# CHAPTER 4: METHODS AND MATERIALS

## CONTACT PHYTOTOXICITY STUDY

### Formulations

Three formulations of triclopyr: Garlon 4 (Triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid), as the butoxyethyl ester (containing 61.6 % a.i., 44.3 % a.e.), Garlon 3A, as the triethylamine salt (TEA) (containing 44.4 % a.i., 31.8% a.e.) and triclopyr TEA (triethylamine salt, containing 32.72 % a.e.) + sequestrant were tested to see if they caused contact phytotoxicity to foliage. These formulations were combined in a factorial experiment, using a range of active ingredient concentrations, with and without the addition of different surfactants. Table 1 lists the surfactants and products used, showing their active ingredient and function. Specific formulations and concentrations used are shown in Table 2. All concentrations are at Garlon 3A acid equivalent.

**Table 1:** Surfactants and products used showing their active ingredient and function.

| Chemical | Active Ingredient | Function |
|---|---|---|
| Silwet 408 | trisiloxane ethoxylate | surfactant |
| Silwet L-77 | trisiloxane ethoxylate | surfactant |
| Polyglycol 26-2 | alkylphenolic glycol ether | wetter/emulsifier in G3A |
| Rhodasurf DA-630 | alcohol ethoxylate | wetter/surfactant |
| Surfadone LP-100 | n-octyl pyrrolidone | wetter/surfactant |
| Garlon 3A | triclopyr amine | herbicide |
| Garlon 4 | triclopyr butoxyethylester | herbicide |
| Triclopyr TEA + seq. | triclopyr amine | herbicide |

### Plant material

*Acer rubrum* (red maple), *Quercus rubra* (red oak) and *Liquidambar styraciflua* (sweetgum) seedlings (~1 m tall, 100 plants/species) were planted (9 *l* buckets with 3 + 1 [ground bark / pumice] coarse mix from Granulated Bark supplies, Putaruru, to which 2.5 kg of Magamp / $m^3$ [a continuous slow release fertiliser containing 7% nitrogen, 17% phosphorous, 5% potassium and 12% magnesium; available from Yates, New Zealand] was added) 6 months prior to use, and grown outside at the NZ Forest Research nursery. Plants were used between the months of December and May. Fully expanded leaves from the mid-section of plants were used for all treatments. Two hours prior to the contact phytotoxicity study, plants were transferred to a

controlled environment cabinet (light intensity 450-500 μmol/$m^2$/s; 70% relative humidity; daylength of 11.5 h plus a 1% red light supplement for 0.5 h either side of the main photoperiod to provide dawn and dusk periods; the temperature was changed smoothly (10 °C/h) between the night (15 °C) and day (20°C) temperatures).

**Table 2 :** Concentration (% product) of solutions used for contact phytotoxicity, adhesion and retention studies.

| **Chemical** | **Additives** | **Herbicide & Adjuvant Concentrations** | | |
|---|---|---|---|---|
| Garlon 4 | Nil | 0.72 % | 3.59 % | 7.18 % |
| Garlon 3A | Nil | 1 % | 5 % | 10 % |
| Triclopyr TEA + seq. | Nil | 1 % | 5 % | 10 % |
| Triclopyr TEA | Nil | 1 % | 5 % | 10 % * |
| + Sequestrant | + alkylphenolic glycol ether | 0.01 % | 0.05 % | 0.1 % * |
| + 0.2% Silwet L-77 | + n-octyl pyrrolidone | 0.01 % | 0.05 % | 0.1 % * |
| | + alcohol ethoxylate | 0.01 % | 0.05 % | 0.1 % * |
| Triclopyr TEA | Nil | 1 % | 5 % | 10 % * |
| + Sequestrant | + alkylphenolic glycol ether | 0.01 % | 0.05 % | 0.1 % * |
| + 0.2% Silwet 408 | + n-octyl pyrrolidone | 0.01 % | 0.05 % | 0.1 % * |
| | + alcohol ethoxylate | 0.01 % | 0.05 % | 0.1 % * |
| Silwet L-77 | Nil | 0.2 % * | | |
| Silwet 408 | Nil | 0.2 % * | | |
| alkylphenolic glycol ether | Nil | 0.1 % * | | |
| n-octyl pyrrolidone | Nil | 0.1 % * | | |
| alcohol ethoxylate | Nil | 0.1 % * | | |

