Spray application factors and plant growth regulator performance: II. Foliar uptake of gibberellic acid and 2,4-D

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Abstract: Effects of droplet size and carrier volume on foliar uptake and translocation of gibberellic acid (GA_3) and 2,4-D were investigated. Simulated spray droplets were applied to primary leaves of 10-day-old *Phaseolus vulgaris* (cv Nerina) in droplet sizes and carrier volumes ranging from 0.5 to 10 µl and 10 to 200 µl per leaf, respectively. Doses of GA_3 (2 µg per leaf) and 2,4-D (100 µg per leaf) were held constant. Total uptake of GA_3 approached a penetration equilibrium within 24 h after application, but uptake of 2,4-D continued to increase. Decreasing droplet size and/or increasing carrier volume increased GA_3 and 2,4-D uptake.

Translocation to stem and roots was positively related to total uptake. A positive linear relationship between the logarithm of the total droplet/leaf surface interface area and 2,4-D uptake or translocation was found, but for GA_3 this relationship was quadratic. Potential mechanisms of the effects of spray application factors on foliar uptake are discussed.

Keywords: gibberellic acid; 2,4-dichlorophenoxyacetic acid; carrier volume; spray volume; droplet size; penetration; translocation

1 INTRODUCTION

Spray application is an effective but often inefficient method for applying agrochemicals to crop plants and thus there is considerable economic and ecological interest in increasing efficacy.[1,2] First, low efficacy of spray application may lead to contamination of the environment. Second, agrochemicals represent a significant portion of production costs. Third, integrated pest management practices require efficient use and judicious placement of agrochemicals to maximize natural control systems.

Spray application is a complex process consisting of a series of sequential transfer stages.[3,4] Droplets generated by spray nozzles must be transferred, impact on and be retained by the target. For growth regulators and other systemic agrochemicals, the active ingredient (AI) must also be absorbed and transported to the receptor site before a biological response may be induced. Within limits, the final amount of binding to the receptor site is expected to be positively related to performance. Efficient spray application therefore requires optimizing individual transfer processes.[5]

Droplet size and carrier volume are two important application factors affecting efficacy. Both may alter performance by affecting droplet transfer to and/or retention on the plant, but less is known about their effects on foliar uptake and subsequent events.[5,6] Decreasing droplet size and/or increasing carrier volume improved performance of daminozide, gibberellic acid (GA_3) and 2,4-D as measured by biological response.[7] Further, response was closely related to the interface area between droplets and leaf surface.[7] Qualitatively, similar data on carrier volume effects on performance were obtained when daminozide and GA_3 were applied as foliar sprays under field conditions (Bukovac M J unpublished and Reference 3).

The objective of this study was to establish the effects of droplet size and carrier volume on foliar uptake and subsequent translocation of GA_3 and 2,4-D using bean (*Phaseolus vulgaris* L) seedlings as a model system. To permit comparison of these uptake data to biological response, a parallel study was performed using the same experimental design and plant system.[7]

2 EXPERIMENTAL METHODS

2.1 Plant material

Phaseolus vulgaris L (cv Nerina) was sown in plastic pots (9 cm diam., one seed per pot) filled with a mixture of a commercial natural growing medium

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(Triomf potgrond No. 17, Trio bv, Westerhaar, NL) and quartz sand (2 + 1 by volume). Plants were raised in a growth chamber at 23/19 (±2)°C day/night temperature and 75/75 (±5)% relative humidity. Light was provided during a 14-h photoperiod at 400 μmol $m^{-2} s^{-1}$ photosynthetic active radiation (PAR) at the plant level. Plants were selected for uniformity and freedom of defects nine days after seeding and transferred to a growth chamber in the isotope laboratory. Here, growing conditions were similar, except that light intensity was only 200 μmol $m^{-2} s^{-1}$ PAR. Plants were watered daily using a diluted nutrient solution.

2.2 Chemicals

Spray solutions were prepared one day prior to treatment using deionized water and non-labelled GA_3 (GA_3 content > 90%, Sigma Chemical Company, St. Louis, MO 63178, USA) and 2,4-D (99%, Aldrich Chemie Benelux, 1030 Brussel, Belgium). The 2,4-D was converted to the triethanolamine salt by adding an equimolar quantity of triethanolamine (98%, Sigma Chemical Corp.).[8] Spray solutions were radiolabelled with [1,7,12,18-^{14}C] GA_3, (sp act 259 MBq $mmol^{-1}$, radiochemical purity 77.3% by HPLC; Amersham Arlington Heights, IL 60005, USA) and 2,4-dichloro[^{14}C]phenoxy acetic acid (sp act 747 MBq $mmol^{-1}$, radiochemical purity by HPLC > 98%; Sigma Chemical Corp), respectively. Specific activities of spray solutions differed among spray volumes and, to improve counting statistics, were adjusted such that the amount of radioactivity applied per plant was >3500 dpm.

