated rainfall, while the other three did not receive the rain, for the sake of comparison.

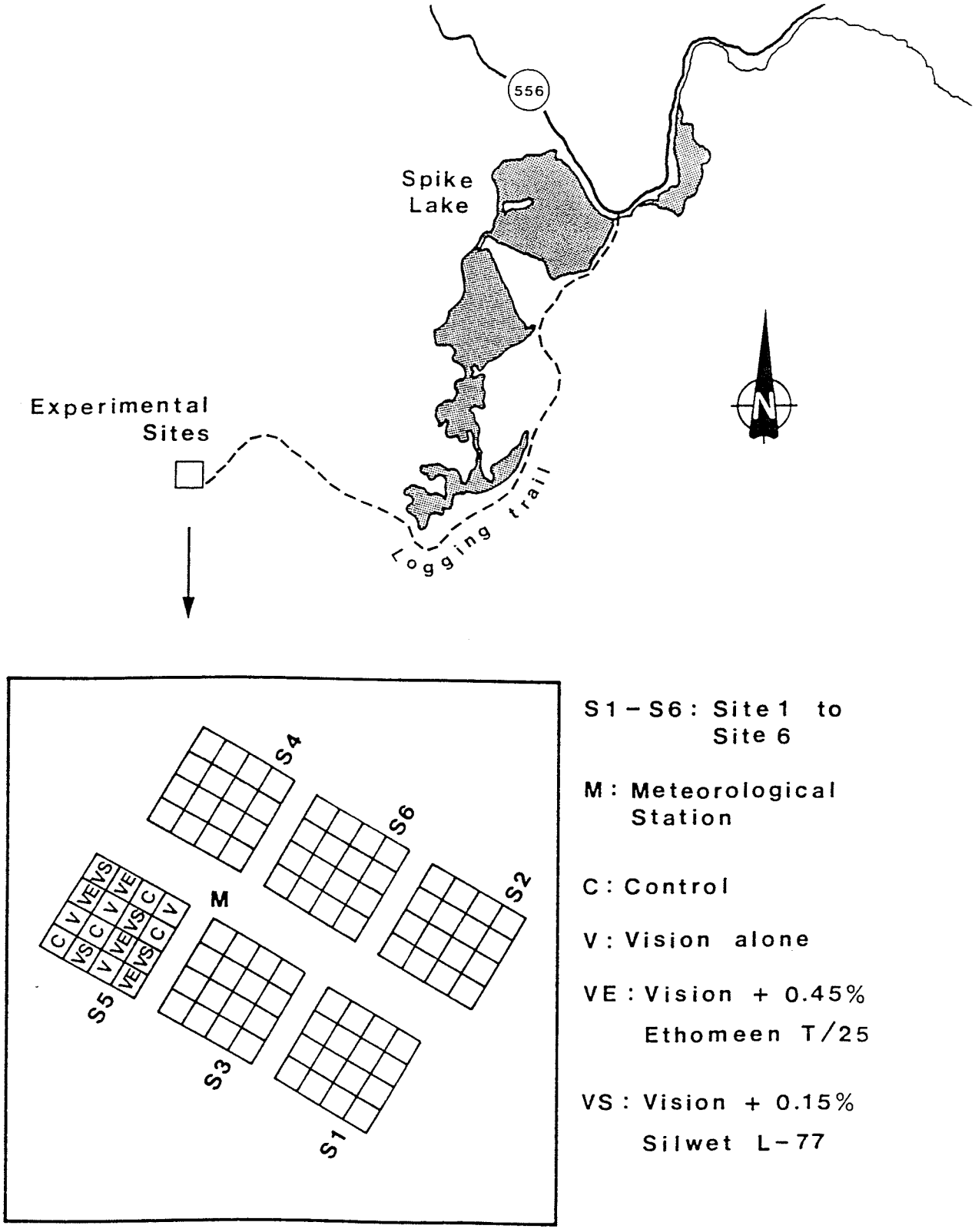

Figure 5.1 The location of the experimental sites and the experimental design fo the rain-washing field trial study.

2.1.3 Photo-stability Study

A total of 48 precleaned microscope slides (each one 75 x 50 mm) were used for this study. Twenty four of these were coated with the epicuticular wax of aspen leaves as described in section 2.1.3 of Chapter 3. Wax-coated glass slides were used instead of live foliage to reduce variability that might have been caused by plant or microbial metabolic activity. These 24 wax-coated glass slides (WCGS) were individually mounted onto the bottom dish of a Pyrex culture petri dish using a piece of double sided tape. They were prepared ahead of time on the night before the field trial spray operation. They were then divided into four groups (i.e., six WCGSs per group), and subsequently three groups received a corresponding treatment (e.g., using a microapplicator, 9 x 0.5 μL drop of the radiolabelled EUM were applied on to the wax surface of each WCGS.) of V, $VE_{0.45}$ or $VS_{0.15}$, while the remaining group, which obtained no EUM treatment, was used for background measurement. Each group was then further subdivided into two sets (i.e., each contained three WCGS). One of these sets was used for sunlight exposure, the other set was covered with Al-foil and maintained in the dark for comparison. The remaining 24 non-wax glass slides were treated in the same manner as WCGSs for comparison to investigate the possibility that the aspen epicuticular wax would be a factor that might alter the photo-stability of glyphosate and cause other undesirable interactions with glyphosate. The experiment was a split-plot design (Table 5.1). After these sampling units were prepared, they were stored in a deep freeze, and were kept in a cooler during transportation to and from the field.

Table 5.1 Experimental design for the photo-stability study

Factors	Block	No. of samples per treatment			
		BG.[1]	V	$VE_{0.45}$	$VS_{0.15}$
Light	Non-wax	3	3	3	3
	Wax	3	3	3	3
Dark	Non-wax	3	3	3	3
	Wax	3	3	3	3

[1]BG. = background

2.1.4 Rain-washing Characteristics Study

2.1.4.1 Selection and Layout of Sample Trees

Within each site, sixteen single trees (or small clusters of trees) of trembling aspen were chosen. They were randomly labeled as T_1 to T_{16}. The trees averaged 1.00 ± 0.05 m in height, with 0.5 ± 0.05 m diameter of mid-crown foliage, and had abundant foliage. The average foliar surface area per leaf was 41 ± 18 cm^2. A Latin Square design was used, in which the treatments (i.e., control, V, $VE_{0.45}$ and $VS_{0.15}$) were arranged in blocks in two different ways, namely, by rows and columns. Each treatment occurred once and only once in each row and column; each row, like each column, was a complete block. The layout of the sample trees, their corresponding treatments and the location of the mechanical weather station (model 1072, Meteorology Research, Inc., Altadena, Calif. 91001) are shown in Figure 5.1. The purpose of using such a design was to remove error that could arise from the

variability due to differences in both rows and columns. Since six sites were selected, a total of ninety-six (6 x 16 = 96) sample trees or clusters were used for the experiment.

2.1.4.2 Sampling Units for Ground Deposit Assessment

The sampling units used to assess ground deposit consisted of two 4.0 x 2.7 cm water-sensitive paper strips (WSPS) (Ciba Geigy, Agrochemicals Division, Basle, Switzerland) and two 75 x 50 mm microscope slides. They were constructed similar to the conventional ground deposit units described by Randall (1980). Two of these sampling units were used per sample tree during the time of spray application (Figure 5.2b). Thus, 6 x 16 x 2 = 192 sets of this ground sampling unit (GSU) were used for the study.

2.1.4.3 The Weather Station, The Spray Atomizer, The Wind Breakers and The Rainfall Applicator

A mechanical weather station was used in the field for monitoring the weather conditions (e.g., rain fall activity, wind speed and direction, temperature, and % relative humidity, Table 5.2) for the entire experiment. Surrounded by three wind breaks (a wind break was constructed by mounting a 1.5 m x 3.0 m plastic sheet onto two wooden posts with the leg of the wooden posts attached to a 0.5 m metal stake which was driven into the forest floor as a supporting mechanism), the previously calibrated (Table 5.2) spinning disc atomizer "Herbi" and "Solo" backpack sprayer (Figures 5.2c & 5.2d) were used to generate a treatment spray volume of 35 L/ha of EUM and 5 mm rain respectively at the height equivalent to the canopy level of the selected sample trees.