* Only used for contact phytotoxicity study

**Contact phytotoxicity**

Treatments (3 replicates) were applied as 10 droplets (each 0.24 and 4 μl, except for adjuvants alone, which were only applied as 4 μl droplets ) per leaf to foliage of all three plant species. Effects were observed over a 2 to 24 h period. Solutions were applied at concentrations equivalent to 0.32, 1.6 and 3.2% a.e. plus other adjuvants. To find out to what extent the additives might contribute to the contact phytotoxicity, Silwet L-77 and Silwet 408 were applied alone at 0.2% (w/v), while n-octyl pyrrolidone, alcohol ethoxylate and alkylphenolic glycol ether were each applied alone at 0.1% (w/v), since these were the highest concentration these additives were used at, and therefore the worst possible case. Phytotoxicity results were scored as nil, mild and severe and given a numerical value (0, 1 and 2) to provide an arbitrary numerical comparison.

Results are the mean of three replicates, where a mean of 0 showed nil contact phytotoxicity, a mean > 0 but ≤ 1 was considered mild, > 1 but < 2 considered high and = 2 considered severe.

**Experimental design and statistical analysis**

Analysis of variance was used to test the significance of six factors: formulation, species, leaf surface, droplet size, concentration and time, together with their interactions. The data was analyzed as a split-plot design because each individual droplet was assessed at five time intervals (2, 4, 6, 8 and 25 hours). Analysis of variance was also carried out in the same manner with each tree species, firstly with both droplet sizes included, then with each droplet size separately, as this was the manner in which results were broken down for discussion, graphs and tables.

## ADHESION AND RETENTION STUDY

**Formulations**

As per contact phytotoxicity study. See Tables 1 and 2.

**Plant material**

As per contact phytotoxicity study. Foliage was gathered directly from plants grown outside.

**Adhesion and retention**

All 3 tree species were studied with the two lower droplet sizes (650 μm, 1000 μm), while only sweetgum was studied using the largest droplet size (2000 μm). All adhesion and retention droplet applications (up to 1000 μm) were made using a piezoelectric droplet generator ( Young, 1986 ). Ten drops per formulation were impacted onto each of five replicate leaves, taken from different, randomly chosen plants, for each leaf angle and leaf surface. Adhesion was determined from visual observation of the droplet's initial impact behavior. If the droplet hit the surface and stuck, then this was defined as adhesion. If a droplet hit, and bounced, regardless of distance, this was defined as no adhesion. In this study, it was arbitrarily decided that retention would be the percentage of solution remaining within a 1 cm radius from where the droplet initially impacted. Where there was not 100% adhesion, it could readily be observed if there was still 100%

retention, or > 95 %. All other values of retention are estimates from observations, hence obviously retention values are not absolute values.
Detached leaves were oriented at 0, 22.5 and 45$^{o}$ from the horizontal on plastic stages. Three droplet sizes were studied (650 μm, 1000 μm and 2000 μm). Drops were permitted to fall a distance of 1485 mm (droplets of 1000 μm or less ). The 2000 μm droplets were permitted to fall a distance of 4.57 m (15 ft) which is the distance required to achieve 95% of the terminal velocity for this size of droplet.

650 μm size drops were chosen as an example of a drop size that would be used for spraying in NZ. 1000 μm size drops are produced by microfoil boom sprayers in America. These two droplet sizes were studied in great detail, while the 2000 μm droplets were studied to a much lesser extent. 2000 μm size drops were chosen because this size of drop is produced by Radiarc sprayers in America for forest site preparation. Smaller droplet sizes (1500 and 1000 μm) are also produced by Radiarc sprayers used for forest site preparation and release sprays.