A polar ^{14}C-contaminant was present in the GA_3 that eluted from a C_{18} reversed-phase column at shorter retention times than GA_3 (solvent system: solvent A: methanol + water (20 + 80 by volume), B: methanol (100%), solvents A and B acidified with 50 μl $litre^{-1}$ of concentrated acetic acid, linear gradient from 100% A to 100% B). Uptake was compared from a purified fraction with the original (as received) in a preliminary experiment using bean leaf discs ($n = 15$) floated on deionized water over 24 h. GA_3 penetration from the non-purified and purified GA_3 averaged 28.8(±1.5) and 30.6(±2.5)%, respectively, and was not significantly different. Hence, no attempt was made to purify the entire GA_3 lot. Due to the limited supply of radiolabeled GA_3, solutions were prepared at one time and used for all GA_3-uptake experiments, which were conducted within a seven-day period. Solutions were stored at 4°C.

2.3 Experiments

Droplets of the spray solutions were applied to one (2,4-D) or both (GA_3) of the primary leaves of 10-day-old bean seedlings using microlitre syringes fitted with mechanical dispensers (Hamilton Bonaduz AF, 7402 Bonaduz, Switzerland). Plants were returned to the growth chamber immediately following treatment. For sampling, treated primary leaves were excised at the base of the petiole. Droplet deposits (droplet residues after drying) were removed from the leaf surface by rinsing with a solvent mixture and carefully brushing the deposit area using a soft camel's hair brush. Since efficacy of deposit removal may be related to the surface area covered by the deposit, the rinsing procedure was optimized. Effects of composition and volume of rinse solution were evaluated and efficiency of recovery, immediately after droplet drying, was monitored in preliminary studies. The following procedure was established. Leaves were rinsed once with acetone + water (7 + 3 by volume; 15 ml).[8,9] A 1-ml aliquot of the rinse solution was used to determine radioactivity by liquid scintillation spectrometry (LSC). The remainder of the plant was excised at the root/hypocotyl transition zone using a razor blade. Roots were removed and cleaned of adhering growing medium by sonication in deionized water for about 30 s. Leaves, stems and roots were dried at 70°C for a minimum of 48 h and oxidized. The evolved [^{14}C]carbon dioxide was trapped in a scintillation liquid containing Carbosorb and radioactivity was quantified. A mass balance was determined on an individual plant basis.

To establish if radioactivity was lost to the root medium, preliminary experiments were conducted in nutrient solution. No radioactivity was found in the nutrient solution, thus subsequent experiments were carried out using a commercial growing medium.

Penetration was followed during a 168-h (GA_3) or a 24-h (2,4-D) time-course. In our previous study on effects of application factors on biological response, performance of GA_3 and 2,4-D was assessed at 168 and 24 h after treatment, respectively (see Fig. 1 in Knoche *et al.*[7]). Hence, these time periods were also selected for the time-course of foliar uptake. Primary leaves were treated with 10 × 1-μl droplets per leaf. Solution concentrations were 0.2 and 10 g $litre^{-1}$ for GA_3 and 2,4-D, respectively. Plants were sampled and radioactivity was determined as described above. Average recoveries of radioactivity were 99.6 and 100.3% for GA_3 and 2,4-D, respectively.

Effects of droplet size and carrier volume on foliar uptake were studied using the same experimental design as previously reported.[7] Briefly, the dose of AI per plant was held constant and thus concentration of simulated spray solutions differed according to carrier volume. GA_3 and 2,4-D doses were 2 and 100 μg per leaf, respectively. Assuming an average leaf size of 50 cm^2 and a leaf area index of one, these dose rates corresponded to 4 and 200 g ha^{-1}. Droplet sizes were 0.5 (2,4-D), 1, 2, 5 and 10 μl (2,4-D and GA_3). Carrier volume was varied from 10 to 100 (2,4-D) and 10 to 200 μl per leaf (GA_3) by varying droplet numbers. The corresponding carrier volume ranges on a per hectare basis were 20–200 and 20–400 litre ha^{-1} for GA_3 and 2,4-D, respectively. Droplets were evenly distributed on adaxial leaf sur-

faces and care was taken to avoid major veins. There was no coalescence of droplets. Plants were sampled 24h after treatment and processed as described above. Average recoveries of radioactivity were 101.5 and 98.0% for GA_3 and 2,4-D, respectively.