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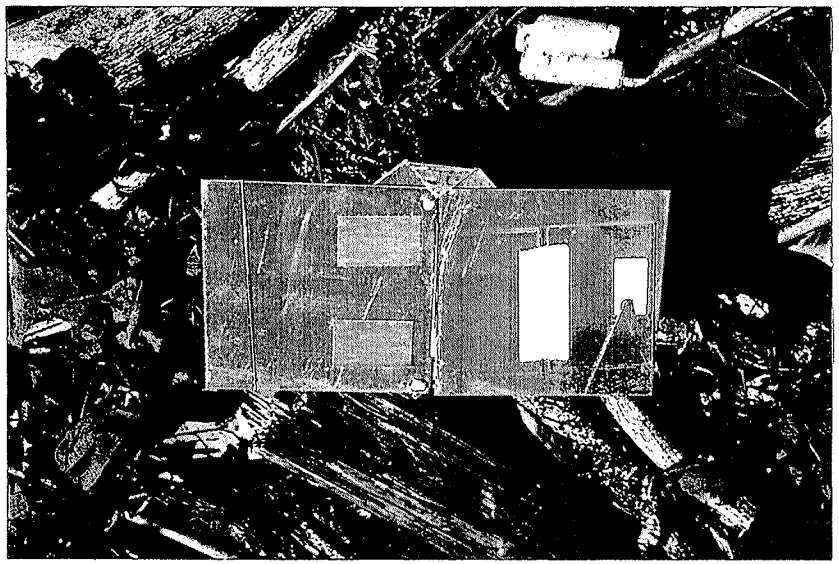

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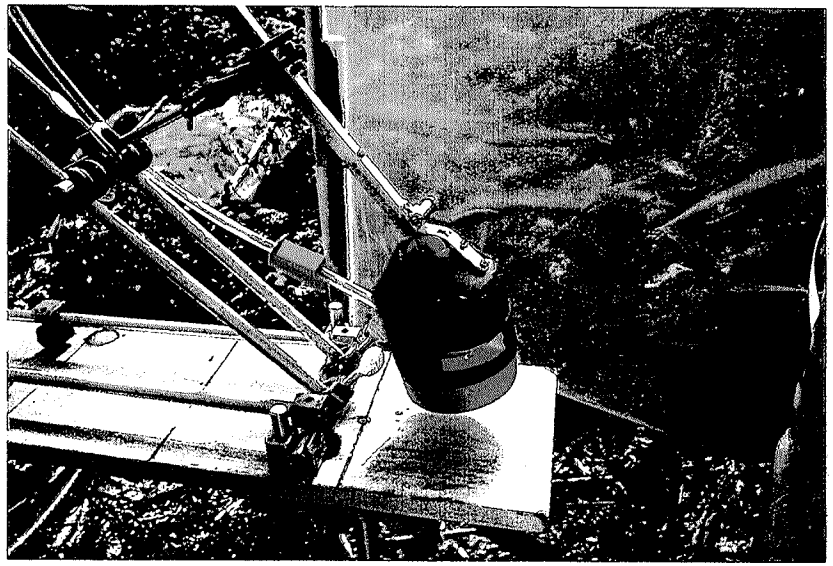

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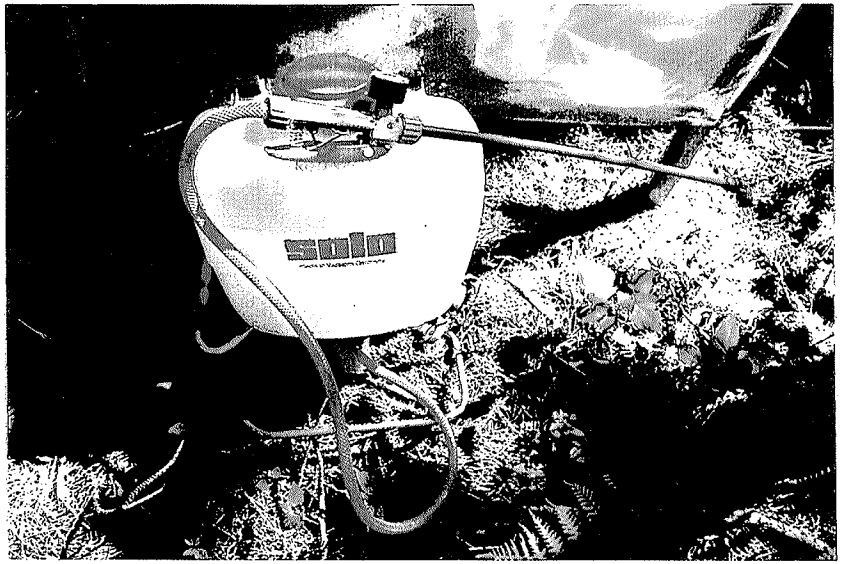

d

Figure 5.2 Field study set ups (a) sample trees with plastic encloser, (b) ground sampling unit, (c) treatment application system and (d) simulated rainfall application system

Table 5.2 Treatment and rainfall application parameters, and meteorological conditions during spray application of the study

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Treatment application system :

Atomizer : Herbi (Micron Corporation)[1]
Date of application : Aug. 11, 1989
Time of application : 0643-1130 h
Spinning disc speed : 1900 ± 20 RPM
Flow rate : 3.45 ± 0.5 mL/sec
Atomizer speed : 15 ± 0.5 cm/5 sec
Spray height : 1.20 ± 0.05 m above ground

Rainfall application system :

Rainfall simulator : "Solo" backpack sprayer[2]
Date of application : Aug. 12, 1989
Time of application : 1900-2030 h
Pump pressure : 24.0 ± 0.2 Psi
Spray height : 1.5 ± 0.1 m above ground
Duration of rain : 30 ± 0.5 sec

Meteorological conditions :

Temp. (°C) : 12-25
RH (%) : 48-70
Wind speed (km/h) : 0.8-4.0
Natural precipitation : no precipitation was detected[3]

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[1]Micron Corporation, Houston, TX, USA.
[2]Forestry Supplier, Inc., Jackson, MS, USA.
[3]within 96 h after treatment, minimum detection limit is 0.254 mm.

2.2 Methods

2.2.1 Photo-stability Study

On the day of the treatment spray application, the petri dish samples of EUMs treated WCGSs and EUMs treated glass slides, with and without Al-foil cover, were placed in the middle of the sampling sites on an open area of the forest floor. They were left there for 36 h (with at least 20 h of sunlight exposure) until the application of simulated rain fall. These samples were then transferred back to the lab and stored at -20°C in the dark until analysis. The light exposed glass slide samples, the light exposed WCGS samples, the dark covered glass slide samples, and the dark covered WCGS samples were all extracted with 2 x 10 mL of distilled water. The extract of each sample was flash evaporated at 60°C to 1 mL and reduced in volume by evaporation under a stream of dry nitrogen to a final volume of 50 μL. The same method that was used to study glyphosate degradation in Chapter 1 was used to measure the amount of [^{14}C]glyphosate remaining on the samples to determine the photo-stability of glyphosate in the EUMs. The results of these measurements are given in Table 5.3.

Table 5.3 Mean (± s.d.) percent recovery of ^{14}C - glyphosate from samples of EUMs with or without the exposure to sunlight irradiation

Sample descriptions		Treatments			
		B.G.[1]	V	$VE_{0.45}$	$VS_{0.15}$
Light	Non-wax	0.0	95 ± 1	92 ± 4	95 ± 8
	wax	0.0	93 ± 6	93 ± 3	94 ± 5
Dark	Non-wax	0.0	100 ± 6	96 ± 9	100 ± 7
	wax	0.0	98 ± 5	92 ± 4	100 ± 5

[1]B.G. = Background

2.2.2 Rain-washing Characteristics Study

2.2.2.1 Site Preparation

After all the sample trees had been selected and labelled, a 1 x 1 m area of forest floor was cleared and a 1-m^3 enclosure was built around each tree using heavy duty polyethylene fixed to four wooden stakes (Figure 5.2a). Two aluminum stands, on which the ground sampling units were mounted, were placed at the opposite sides of the sample tree and were aligned with the treatment spray direction. These experimental setups are illustrated in Figure 5.3a.

2.2.2.2 Spray Application

Just before the EUMs were applied, the enclosure top was removed and pre-spray samples of untreated foliage (PrSS) were collected into 125 mL capacity wide-mouth jars for background measurement. Soon after the ground sample units (GSU) and the wind breaks were in place, the spray operation was undertaken. Once the spray operation had been completed, the enclosure top was immediately replaced to protect the spray deposits against from any undesired natural rainfall activity. These procedures were repeated for every sample tree (Figure 5.3b).