Some method development was required to produce drops of about 2000 μm in size.
This work was carried out in a growth room to enable droplets to fall 15 feet, allowing them to be near terminal velocity on impacting the target foliage. Dry nitrogen was used to move the solution up the required height and produce droplets at the “nozzle”, which was a piece of capillary tubing. Due to the different surface tensions of the solutions, different sizes of capillary tubing were required to produce droplets close to 2000 μm.

The size of the drops (mono-sized) was determined by a colorimetric method. Blankophor P ( 0.5% w/v; Ciba-Geigy ) was used as the UV fluor, incorporated into the solution to allow quantitation and visualization of drops. A known numbers of drops were collected into a vial, a standard volume of water added and the absorption of the dye determined (276 nm). From a standard curve of volume vs. absorbance (produced using a 50 μ*l* syringe (Hamilton Company, Reno, Nevada) with dispenser, to add 2 μ*l* at a time of the solution to a cuvette for the absorbance to be measured), the size of the drops were calculated.

### Experimental design and statistical analysis

Ten drops were impacted onto each of five replicate leaves, taken from different, randomly chosen plants, for each formulation / concentration / species / leaf angle / leaf surface / dropsize combination. Logistic regression analysis was carried out with the statistical package Genstat 5 on the entire adhesion data set, and on the entire retention data set independently. An analysis of deviance was used to measure the effectiveness and statistical significance of each term in the model. Deviance is a measure of variation used in logistic regression models, and analysis of deviance is equivalent to the analysis of variance of a conventional regression model. Predictions (estimated mean proportions) were calculated from the regression models.

## SPRAY RETENTION UNDER FIELD AND TRACK-SPRAYER CONDITIONS

### Spray Retention under Field Conditions

### Formulations

The spray formulations and droplet size used for this study were decided upon at a meeting with the sponsors of this research (i.e. DowElanco and OSi Specialties), after reviewing the results from all earlier work in this study.

An initial test was carried out prior to the field study, which showed 100 % recovery of tartrazine (1% tartrazine dye was added to the spray formulations in order to quantify by colorimetry the amount of solution retained by the leaves) when 0.1% Silwet L-77 was used, but loss of tartrazine on the abaxial surface, presumably due to infiltration, when 0.2% Silwet L-77 was used. For this reason, 0.1% instead of 0.2% Silwet 408 was used in the field trials for the retention study.

A list of formulation combinations used is given in Table 3.

**Table 3 :** Concentration of solutions used for spray retention study.

| Chemical | Additives | Herbicide & Adjuvant Concentrations (% Product) | |
|---|---|---|---|
| Garlon 4 | Nil | 0.72 % | 3.59 % |
| Triclopyr TEA<br>+ Sequestrant<br>+ 0.1% Silwet 408 | Nil<br>+ alcohol ethoxylate | 1 %<br>0.01 % | 5 %<br>0.05% |

**Field site and plant material**

The field experiment was conducted on a right-of-way site in North Anna, Virginia, USA, in June 1996. This site contained the species to be studied - *Acer rubrum* (red maple), *Quercus rubra* (red oak) and *Liquidambar styraciflua* (sweetgum). The average height of red maple and red oak was 1.0 m and the average height of sweetgum was 1.4 m. Trees were tagged prior to spraying, but not all plots contained at least 5 trees of each species.

**Spray retention**

A Radiarc sprayer (Waldrum Specialties, Inc.) equipped with 0.508 mm nozzles (producing approx. 1000 μm droplets) was used to apply 140 l/ha of spray solution. The Radiarc sprayer was attached to a Kubota B6100 4WD tractor. To produce the desired rate of application, the swath width was measured at an approx. 40 psi setting, the volume of spray liquid dispensed per minute was measured, the speed of the tractor was measured in each gear, and a suitable gear / speed chosen ($2^{nd}$ gear, 75 ft/min). Photo's taken in the field are shown in Figure 4.

