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Fluorescent tracer technique for measuring total herbicide deposits on plants

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Summary

A tracer technique similar to that developed by C. R. Merritt at the Weed Research Organization for measuring spray deposits on plants is described and evaluated. Fluoresceine is used as the tracer and washed off plants with 0.005 M sodium hydroxide. Washing volume was not critical and loss of tracer from plant surfaces was evaluated indoors and outdoors and shown to be small.

Introduction

Retention and interception of spray droplets is affected by the nature of the leaf surface and the volume rate (Holly, 1964), as well as by the physical characteristics of the spray such as droplet diameter, surface tension, angle of incidence, velocity and viscosity (Hartley and Brunskill, 1958; Brunskill, 1956). To evaluate the effect of these parameters on total herbicide retention a fast and accurate technique for measuring spray deposit is needed. Fluorimetric techniques offer the research worker such a method. Goering and Butler (1974) describe a system using Brilliant Sulfo Flavine and Rhodamine B tracers which were washed off mylar targets with ethyl alcohol and measured in a fluorimeter. Sharp (1976) described a water soluble fluorescent dye (7-hydroxy-4-methylcoumarin) that is compatible with paraquat, while Lake and Taylor (1974) used the fluorescent dye Saturn Yellow (MF series) which they washed off plants with acetone to measure retention. This paper describes and evaluates a technique similar to that developed at the Weed Research Organization by C. R. Merritt (personal communication) using fluoresceine as a tracer to estimate total spray deposits on plant surfaces.

Experimental

Plant propagation

Wheat (*Triticum aestivum* L. (cv. Olympic)), Wimmera ryegrass (*Lolium rigidum* Gaudin) and radish (*Raphanus sativus* L. (cv. Fireball)) were chosen to simulate a wheat–weed complex for spray retention studies. The seeds were planted on the same day in a commercial peatmoss and nutrient mixture, germinated in a glasshouse and then moved to a shadehouse. When wheat reached the 2- to 5-leaf stage four plants of each species were arranged in a 345 mm×280 mm tray to form one replicate for spraying.

Fluorescent tracer technique

Fluoresceine was used as a tracer to measure the amount of spray solution retained by plant surfaces. A solution of 0.01% w/v fluoresceine in distilled water was either sprayed directly on to plants or mixed with compatible herbicides or additives before spraying. Ulvapron, a paraffinic emulsifiable petroleum oil produced by B.P. Australia Pty Ltd, was used to form an emulsion in these experiments thus simulating herbicide spraying. The tracer was washed off the plants, which were cut off at ground level, by shaking them in a plastic bag for 30 seconds with 30 mL of a solution of 0.005 M sodium hydroxide.

A Pye Unicam SP8–100 spectrophotometer fitted with a fluorimeter attachment and an autocell for rapid sample handling was used to measure the concentration of fluoresceine. The exitation wavelength was 495 nm and a cut-off filter (λ_F Filter transmittance 10% at 545 nm) was placed between the sample and the detector to eliminate the exitation light.

Leaf area measurement

The leaf areas of sprayed plants were measured after the plants were washed using a Paton Electronic Planimeter. This enabled the amount of spray solution deposited per square centimetre to be calculated and statistical calculations were made on this figure.

Effect of washing volume

The volume of washing solution that will efficiently remove the tracer was determined by washing groups of four plants of each species in different volumes. Wheat, Wimmera ryegrass and radish each at the 2-leaf stage were sprayed with 0.01% fluoresceine and 2.0% Ulvapron at 117 L ha^{-1} using Spraying Systems 80015 brass nozzles operating at 240kPa.