2.2.2.3 Sampling Procedures after Glyphosate Application

One HAT, the GSU were collected and put in wooden boxes, while the post-spray foliar samples (PoSS) were collected and stored in coolers for transportation back to the lab. In the lab, the WSPSs were removed from the GSU and stored in a desiccator for later assessment of the droplet size spectra. The glass slides were put into slots of microscope slide boxes and were placed in a refrigerator for later analysis. The foliage and the spiked foliar samples were stored at -20°C in

a b

Figure 5.3 (a) Location of the ground sampling units, (b) Treatment application

a freezer. Foliar surface area was measured before being extracted (2 min) with 2 x 50 mL distilled water. These extracts were passed through a double Whatman No.1 filter paper to remove any particulates, and were stored in amber-colored bottles at 2°C until analyzed.

2.2.2.4 Sampling of Foliage at 36 HAT, and Simulated Rainfall Application

At 36 HAT, the enclosure top over the sample trees was once again removed and the pre-rain foliar samples (PrRS) were collected. A previously calibrated "Solo" backpack sprayer (Forestry Supplier, Inc., Jackson, MS, USA) was then used to apply 5 mm simulated rain onto the EUM-treated sample trees of sites of S1, S5, and S6. As before, the enclosure tops were replaced to prevent any undesired natural rainwash until four days after treatment, then the enclosures were permanently removed from all sample trees.

2.2.2.5 Sampling Procedure after Exposure to Rainfall

One hour after rain (1 HAR), a foliage sample was collected from each sample tree that had been rained on. These samples were called the post-rain samples (PoRS). The samples of PrRS and PoRS were handled and processed in the same manner as those for PrSS and PoSS samples, mentioned above in section 2.2.2.3. In addition, four petri dishes were placed underneath the four quarters of the sample trees and an attempt was made to collect the foliar runoff. We later discarded this idea because of the great variability in the collecting volume between dishes.

2.2.2.6 Analysis of Ground Samples and Foliar Samples

2.2.2.6a The Water Sensitive Paper Strip (WSPS)

The spray deposits on the WSPSs were analyzed much as described by Sundaram et al. (1985a, 1985b) and Buisman et al. (1989), except that 1 cm^2 in the center of every WSPSs was used for the assessment. For each EUM, the data obtained from the two WSPSs from each treatment were pooled and grouped according to droplet diameter classes to evaluate the number median diameter (NMD), volume median diameter (VMD), D_{max}, D_{min}, droplet density (droplets/cm^2). The mean and standard deviations (s.d.) were calculated using the data obtained for site replicate measurements to assess the overall value for each EUM. The droplet data are given in Table 5.4 and the overall number and volume distribution percentages are presented as histograms in Figure 5.4.

2.2.2.6b The Spray Deposit on Microscope Glass Slides (GS)

The two glass slides corresponding to each single tree spray operation were eluted with 15 mL of distilled water into a graduated centrifuge tube. For each of the V, $VE_{0.45}$, and $VS_{0.15}$ EUMs, samples treated with the same EUM within each experimental site were pooled in a 250 mL round bottom flask (i.e., one pooled sample per each EUM per site). The amount of spray deposit (L/ha) was measured by using a spectrofluorometric technique (as in Appendix II). The mean and s.d. (Table 5.4) were calculated using the data obtained for the six sites to determine the overall spray deposit for each EUM.

2.2.2.6c The Spray Deposit on Foliar Samples

Similar to the GS, the same spectrofluorometric tech- nique (Appendix II) was used to measure the amount of EUM on samples of PrSS, PoSS, PrRS and PoRS. Because the PrSS was collected only for background checking, only the sub-samples that represented each experimental site were examined. The PoRSs were pooled (in the same way as the GS) before being analyzed, because after 5 mm of rain washing, the amount of remaining residue of the spray deposit on the foliar surface would be expected to be very small. Sufficient foliage (at least 15 to 20 leaves) was collected for extraction of the deposits, to ensure that the glyphosate levels would be at least 3 to 4 times greater that the minimum detection level of the analytical technique. The data (mean ± s.d.) on spray deposit on foliar samples are also presented in Table 5.4 for the purposes of comparison.

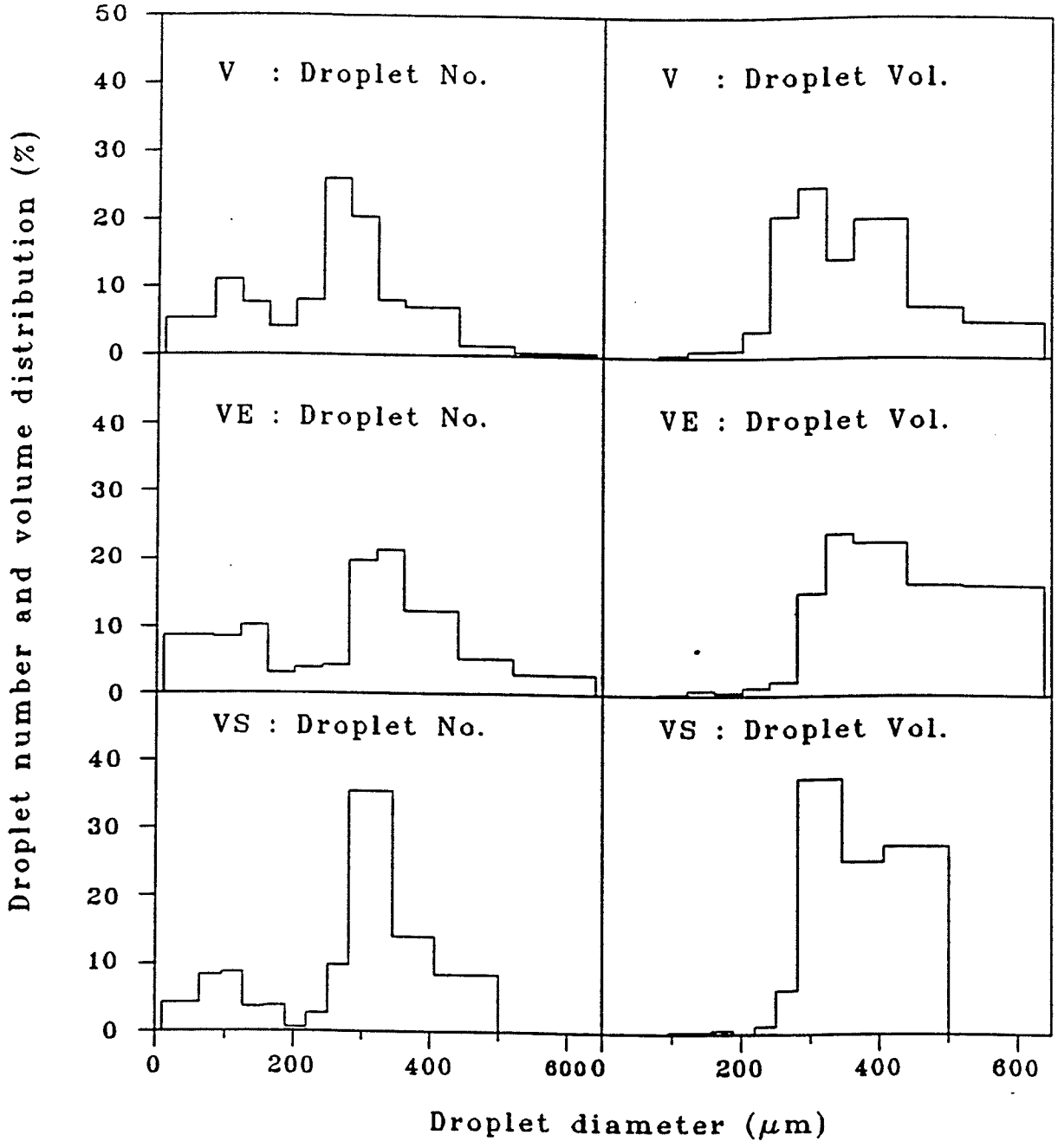
2.2.2.7 Herbicidal Response of Trembling Aspen after Treatment Application and Exposure to Simulated Rainfall Activity

The sample trees were observed to determine percent phytotoxic injury 10 and 30 d after treatment. They were also examined a year later for possible regeneration. These observations were made to determine the effectiveness of using adjuvants in the EUMs for rainwash characterization and for control of trembling aspen under a normal field conditions. The results are given in Table 5.5.