2.4 Terminology

The amount of radioactivity recovered from the stem and root fraction is referred to as 'translocation'. No attempt was made to characterize the nature of the molecules associated with the radiolabel and, hence, the radiolabel may be associated with the original molecule, metabolites or both. Droplet residues formed after evaporation of the aqueous phase are referred to as deposits. However, the apparently dry deposit may be hydrated to some degree.

2.5 Statistics

Complete randomized experimental designs were used. Time-course penetration studies were conducted with a minimum of eight replications, while experiments on droplet size and carrier volume effects were performed with three replications per experiment and each was repeated three times ($n = 9$). Data are presented as means $\pm$ standard errors. Where standard errors are not shown in figures, they were smaller than data symbols. Foliar uptake data, expressed as percentage of applied, were transformed (arcsine) and subjected to analysis of variance. Treatment means were compared at $P = 0.05$ using Duncan's multiple range test. Unless specified otherwise, regression analysis was carried out using treatment means. Significance of regression coefficient at $P = 0.05$, 0.01 and 0.001 is indicated by *, ** and ***, respectively.

3 RESULTS

Time-courses of GA_3 and 2,4-D penetration were characterized by rapid initial uptake followed by uptake at decreasing rates (Fig 1). Initial rates (0–8h) of penetration estimated by linear regression analysis were 1.1 and 2.1% h^{-1} for GA_3 and 2,4-D, respectively. However, droplets appeared dry within 1h after application and uptake after 1h averaged 2.4 and 1.9% for GA_3 and 2,4-D, respectively. This corresponded to 11.4 and 6.9% of final uptake, respectively, and, hence, most uptake occurred from the apparently dry deposits. Total penetration averaged 20.7% for GA_3 (168h) and 27.0% for 2,4-D (24h). GA_3 penetration approached an asymptote within 24h, but uptake of 2,4-D continued to increase at this time.

2,4-D translocation to stem and roots increased throughout the experimental period, averaging 7.1% of the amount applied at 24h (corresponding to 26% of uptake at 24h), but GA_3 translocation approached an asymptote after 168h at 7.9% (corresponding to 37.9% of uptake at 168h; Fig 1).

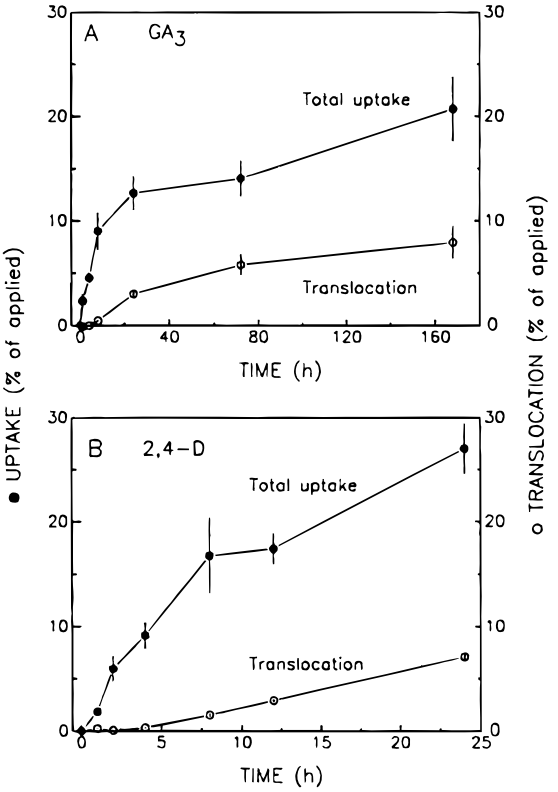

Figure 1. Time-course of uptake and translocation of foliar-applied (A) GA_3 and (B) 2,4-D in *Phaseolus vulgaris*.

Droplet size and/or carrier volume significantly affected uptake of both GA_3 and 2,4-D 24h after treatment (Tables 1 and 2). There were no significant interactions among application factors. GA_3 uptake was greatest at a carrier volume of 100 µl per leaf, but droplet size had no significant effect (Table 1). In contrast, 2,4-D uptake was affected by both droplet size and carrier volume, uptake increasing with decreasing droplet size and increasing carrier volume (Table 2). Qualitatively similar relationships have been observed for the amount of radiolabel translocated to stem and roots (Knoche M, unpublished).

Plotting uptake against translocation yielded positive linear relationships for GA_3 and 2,4-D (Fig 2A and B; Table 3). Similarly, translocation efficacy, calculated as translocation in percentage of uptake, was positively related to uptake for GA_3 and 2,4-D (Table 3).