Tartrazine at 10 g/l (1%) was added to the spray mixes. Ten fully exposed leaves were detached from each tagged tree and kept together on a copper wire for processing ( it was initially calculated that 10 leaves could be required to attain a dye concentration which would give an absorbance value that would be easily read by colorimetry, however it was found that 5 leaves were sufficient, so each group of 10 leaves was split into two sets of 5 leaves and the results averaged in the final analysis). The adaxial surface of each batch of 5 treated leaves was washed into a beaker using a washbottle. The adaxial leaf surface was washed well past the point at which the wash from the leaf ran clear. The liquid in the beaker was transferred to a measuring cylinder where the volume was made up to 500 mls. The set of 5 leaves were then placed into another

beaker containing 500 mls of water to collect the tartrazine remaining on the abaxial surfaces. All of the samples were analyzed by colorimetry (427 nm), and the volume of spray deposit calculated. Leaf areas of the collected leaves were determined using a Licor Leaf Area Meter so that the volume of spray per unit of leaf area could be calculated.

**Experimental design and statistical analysis**

A randomized complete block design with 3 blocks was used for the field trial. Each of the six formulation treatments appeared once in each block, i.e. there were six plots/block. Each of the 18 plots contained all three tree species (5 trees of each species / plot). Statistical analyses were run separately for spray retention by the adaxial leaf surfaces, abaxial leaf surfaces, total deposition, and abaxial retention as a percentage of total deposition. Square root transformations were carried out on abaxial, adaxial and total deposition data sets, while a log transformation was carried out on the percentage abaxial retention data set.

As there were many missing values, the SAS procedure, proc mixed, was used to analyze the data. Differences of least squares means was carried out to show any significant differences between treatments.

**a.** Field Site

**b.** Spraying plants in the field

**c.** Tractor with radiarc sprayer

**d.** Spray equipment

**e.** Radiarc sprayer

**Figure 4:** Photos taken at the field site in North Anna, Virginia, USA

## Spray Retention using a Track-Sprayer

### Formulations

The formulations used were the same as for the field study. The formulations and concentrations used are shown in Table 4.

**Table 4**: Concentration of solutions used for track sprayer study

| **Chemical** | **Herbicide & Adjuvant Concentrations (% Product)** | | |
|---|---|---|---|
| Garlon 4 | 0.72 % | 3.59 % | 7.2 % |
| Triclopyr TEA+ Seq<br>+ alcohol ethoxylate<br>+ Silwet 408 | 1 %<br>0.01 %<br>0.1% | 5 %<br>0.05%<br>0.1% | 10 %<br>0.1%<br>0.1% |

### Plant material

*Liquidambar styraciflua* (sweetgum) seedlings growing in 3 + 1 (ground bark / pumice) coarse mix to which 2.5 Kg of Magamp / $m^3$ had been added, were grown outdoors in the NZ Forest Research nursery. Plants were used in February (mid-Summer).

### Spray retention

A track-sprayer constructed at the NZ Forest Research Institute (Figure 5), consisting of a purpose built conveyor belt and spray application system, enclosed in a ventilated cabinet, was used for the simulated field studies. Two droplet sizes were used for this trial - ~650 μm (nozzle Turbo teejet 110-05), and ~1000 μm (nozzle Turbo floodjet TF5 ). The application volume was the same as for the field trial ( i.e. 140.3 *l*/ha ). Spray retention analysis was carried out as for the field trial except that the leaf areas of the collected leaves were determined using image analysis equipment.