Table 5.4 Spray deposits on water sensitive paper strips, glass slides and foliar surfaces of three EUMs

Parameters	Treatments V	$VE_{0.45}$	$VS_{0.15}$
Water sensitive paper strips :			
NMD[1] (μm)	244	278	258
VMD (μm)	300	352	348
D_{min} (μm)	56	56	60
D_{max} (μm)	640	640	680
Drops/cm^{2*}	28 ± 7	17 ± 9	23 ± 4
Glass slides* :			
Deposit density (L/ha)	33 ± 5	32 ± 4	32 ± 4
Percent deposition	94 ± 16	92 ± 12	92 ± 12
Foliar samples* :			
Residue deposit density (L/ha)			
1 HAT	33 ± 3	33 ± 2	33 ± 2
36 HAT	22 ± 4	17 ± 4	16 ± 2
1 HAR	2.4 ± 0.8	3.2 ± 0.9	3.3 ± 1.5
Washoff	19 ± 1	14 ± 3	13 ± 2
Percent residue deposition			
1 HAT	95 ± 7	93 ± 6	93 ± 7
36 HAT	62 ± 10	49 ± 11	45 ± 7
1 HAR	6.9 ± 2.4	9.2 ± 2.6	9.5 ± 4.3
Washoff	55 ± 2	40 ± 8	36 ± 4

*Data presented as Mean (± s.d.)
[1]NMD, VMD: number median and volume median diameter.



V : Vision alone

VE : Vision + 0.45% of Ethomeen T/25

VS : Vision + 0.15% Silwet L-77

Figure 5.4 Droplet number and volume distribution according to size category for the three EUMs of the study

Table 5.5 Assessment* of the control of trembling aspen at various time periods after treatment applications.

Periods	Control	Treatments V	$VE_{0.45}$	$VS_{0.15}$
No Rain :				
10d	0	12 ± 11	16 ± 15	20 ± 29
30d	0	90 ± 21	90 ± 25	92 ± 20
1 year	Y	N	N	N
5 mm Rain :				
10d	0	3.9 ± 2.8	6.5 ± 3.4	6.8 ± 5.6
30d	0	79 ± 28	84 ± 29	90 ± 15
1 year	Y	N	N	N

*% foliar phytotoxicity was used as the parameter for the control assessment of 10 and 30 d observations, and the mean ± s.d. were calculated from 12 replicate trees. Regeneration of new buds was used as the parameter for the control assessment of 8 months observation, where the abbreviations of "Y" represents the presence of new buds after regeneration, and "N" represents the absence of new buds on the treated sample trees.

3 RESULTS AND DISCUSSION

3.1 Photo-stability Study

A compound need not absorb radiation energy directly in order to undergo photochemical processes. If an acceptor molecule is in contact with an excited donor species, the donor may transfer its excitation energy to the acceptor. The acceptor then undergoes photochemical reaction. This process is known as photosensitization and the donor is called photo-sensitizer, whereas the acceptor is called photo-quencher. However, glyphosate appears to be very photostable when released into the environment. Duke (1988) mentioned that significant photodegradation of glyphosate occurring on leaf surfaces is unlikely, since metabolism studies with plants have often revealed little or no degradation. The extensive investigations by Rueppel et. al. (1977) found only 2% degradation of glyphosate solution in a Crosby reactor over a period of 16 d with 8 h of sunlight per day. From the data in Table 5.3, it is evident that more than 90% of the AI ([^{14}C]glyphosate) was recovered from all samples of the photo-stability study. As no significant differences (S-N-K's test, at α = 0.05) were found between any of these samples (e.g., EUMs of V, $VE_{0.45}$ and $VS_{0.15}$ without concern for light or dark, wax or non-wax, and the combination of the two), this implies that the in-flight spray droplets, the short term spray deposit of the three EUMs, their photo-stability of glyphosate were little changed by the addition of 0.45% of Ethomeen T/25 or 0.15% of Silwet L-77. These findings do not provide any evidence to support the concern that Ethomeen or Silwet could have a role as photo-quenchers or photo-sensitizers of the AI, which might have had an effect on the analysis of glyphosate measurement in the rain-washing study.

3.2 Rain-washing Characteristics Study

3.2.1 Droplet Spectra and Spray Deposits on Water Sensitive Paper Strips (WSPS)

Table 5.4 and Figure 5.4 indicate that the droplet size characteristics of the three EUMs on WSPS were similar to one another despite the D_{max} of $VS_{0.15}$ (680 μm) being larger than that of V (640 μm) and $VE_{0.45}$ (640 μm). The spray droplet density results appear varied among V, $VE_{0.45}$, and $VS_{0.15}$ (their mean values measured 28 ± 0 7 drops/cm^2, 17 ± 9 drops/cm^2, and 23 ± 4 drops/cm^2 respectively). Differences were statistically insignificant ($\alpha = 0.05$) because of relatively large s.d. within each EUM. Such large variations in droplet deposits on Kromekote® cards and water-sensitive papaer strips have been observed previously (Himel et al., 1987; Hurtig et al., 1953; Sundaram et al., 1986). The possible reason for the large variation in deposits is the difference in micrometeorological conditions that existed at the different times of treatment spray application (Joyce et al. 1977; Sundaram et al., 1985a, 1985c, 1985d; Sundaram and Nott, 1985; Randall, 1969).

3.2.2 Spray Deposits on Glass Slides (GS)

The glyphosate present in the spray deposits on glass slides of the three EUMs was quantified by a spectrofluorometric technique (Appendix II). The amount of glyphosate recovery was comparable to the initial dosage rate, with corresponding deposit densities (Table 5.4) for V, $VE_{0.45}$ and $VS_{0.15}$ of 33, 32 and 32 L/ha respectively. This is equivalent to 94, 92 and 92% of the dosage rate that was initially applied, with the standard deviations (s.d.) ranging from 12 to 16%. These results thus indicate almost complete recovery of the applied spray volume. The reason for this lies in the droplet sizes delivered, and in the location chosen for placing the

ground sampling units. For example, the VMDs of the three EUMs ranged from 300 to 352 μm. This indicates that the spray cloud consisted of large droplets (compared to about 50 μm droplets delivered in the insecticide treatments). Because of the high sedimentation velocities of these large droplets, their deposition efficiencies on the glass slides would be high, thus resulting in an almost complete recovery of the spray mass. Secondly, since the ground sampling units were placed in the open areas between two adjacent seedlings, there was little filtration of the spray droplets by the aspen canopies, thus resulting in an almost total recovery of the spray mass.

3.2.3 Spray Deposits on Foliar Samples

3.2.3.1 Residue Deposit on Foliar Surfaces at 1 HAT

The same technique described above was used to quantify the glyphosate residue in spray deposits remaining at 1 HAT on the foliar surfaces. The data of residue deposit density (L/ha) data for the 1 HAT samples of the three EUMs showed that they were comparable to the initial volume rate. The percent residue deposition ranged from 93 ± 6% to 95 ± 7%, and statistical tests found no significant difference (using S-N-K's test, $\alpha = 0.05$) between them, which suggests that the precision involved in calibrating the parameters of the spray delivery system to provide the same AI deposits on the target area was achieved.

3.2.3.2 Deposit Residue Remaining on Foliage at 36 HAT

For the samples for 36 HAT, the residue deposit densities (Table 5.4) that were analyzed from the foliar surface for V, $VE_{0.45}$ and $VS_{0.15}$ were 22 ± 4, 17 ± 4 and 16 ± 2 L/ha respectively. Compared to the dosage rate, these values are equivalent to 62 ± 10%, 49 ± 11% and 45 ± 7% of the initial volume that was originally applied. Using the same test as for the 1 HAT samples, the statistical treatement of results suggest that the amount of AI which was available for rain washoff from the foliar surface for V is significantly higher than that of $VE_{0.45}$ and $VS_{0.15}$, whereas such a difference between $VE_{0.45}$ and $VS_{0.15}$ was not significant. This suggests the rates of uptake and/or translocation of the AI into the plant system in EUMs of $VE_{0.45}$ and $VS_{0.15}$ were significantly higher than that of V. The reason behind this phenomenon may be attributed to the spreading and drying characteristics of the EUMs which is discussed in Chapter 3 of this thesis.