In our earlier study we determined effects of droplet size and carrier volume on the interfacial area between spray solution and leaf surface.[7] Since the experimental system was identical to that in the present study (AIs, concentrations, droplet sizes, carrier volumes, plant species and cultivar), these data were employed to investigate the relationship between interfacial area and uptake or translocation (Fig 3). The analysis revealed that effects of application factors on uptake and translocation were related to their effect on the interfacial area between spray solution and leaf surface (Fig 3). For GA_3, an

Droplet Size (*μl*)	*Uptake* (% *of applied*)					
	Volume (*μl per leaf*)					
	10	*20*	*50*	*100*	*200*	*Mean*[a]
1	15.3	34.1	30.0	35.0	24.3	27.8a
2	14.1	28.1	30.3	40.2	31.5	28.8a
5	34.6	29.1	25.6	36.3	26.5	30.4a
10	13.8	17.6	31.5	29.9	29.3	24.4a
Mean[a]	19.5c	27.2b	29.4ab	35.4a	27.9b	

[a] Means followed by the same letter are not significantly different at $P = 0.05$, Duncan's multiple range test.

Table 1. Effect of droplet size and application volume at constant GA_3 dose (2 μg per leaf) on GA_3 uptake by *Phaseolus vulgaris*

Droplet Size (*μl*)	*Uptake* (% *of applied*)				
	Volume (*μl per leaf*)				
	10	*20*	*50*	*100*	*Mean*[a]
0.5	20.4	30.2	36.9	41.4	32.2a
1	15.4	29.5	36.7	39.1	30.2a
2	17.4	18.5	32.8	36.6	26.3b
5	14.3	17.9	27.0	33.8	23.3bc
10	15.2	13.7	25.3	30.2	21.1c
Mean[a]	16.5d	21.9c	31.7b	36.2a	

[a] Means followed by the same letter are not significantly different at $P = 0.05$, Duncan's multiple range test.

Table 2. Effect of droplet size and application volume at constant 2,4-D dose (100 μg per leaf) on 2,4-D uptake by *Phaseolus vulgaris*

optimum-type relationship of uptake with the logarithm of the interfacial area was found, while 2,4-D uptake was linearly related to log interfacial area. Regression equations for the relationship between the logarithm of the total droplet/leaf interfacial area (mm^2) and total uptake (% of applied) were:

$$\text{Uptake} = -57.6 + 71.0 \times (\log \text{area}) - 14.1 \times (\log \text{area})^2,\ r^2 = 0.425^{**}$$

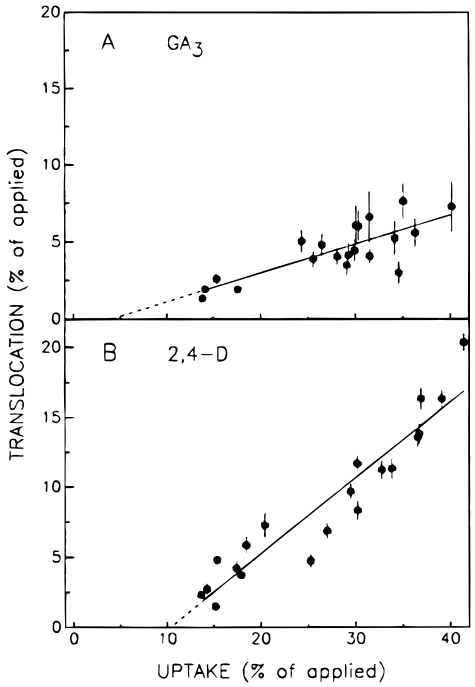

Figure 2. Translocation of foliar-applied (A) GA_3 and (B) 2,4-D to shoot and root of *Phaseolus vulgaris* as a function of uptake.