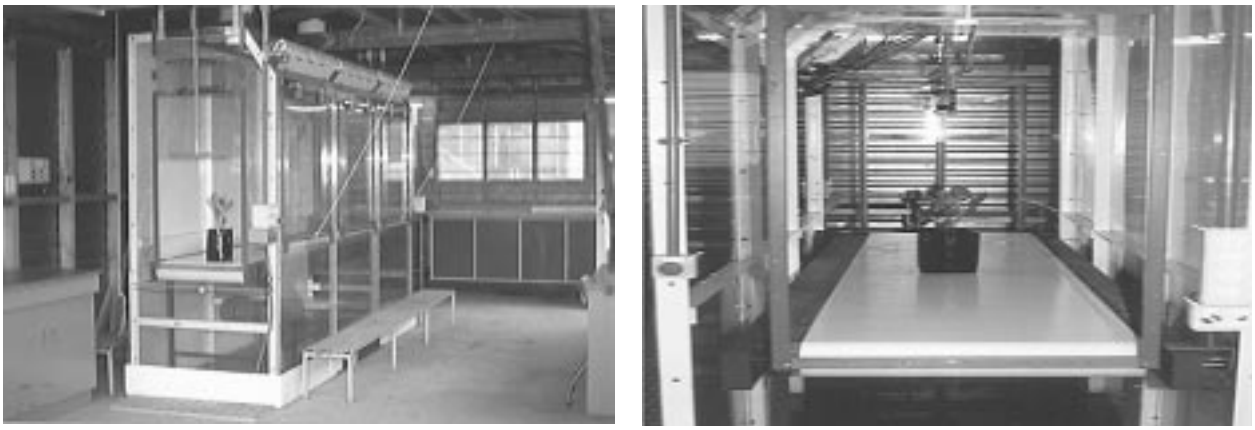

**Figure 5:** NZ Forest Research Institute Track-Sprayer used for spraying sweetgum in the Track-Sprayer spray retention trial.

**Experimental design and statistical analysis**

A completely random design was used. Ten trees were sprayed for every treatment.
Statistical analyses were run separately for spray retention by adaxial leaf surfaces, abaxial leaf surfaces, total deposition and abaxial retention as a percentage of total deposition. Square root transformations were carried out on all of the data sets in order to remedy departures from normality. The SAS general linear models (GLM) procedure was used to analyze this data.
The data was also analyzed as a complete set using the SAS procedure, proc mixed.

Two initial studies were carried out under the track-sprayer conditions.
In the first small track-sprayer experiment, a formulation containing 5% TTEA + 0.1% 408 + DA6 was sprayed onto sweetgum leaves using the nozzle which produced 650 μm droplets. The leaves were washed as normal, at 1 hr, 24 hours and 48 hours, to check that there was no loss of tartrazine. There was no significant difference ($p=0.0922$) in retention by the adaxial surface between 1 hour and 48 hours ( 48 hours actually gave a slightly higher deposition value than 1 hr, showing that there was no loss of tartrazine). There was no significant difference ($p=0.8574$) in retention by the abaxial surface between the 1 hour and 48 hour wash off. Total deposition gave a slightly higher value at 48 hour than at 1 hour, again confirming that there was no loss of

tartrazine. There was no significant difference (p=0.4241) in retention by the abaxial surface as a percentage of total deposition between the 1 hour and the 48 hour wash off.
The second small experiment carried out was a comparison between the formulation containing 5% TTEA plus 0.05% DA6 plus 0.1% 408, and a formulation containing 5% TTEA plus 0.05% DA6 plus 0.2% 408, to investigate whether an increase in surfactant concentration would increase the retention of the spray formulation. The nozzle producing 1000 μm droplets was used. There was no significant difference ($p > 0.1$) in the retention by the adaxial or abaxial leaf surfaces, or in the total deposition or percentage abaxial retention.

## LEAF CHARACTERISTICS ( WAX CHARACTER AND LEAF ANGLE )

### Plant material

As per contact phytotoxicity study. Foliage used for the scanning electron microscopy study was gathered directly from plants grown outside.

### Scanning electron microscopy (SEM)

Samples were prepared from freshly collected foliage (adaxial and abaxial leaf surfaces of the three tree species). They were mounted on aluminium specimen stubs with double sided adhesive tape, coated with a 400 Å layer of gold in a sputter coater (Polaron coating unit E5000 at 0.1 torr) and examined in a Philips PSEM 500 scanning electron microscope.

### Leaf angle determinations

The mean and extremes of leaf angle for each of the three tree species was measured using a square protractor. The angle of the leaves, attached to the tree, were measured from the horizontal.