3.2.3.3 Deposit Residues Remaining on Foliage at 1 HAR

The residue deposit densities recovered from the foliar surfaces at 1 HAR (Table 5.4) were 2.4 ± 0.8, 3.2 ± 0.9 and 3.3 ± 1.5 L/ha (i.e., equivalent to 6.9 ± 2.4%, 9.2 ± 2.6% and 9.5 ± 4.3% of the initial dosage volume) for V, $VE_{0.45}$ and $VS_{0.15}$ respectively. Although the remaining values after rainwash seem slightly greater for $VE_{0.45}$ and $VS_{0.15}$ than for V, they were statistically (S-N-K's test, α = 0.05) insignificant from one another. This indicates that the rainfall activity reached the level at which the differences in the adhesive forces were overcome for keeping the spray deposit remain intact on foliar surfaces between the three EUMs against rain washoff.

3.2.3.4 Rain Washing Characteristics of The EUMs

The differences in residue deposit density between the results of 36 HAT and 1 HAR were calculated to obtain the wash-off deposit density values in the 5 mm rainfall. The values (Table 5.4) were 19 ± 1, 14 ± 3 and 13 ± 2 L/ha or 55 ± 2, 40 ± 8 and 36 ± 4% for V, $VE_{0.45}$ and $VS_{0.15}$ respectively. Statistical analysis (S-N-K's test, $\alpha = 0.05$) of the data showed that the residue deposit of V got washed off from the foliar surfaces significantly more than those of $VE_{0.45}$ and $VS_{0.15}$; whereas no significant difference was detected between the latter two. The EUMs containing 0.45% Ethomeen T/25 and 0.15% Silwet L-77 showed significantly lower (i.e., nearly 15.0% for $VE_{0.45}$ and 19.0% for $VS_{0.15}$) wash-off from the foliar surface than Vision alone.

In general, the residue deposit densities (L/ha) on the glass slides and foliage (1 HAT) were similar, with a recovery of more than 90% of the applied volume on both surfaces. Any differences that were observed were statistically insignificant (S-N-K's test, $\alpha = 0.05$).

3.2.4 Field Efficacy Assessment of Trembling Aspen Control

The data in Table 5.5 show the response of the sample trees to the treatments of the three EUMs with or without exposure to simulated rainfall.

3.2.4.1 The No-Rainwash Group

Ten days after treatment application, the sample trees that were not exposed to the 5 mm rain showed the following herbicidal activity (in %phytotoxicity): $VS_{0.15}$ (20 ± 29 %), which was greater than $VE_{0.45}$ (16 ± 15%), which was greater than V (12 ± 11%). These values, however, are not significantly different from one another. The 30 d of observation indicated that all sample trees reached a similar level of response (90 ± 21, 90 ± 25 and 92 ± 20% for V, $VE_{0.45}$ and $VS_{0.15}$ respectively). To interpret these results, one must realize that the herbicidal activity relates directly to the amount of AI penetrated into the sample tree. Thus, an estimation (since the loss of AI by microbial activity can not be accounted for) for the amount of deposit (since it is AI related) present in the sample trees at 36 HAT can be computed by subtracting the residue deposit density of 36 HAT from the residue deposit density of 1 HAT (Table 5.4). The estimated deposits that were considered to be present in the trees at 36 HAT were computed as 33, 44 and 48% for V, $VE_{0.45}$ and $VS_{0.15}$ respectively. Therefore, an increase in the amount of AI uptake increased the level of response during the 10 d of observation. These data and the data of the herbicidal activity (% phytotoxic response at 10 d with no rain wash) were also found to be highly related, as is shown by the evaluated correlation coefficient 'r' value equal 0.980. Since rainwash did not occur in this group of samples, and the same initial dosage rate of AI was used for the three EUMs, ultimately the AI uptake of V, and $VE_{0.45}$ reached the same maximum effect level as $VS_{0.15}$. Therefore the similar results observed in the three EUMs at 30 d observation are expected (Table 5.5). These results agree with the literature data that increased amount of deposit penetration (or rate of AI uptake) increases herbicidal response (Bishop and Field,

1983; Sprankle et al., 1975c).

3.2.4.2 The Rainwash Group

Exposure to 5mm simulated rain at 36 HAT led to the phytotoxicity values (at 10 d) of 6.8 ± 5.6 % for $VS_{0.15}$, 6.5 ± 3.4 % for $VE_{0.45}$, and 3.9 ± 2.8 % for V. At 30 d, a similar trend was visible: $VS_{0.15}$, 90 ± 15 %; $VE_{0.45}$, 84 ± 29 %; and V, 79 ± 28 %. These values are lower than the corresponding no-rain values (Table 5.5) especially at the 10 d observation. This trend and the extended reduction of herbicidal activity response from 10 to 30 d suggest that a significant amount of the deposit was washed off from the foliar surface by the 5mm rainfall. Within the observation of the same time period, no significant difference was noted (S-N-K's test, $\alpha = 0.05$) between the no-rainwash group and the rainwash group, or between samples of the different EUMs. Furthermore, good correlations were also found between data of the phytotoxic response and the amount of deposit retained in/on the sample trees (by subtracting the washoff values from the 1 HAT, Table 5.4). The computed 'r' values were 0.992 and 0.933 for 10 and 30 d respectively.

4 CONCLUSION

In summary, the photo-stability study indicate that neither Ethomeen T/25 nor Silwet L-77 has any effect on the photo-stability of glyphosate in Vision EUM. The results of the rain-washing characteristics study are clearly verified by the measurement of residue deposits (i.e., before and after rain) on foliar surfaces and supported the findings of the lab study (Chapter 4), particularly in comparison of the data of the amount of spray deposit being taken up and translocated by the trembling aspen and being washed off by 5mm rain at 36 HAT. Although the washoff values of the field study (36 to 55%, Table 5.4) appears markedly lower than the RW values of the lab study (60 to 83%, Chapter 4, Table 4.8), this appears to be due to the field trembling aspen seedlings being more mature and larger, and therefore capable of speeding up the uptake and translocation processes and reducing the amount of the spray deposit available for rainwash activity. This phenomenon can also be illustrated by comparing the values of spray deposit retained on target after rainwash of the field study (from 40 to 57%) with the corresponding values of the sum of UTP and TLR at 36 HAT (from 17 to 40%, Chapter 4. Table 4.8) of the lab study. Likewise, statistical analysis (S-N-K's test, $\alpha = 0.05$) was used to test the data between the three EUMs and the results substantiated the concept of using the additional 0.45% of Ethomeen T/25 and 0.15 % Silwet L-77 in the EUM of Vision to improve the uptake and translocation processes and reduced the amount of spray deposit being washed off from foliar surface in both field and lab studies. Furthermore, the results of the treatment were identical for the two groups (i.e., rainwash or no rainwash) of sample trees one year later (Table 5.5), which indicated

that after 5 mm rain washing, the amount of spray deposit retained (in and on trembling aspen seedling) is still high enough to have a good control of the target species under field conditions.

The findings of the field study indicated that the dosage rate of 1 kg of glyphosate/ha used is so high that any reduction in foliar deposits observed during the 5 mm rainfall, failed to inhibit the herbicidal activity response over a long term basis. This is because all the three EUMs provided adequate herbicidal activity (measured in percent phytotoxicity) after 30 d observation. This finding was further confirmed by the lack of the regeneration capability of the seedlings in the spring, the following year. Further investigations are required using lower dosage rates (i.e., 0.5 kg AI/ha), to examine whether the two adjuvants would provide adequate rain-protection at the lower glyphosate levels of field application. Secondly, the young age of the seedlings could also have contributed to high susceptibility of the seedlings to the dosage rate used, and consequently, any loss of deposits by rainfall failed to reduce the herbicidal activity response in the seedlings.

GENERAL DISCUSSION AND CONCLUSION

Over the years, many pesticide researchers have realized that a sound understanding of the physical and chemical principles upon which pesticide formulation science is based are just as important as the development of new pest control agents. Formulation technology involves different approaches to agricultural and forestry uses because of the differences in application technologies, especially in the use of high volume rates and large droplet sizes in agriculture as opposed to ultra-low-volume rates and small droplet sizes in forestry, and the types of terrain encountered. It is necessary to optimize formulation properties and ingredients so that pesticidal activity can be enhanced and adverse environmental effects can be reduced. For chemical and biorational control agents, low application rates and fine atomization are used, and optimum physicochemical properties of EUMs are of vital importance to maximize droplet target-ability, spreading, adhesion, wetting, penetration and rain protection.