Table 1 shows that 80 mL of washing solution recovered significantly (P = 0.05) more tracer than volumes of 40 mL or less on wheat and ryegrass but not on radish. Mean leaf area per replicate remained reasonably constant within species but varied considerably between species and did not appear to

Table 1 Effect of washing volume on recovery of spray solution. Each result is the mean of eight replicates. Mean leaf area (cm^2) washed per replicate is shown in parentheses

Washing volume (mL)	Recovery μL cm^{-2} leaf area		
	wheat	ryegrass	radish
5	0.325 (29.8)	2.581 (3.6)	1,237.(44.3)
10	0.321 (28.3)	2.848 (3.4)	1.212 (43.1)
20	0.289 (26.9)	2.732 (3.3)	1.303 (40.5)
40	0.300 (25.8)	2.573 (3.1)	1.161 (41.9)
80	0.373 (31.2)	4.652 (2.4)	1.095 (46.2)
LSD (P = 0.05)	0.051 N.S.	1.106 N.S.	N.S. N.S.

be associated with recovery from ryegrass or radish, although there was some association with wheat. This indicates that washing efficiency is not greatly affected by washing volume and leaf area. A standard volume of 30 mL was used in subsequent experiments.

Fluoresceine absorption and degradation

The degradation of fluoresceine on the plant surface or its absorption by the plant can be estimated by varying the time and storage conditions between spraying and washing. Trays containing 40 wheat (3-leaf stage), Wimmera ryegrass (2-leaf stage) or radish plants (2-leaf stage) were sprayed with a 0.01% fluoresceine and 2% Ulvapron mixture as above. After spraying, the plants remained in the spray room (21° ±1°C) in diffuse light near north facing windows. Replicates of four plants were chosen at random from the sprayed plants and washed with 30 mL of washing solution at intervals 0–23 hours after spraying. The amount of fluoresceine recovered is shown in Table 2.

To examine degradation in sunlight the experiment was repeated in midsummer with wheat and ryegrass with 4-6 leaves and radish with 2-4 leaves. The plants were sprayed at 9.00 a.m. and left outside in direct sunlight (12 000 lux at noon) and a noon temperature of 28° for up to 24 hours before washing. As there were no cloudy periods this would approximate the worst case likely to be experienced in the field. Results of this experiment are given in Table 3.

The results show that in diffuse light there were no significant differences (P=0.05) between the initial level of fluoresceine on wheat and radish and the amount recovered up to 4 and 8 hours respectively after spraying. There were no significant differences at all on ryegrass. The differences were more pronounced when plants were exposed to direct sunlight, with significant losses of tracer (P=0.05) being detected at 1, 4 and 1 hour respectively after spraying on wheat, ryegrass and radish. This indicates that fluoresceine should be washed off the plants within 2 hours for indoor experiments and well within 1 hour for outdoor experiments if significant, although small, loss of tracer is to be avoided.

Discussion

This technique provides a fast, sensitive and reliable way of measuring total spray deposits on plants. C. R. Merritt (personal communication) demonstrated the importance of an alkaline washing solution in obtaining complete recovery of fluoresceine. The experiments demonstrated that washing volume can affect recovery of fluoresceine from plants sprayed with an emulsion. Recovery did not vary significantly when the plants were washed with less than 40 mL.

There is some loss of fluoresceine from leaf surfaces after spraying, which is accelerated by exposure to direct sunlight. Thus for indoor experiments there is ample time for washing plants after spraying while for outdoor experiments care must be taken to avoid prolonged exposure to sunshine.

This technique has been used extensively for measuring spray deposits on potted plants and field grown plants with essentially no problems.

Table 2 Effect of delay in washing after spraying on recovery of fluoresceine from wheat, Wimmera ryegrass and radish kept in diffuse light. Each result is the mean of four replicates

Time delay (h)	Recovery μL cm^{-2} leaf area		
	wheat	ryegrass	radish
0	0.417	2.425	0.945
1	0.423	2.904	1.008
2	0.441	2.671	1.029
4	0.350	2.927	0.836
8	0.360	2.981	0.768
23	0.323	2.129	0.595
LSD (P=0.05)	0.041	N.S.	0.232

Table 3 Effect of delay in washing after spraying on recovery of fluoresceine from wheat, Wimmera ryegrass and radish kept in direct sunlight. Each result is the mean of ten replicates

Time delay (h)	Recovery μL cm^{-2} leaf area		
	wheat	ryegrass	radish
0	0.679	1.884	1.155
1	0.543	1.730	0.912
2	0.490	1.595	0.832
4	0.460	1.205	0.680
7	0.346	1.315	0.616
24	0.305	0.774	0.462
LSD (P=0.05)	0.103	0.329	0.174

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