With herbicides, however, higher volume rates and coarser atomization are used, and optimum physicochemical properties are required to minimize droplet reflection and to enhance foliar retention. Also, adjuvants are often necessary to enhance droplet spreading/wetting, adhesion, penetration, translocation, and rainfastness. Before considering the addition of an adjuvant, the compatibility of the EUM with the adjuvant needs to be resolved.

The logic of this thesis is derived from the questions normally encountered in developing a new pesticide EUM for spray application of forest pest management. Chapter 1 and Chapter 2 focused on the compatibility of AI with adjuvants and on

the selection of an optimum concentration of either Ethomeen T/25 or Silwet L-77 in EUMs of Vision formulation. The methods described in these studies were considered the simplest techniques for screening numerous adjuvant candidates and various concentration levels to ensure the provision of a stable and homogeneous mixture for treatment application during the rainfastness study.

Several studies have found that if rainfall occurs shortly after pesticide treatment, a significant amount of deposits can be washed off from the treated foliage (Bovey and Diaz-Colon, 1969; Anderson and Arnold, 1985; Upchurch et al., 1969; Weaver et al., 1946). Some researchers also believe that if a suitable adjuvant is added to accelerate the rate of uptake and translocation into active sites, then the foliar deposits will be less vulnerable to washing-off by rainfall (Sundaram, 1990a, 1990c, 1991; Saunder, 1986; Taylor and Matthews, 1986). Chapter 3 offered a possible answer to the question of what mechanisms of Ethomeen T/25 and Silwet L-77 are responsible for the acceleration of uptake and translocation of AI into the active sites. This is important in understanding the role of adjuvants on the rainfastness of a pesticide formulation.

The laboratory rainfastness study in Chapter 4 provides information on the dynamic movement of glyphosate spray deposits and the phytotoxic effect of glyphosate remaining on trembling aspen seedlings after exposed to 5 mm simulated rain in relation to different RFPs. The AI uptake and translocation data suggest that with a minimum RFP of 36 h, Ethomeen T/25 and Silwet L-77 significantly reduced the amount of glyphosate washed off from the treated foliage, compared to the amount washed off with Vision alone. The bioassay results show that even with an RFP of 8 h, both adjuvants significantly improved the effectiveness of glyphosate

against 5 mm of rain-wash compared to Vision alone. This information demonstrates the rain protection potential of Ethomeen T/25 and Silwet L-77.

The laboratory studies have the advantage of generating accurate quantitative data. However, these data can rarely be accurately applied to real pest control practices. Environmental effects of forest pesticides are imperfectly understood. For example, factors such as rain, dew, UV light, temperature and wind, individually and in combination, can reduce the persistence of AI on target surface (Coupland, 1986, 1987; Phillips, 1968). Chapter 5 describes the field study portion of this thesis. The field study has the benefit of using the natural environment to produce information that will provide a better understanding of the concerns mentioned above and produce more realistic results. The findings of Chapter 5 support and verify the laboratory results that Ethomeen T/25 and Silwet L-77 can be used as rain protection agents in the EUM of Vision formulation.

This thesis introduced a way of looking into a model to resolve a highly complex rain wash problem in pesticide spray application for pest management. Although glyphosate, Ethomeen T/25 and Silwet L-77 were the only AI and adjuvants used in the experiments of this thesis, the methods described here could be applied to the determination of the rainfastness of other adjuvants and pesticide formulations. Furthermore, one must realize that precision and accuracy arise from ideal procedures, and the results that are presented in this thesis are limited by its experimental design. Variability in the areas of, for examples, i) types of formulations (aqueous/non-aqueous based), ii) delivery of spray deposits on target by using different spray delivery systems (e.g., aerial spray *vs* ground application, nozzle types etc.), iii) stage and development of the target species (e.g., canopy

density and maturity of the tree or weed), iv) micro- and macro-meteorological conditions during spray operation, may still exist. Therefore, much more in-depth studies on many other factors (e.g., various pesticides and adjuvants with respect to their chemical nature, different target species including the stages of development and the type of surface which the spray deposits will be associated with, different quantities of rainfall with respect to the dosage rates of the pesticide applied, various environmental parameters that may alter the chemical nature of the components in the EUM or the physiological response of the target organism, and the interaction of these factors, etc.), would be required to generate enough data to sustain and improve the model for predicting the use of an adjuvant as a rainfastening agent.

Finally, the results of this thesis (section 3.2.3.4 of Chapter 5) indicate that Ethomeen T/25 and Silwet L-77 respectively reduce 15.0% and 19.0% of the amount of the spray deposits that would be washed off from the foliar surface compared to Vision alone. And, if 1 kg (A.E.)/35L per ha was the dosage rate for all glyphosate used in Canada in 1988, for 176,536 ha (Campbell, 1990), 26,480 kg (A.E.) or 33,542 kg (A.E.) of glyphosate would have been protected from being washed off by using 0.45% of Ethomeen or 0.15% of Silwet if 5mm rainfall occurred 36 hours after treatment. Thus, using that small amount of adjuvant to protect a significant amount of spray deposit from being lost is justified for environmental reasons as well as from an economic point of view.

GLOSSARY

A.E. or **AI**	:	acid equivalent or active ingredient
AMPA	:	aminomethylphosphonic acid
Bq	:	becquerel, an unit of radioactivity measurement, it is equivalent to one disintergation per second
cP	:	centiPoise, unit of dynamic viscosity
$\mathbf{D_{max}}$ / $\mathbf{D_{min}}$	:	maximum diameter or minimum diameter
EAR	:	Erio acid red
EUM	:	end-use mixture
FA	:	fluorimetric analysis
GS	:	glass slide
GSU	:	ground sampling unit
HAR / HAT	:	hours after rain or hours after treatment
kPa	:	kiloPascal, unit of pressure
LSC	:	liquid-scintillation counting
MDL	:	minimum detection limit
NMD	:	number median diameter
NVC	:	non-volatile component
OAC	:	optimum adjuvant concentration
OPA	:	o-Phthalaldehyde, a primary amine fluorogenic reagent
PoRS	:	post-rain foliar samples
PoSS	:	post-spray foliar samples
PrRS	:	pre-rain foliar samples
PrSS	:	pre-spray foliar samples

R	:	roots
R(Evap)	:	rate of evaporation
RFP	:	rain-free periods
RSA	:	relative spread area
RW	:	rain water
s.d.	:	standard deviation
SF	:	spread factor
S-N-K's test	:	Student-Newman-Keuls' test
SP	:	stem and petioles
SRS	:	simulated-rainfall system
TAL	:	trembling aspen leaf
TL	:	treated leaves
TLC	:	thin-layer chromatography
TLR	:	treated leaves residue
TLW	:	treated leaves wash
TWV	:	tap water in vial
UTL	:	untreated leaves
UTP	:	untreated parts
V	:	Vision alone (1 kg AE per 35 L)
$VE_{0.45}$	:	V plus 0.45% (v/v) Ethomeen T/25
$VS_{0.15}$	:	V plus 0.15% (v/v) Silwet L-77
VMD	:	volume median diameter
WCGS	:	wax-coated glass slide
WSPS	:	water sensitive paper strip

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APPENDIX I

Table I. The growth height (cm)[1] of trembling aspen seedlings - The bioassay study.

EUMs & RFPs	Days after treament												
	1	2	3	4	6	7	8	9	10	13	15	17	20
Control[2]	0.0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	3.5	4.5	5.5	6.0	6.3
V-0h	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.5	0.5	0.5
VE-0h	0.5	1.0	1.0	1.0	1.5	1.5	1.5	2.0	2.0	2.0	2.0	2.0	2.0
VS-0h	0.5	0.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
V-8h	0.0	0.5	0.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
VE-8h	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.5
VS-8h	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
V-24h	0.0	0.0	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
VE-24h	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
VS-24h	0.0	0.5	0.5	0.5	0.5	0.5	0.5	1.0	1.0	1.0	1.0	0.5	0.5
V-36h	0.0	0.0	0.0	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1.0	1.0
VE-36h	0.5	0.5	0.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
VS-36h	0.0	0.0	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
V-48h	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
VE-48h	0.0	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
VS-48h	0.0	0.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
V-72h	0.0	0.0	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
VE-72h	0.0	0.0	0.0	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
VS-72h	0.0	0.0	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
V-96h	0.0	0.0	0.0	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
VE-96h	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
VS-96h	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

[1] : Values represent the mean of three sets of data obtained from three relpications of the study.
[2] : Values represent the mean of nine sets of data obtained from C1, C2 and C3 of the study.

Table II. Percentage phytotoxicity[1] of trembling aspen seedlings - The bioassay study.

EUMs & RFPs	Days after treament												
	1	2	3	4	6	7	8	9	10	13	15	17	20
Control[2]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
V-0h	0.2	0.3	2.0	3.3	3.5	3.6	3.7	3.7	3.7	3.7	3.7	2.0	1.0
VE-0h	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	1.0	1.0	1.0	1.0
VS-0h	0.1	0.1	0.1	0.1	2.0	2.1	2.5	2.5	2.6	3.0	3.0	3.0	3.0
V-8h	0.0	0.0	0.5	0.5	0.6	1.0	2.5	2.5	2.5	2.6	2.6	4.0	8.0
VE-8h	0.2	0.2	0.2	0.2	0.2	0.4	0.4	0.5	0.5	1.0	1.0	50.0	75.0
VS-8h	0.0	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.3	5.0	5.0	70.0	85.0
V-24h	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.5	1.1	2.5	2.5	4.0	10.0
VE-24h	0.0	0.0	6.0	6.0	6.0	6.3	6.4	6.4	19.0	50.0	50.0	80.0	100.0
VS-24h	0.1	0.2	0.6	0.7	0.7	0.7	0.7	0.7	1.0	2.0	60.0	100.0	100.0
V-36h	0.0	5.0	5.6	6.0	6.1	3.8	4.5	4.8	5.0	45.0	50.0	60.0	75.0
VE-36h	0.0	0.0	0.2	0.3	0.3	0.3	0.4	7.0	14.0	55.0	90.0	100.0	100.0
VS-36h	0.0	0.0	0.7	0.8	4.5	5.5	5.5	5.5	5.5	22.0	80.0	100.0	100.0
V-48h	3.5	3.7	4.3	5.0	5.0	5.0	5.0	5.1	5.1	40.0	40.0	50.0	80.0
VE-48h	0.3	0.5	2.6	3.0	3.8	4.0	4.0	4.0	4.0	4.0	4.0	100.0	100.0
VS-48h	0.2	0.2	0.3	0.3	0.3	0.3	0.4	0.4	4.0	4.0	5.0	100.0	100.0
V-72h	0.3	1.4	6.0	6.1	6.7	6.7	6.7	6.7	6.7	7.0	7.0	90.0	100.0
VE-72h	0.1	0.3	4.0	5.0	5.0	5.5	5.0	6.1	6.2	45.0	60.0	95.0	100.0
VS-72h	0.1	1.7	3.0	3.1	4.0	6.0	6.0	6.0	6.0	33.0	44.0	100.0	100.0
V-96h	0.0	0.1	0.2	0.3	0.7	4.5	8.5	31.0	40.0	80.0	90.0	100.0	100.0
VE-96h	0.0	2.0	3.6	6.0	6.7	7.9	8.2	20.0	24.0	45.0	80.0	100.0	100.0
VS-96h	0.0	0.1	0.2	0.2	0.2	0.4	1.7	27.0	45.0	100.0	100.0	100.0	100.0

[1]: Values represent the mean of three sets of data obtained from three relpications of the study.
[2]: Values represent the mean of nine sets of data obtained from C1, C2 and C3 of the study.

APPENDIX II

Measurement Of Glyphosate Deposits On Ground Sampling Unit And Foliar Surface Using A Spectrofluorometric Technique

INTRODUCTION

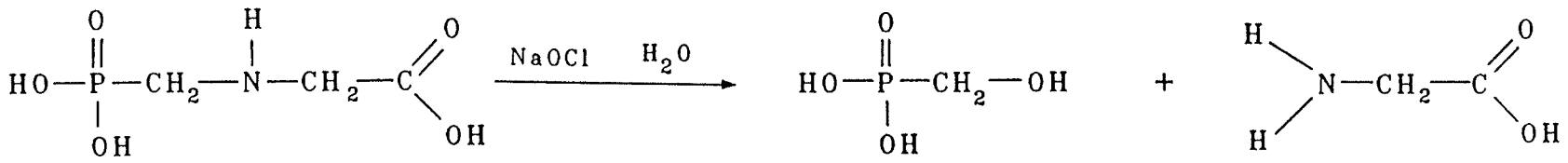
HPLC and GC are the current techniques used for glyphosate analysis (Glass, 1983; Guinivan et al., 1982; Lundgren, 1986; Miles and Moye, 1988). However, these techniques are expensive and time consuming. The objective of this study was to develop a simple and an economical method for measuring glyphosate in sample extracts on glass slides and foliar surface deposits. Based on the principle of oxidizing glyphosate to a primary amine prior to a fluorimetric o-phthalaldehyde (OPA) derivatization (Cowell, 1986; Moye and St. John, 1980), a method of spectrofluorometry for measuring the wash-off of glyphosate spray deposits was developed. The reaction scheme of glyphosate with OPA reagent is illustrated in Figure I.

MATERIAL AND METHODS

Spectrofluorometer

A Turner Fluorometer Model III (G.K. Turner Associates, 2524 Pulgas Avenue. Palo Alto, California, 94303) equipped with the primary filter No. 5860 (excitation energy of 360 nm) and the secondary filter No. 47B (emission energy of 430 nm) was used for detecting the fluorescent derivative of glyphosate.

Step one : Oxidation

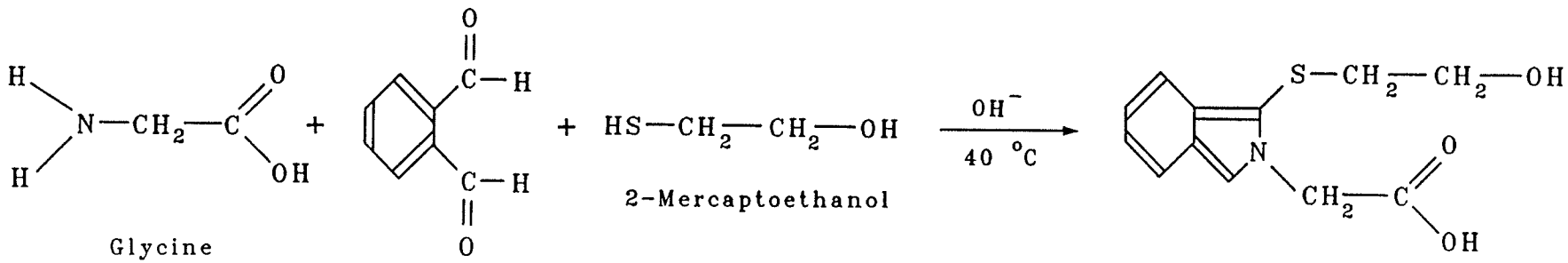


Glyphosate

Hydroxy methyl phosphonic acid

Glycine

Step two : Derivatization



Glycine

OPA

2-Mercaptoethanol

(1-Hydroxy-ethylthio) 2 acetic isoindole

Ex 360 nm / Em 430 nm

Figure I. Reaction scheme of glyphosate with OPA reagent

Chemicals

(a) Glyphosate end-use mixtures: The EUM of V, $VE_{0.45}$ and $VS_{0.15}$ (see section 2.1.1, Chapter 3 for details) were further diluted with deionized water to produce a series of standard (STD) stock solutions with concentrations of 0, 5 10, 20, 30 and 40%.

(b) Buffer solution: A solution of 0.005 M of KH_2PO_4 was prepared in 4% (v/v) of methanol in deionized water with concentrated phosphoric acid to adjust the acidity to pH 2.1.

(c) Oxidation reagent: A solution of 1.36 g of KH_2PO_4, 11.6 g of NaCl, and 0.4 g of NaOH in 500 mL of deionized water was prepared. This solution then mixed with 500 mL of *ca.* 0.4 to 0.6 % sodium hypochlorite (NaOCl) in deionized water.

(d) o-phthalaldehyde (OPA) solution: The commercially available o-phthalaldehyde (OPA) diluent potassium borate pH 10.4 (1.0M) was obtained from Varian (Varian instrument group, Customer Service Center, Sunnyvale, CA, USA, 94086).

Solution Of Foliar Wash-off

The foliar wash-off for each sample was produced by eluting five untreated trembling aspen leaves (each *ca.* 41 cm^2) with 2 x 20 mL of distilled water. A total of 2160 mL of the foliar wash-off was required for 54 samples (see experimental design) of this study.

Experimental Design

Six spiking concentration levels (e.g., 0, 5, 10, 20, 30 and 40%), and triplicate samples for each EUM were used for this investigation.

Procedure

(a) To represent the known amount of deposit washoff, 0.5 mL of the STD stock was transferred into a 250 mL round bottom flask, which contained 40 mL of foliar wash-off.

(b) The solution was then passed through a Whatman FP No. 1 filter paper under reduce pressure to remove any particulates and flash evaporated to dryness at 60°C.

(c) 2.5 mL of the pH 2.1 KH_2PO_4 buffer was added to recover all the residue, and transferred quantitatively to a 10 mL test tube.

(d) The test tube containing the extract was then placed in a 40°C water bath and gently shaken for 10 min.

(e) One mL of the oxidation reagent then 1.5 mL of the OPA solution were added, and the final extract was further shaken at 40°C for another 10 min.

(f) This extract was then centrifuged at 7100 RPM for 10 min, and 3 mL of the top aliquot was used for fluorimetric analysis of glyphosate.

(g) The emission intensity for the samples were measured and recorded (Table I), the calibration standard curves were thus constructed. These are presented in Figure II.

Table I. Emission intensity (Mean ± s.d.) of the glyphosate derivative in EUM STD stock of the study.

Conc. of the EUM STD Stock (% v/v)	Treatments V	$VE_{0.45}$	$VS_{0.15}$
0%	0.0	0.0	0.0
5%	13.7 ± 2.9	13.0 ± 3.6	7.8 ± 2.0
10%	17.5 ± 3.2	25.0 ± 2.6	11.0 ± 1.7
20%	24.5 ± 1.8	33.3 ± 2.0	18.3 ± 3.0
30%	30.5 ± 4.6	46.0 ± 2.5	25.5 ± 1.3
40%	38.0 ± 0.7	62.8 ± 2.6	31.0 ± 2.1

RESULTS AND DISCUSSION

The data shown in Table I illustrate that for all treatment EUMs, an increase in percent concentration of EUM linearly increases the emission intensity value. The linear regression equation and the correlation coefficient (i.e., 'r^2s' of V, $VE_{0.45}$ and $VS_{0.15}$ were 0.998, 0.978 and 0.997 respectively, Figure II) also demonstrated this positive relationship. With the s.d. of 21, 28 and 26% for V, $VE_{0.45}$ and $VS_{0.15}$, the minimum detection limit (MDL) of this method was 145 µg/mL in the cuvette. However, the reproducibility of the method is still considered acceptable because the overall variability between replicate samples is reasonably small compared to the mean value (i.e., the overall standard deviation s.d. is average 12.5% with respect to

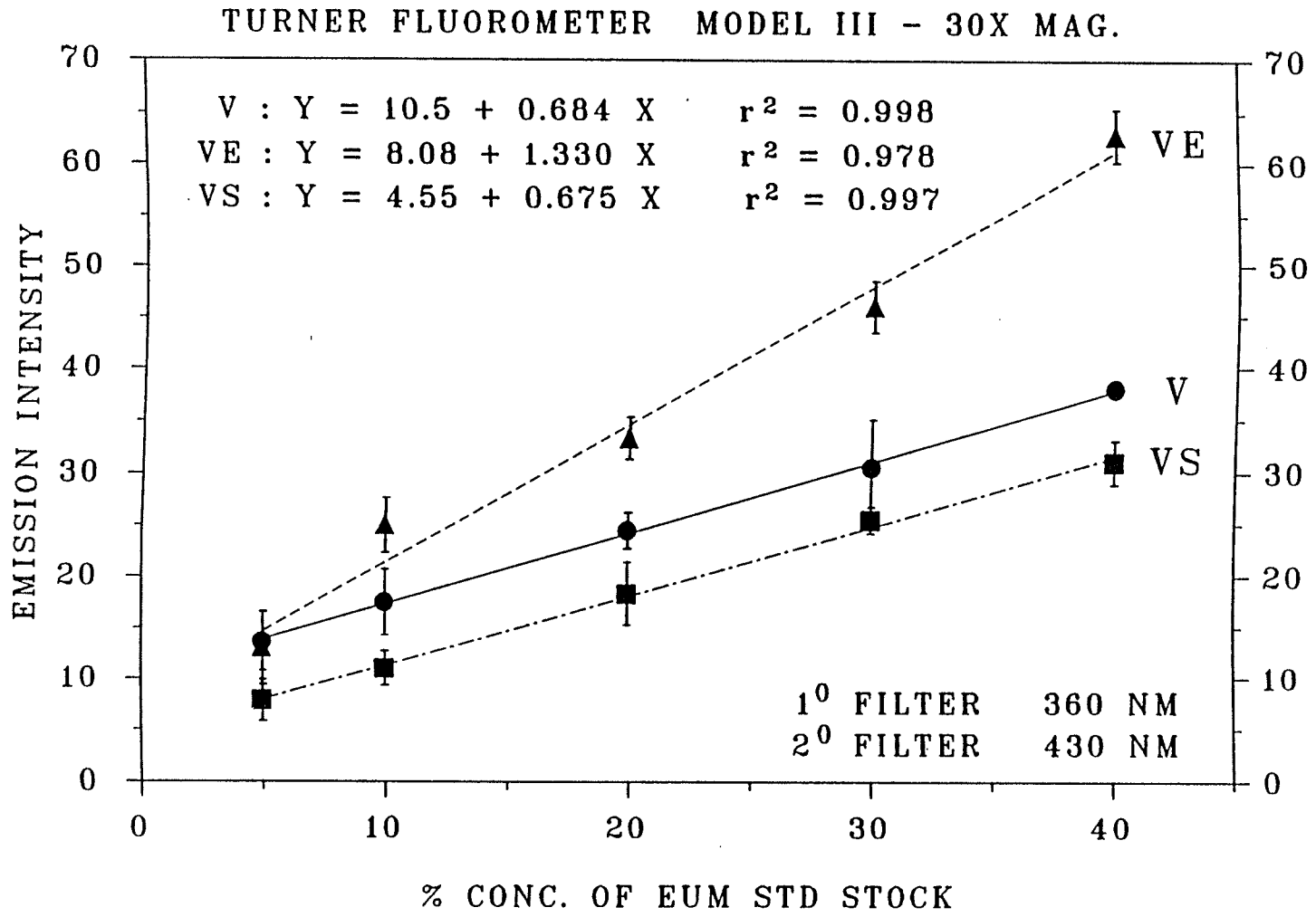


Figure II. The calibration standard curves of EUMs of the study

the mean).

For a volume rate of 1 kg glyphosate A.E. (acid equivalent) per 35 L (i.e., equivalent to 0.0286 g glyphosate A.E. per mL), the amount of A.E. in 0.5 mL of the 5% STD stock is equal to 714.3 μg. As the average foliar surface area per trembling aspen leaf is 41 cm^2 (see section 2.1.4.1 of Chapter 5), the surface area for five leaves is 205 cm^2. If the dosage rate is 1 kg A.E. per ha (or 10 μg A.E. per cm^2), there should be 2050 μg of A.E. deposited on five trembling aspen leaves. This amount of A.E. is approximately three time greater than the amount of A.E. (714.3 μg) in our MDL sample mentioned above.

CONCLUSION

In conclusion, the linear response of the emission intensity of the EUM STD stocks with respect to their concentration levels and the reproducibility of replicate samples indicate that the fluorimetric analysis (FA) method was efficient and consistent at the level of this study. The sensitivity of the FA method (>750 μg) is lower than that of GC and HPLC (1 to 25 ng). However, the cost of materials for analyzing a foliar wash-off sample with the techniques of GC or HPLC was estimated at two hundred dollars per sample while the fluorimetric analysis (FA) method only cost a small fraction (approx. five dollars per sample) of that amount. Furthermore, if the amount of glyphosate spray deposit is relatively high, or if a few more trembling aspen leaves can be collected to increase the sample size if the deposit is low, it is justifiable to choose a much simpler and more economical method like the FA over the more expensive and labor intensive methods of GC or HPLC.

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