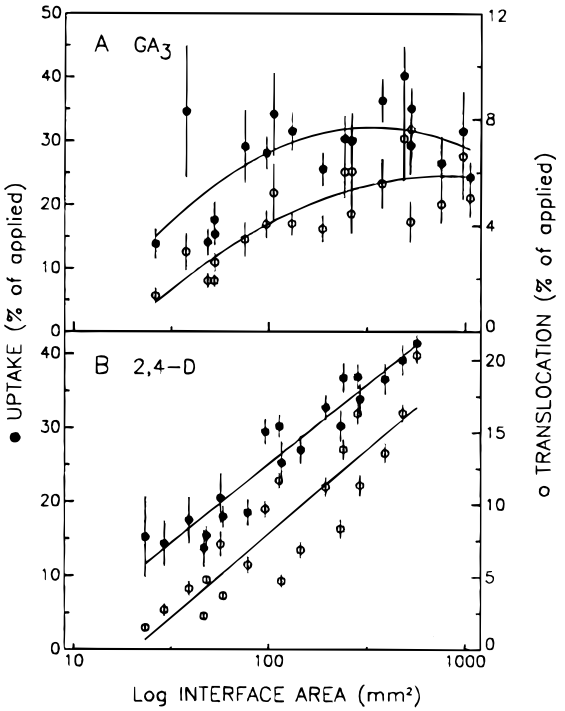

Figure 3. Uptake and translocation of foliar-applied (A) GA_3 and (B) 2,4-D to shoot and root of *Phaseolus vulgaris* as affected by the droplet/leaf interfacial area.

Fraction (% of applied)	Coefficient of correlation[a]				
	Fraction (% of applied)				(% of uptake)
	Leaf	Stem	Root	Stem + Root	Stem + Root
GA_3					
Total	0.99***	0.77***	0.67***	0.77***	0.15*
Leaf		0.66***	0.57***	0.66***	0.02
Stem			0.84***	0.99***	0.62***
Root				0.90***	0.72***
Stem + Root					0.66***
2,4-D					
Total	0.92***	—[b]	—	0.78***	0.12
Leaf			—	0.47***	−0.25***
Stem + Root					0.66***

Table 3. Correlations among various GA_3 and 2,4-D uptake fractions

[a] Significance of the coefficient of correlation at $P = 0.05$, 0.01 and 0.001 is indicated by *, ** and ***, respectively ($n = 180$ within growth regulators).
[b] Not determined.

and

$$\text{Uptake} = -17.8 + 21.4 \times (\log \text{area}),\ r^2 = 0.917***$$

for GA_3 and 2,4-D, respectively.

Similarly, translocation of radiolabel to stem and roots was related to interfacial area (Fig 3) as was efficiency of translocation (Fig 4). Regression equations for the relationship between the logarithm of the interfacial area (mm^2) and translocation efficiency (% of uptake) were:

$$\text{Translocation} = 4.3 + 5.0 \times (\log \text{area}),\quad r^2 = 0.599***$$

and

$$\text{Translocation} = 3.4 + 14.1 \times (\log \text{area}),\quad r^2 = 0.501***$$

for GA_3 and 2,4-D, respectively.

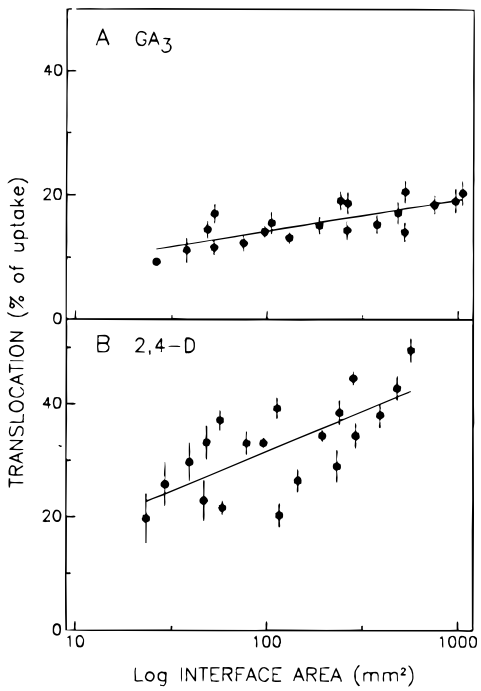

Figure 4. Effect of droplet/leaf interfacial area on the efficiency of translocation of foliar-applied (A) GA_3 and (B) 2,4-D. Translocation efficiency was calculated by dividing quantity translocated (% of applied) out of treated leaves by uptake (% of applied).

4 DISCUSSION

Our data established that droplet size and/or carrier volume affected foliar uptake, translocation and biological response, and that their effects were related to the interfacial area between spray solution and leaf surface.[7] These findings were derived under highly controlled conditions in the absence of confounding factors. Clearly, the experimental conditions are somewhat remote from spray application in the field. First, droplet numbers per unit area applied by a hand-held microsyringe are significantly lower than those applied by spray nozzles. Second, only the smaller droplets in our studies are of comparable size to some droplets in the spray pattern created by high-volume orchard sprayers or modern anti-drift nozzles (for example for TurboDrop Nozzles operated at 275 kPa, D_{90} values ranging from 909 to 1147 μm have been reported).[10] Third, our droplets were carefully placed on the leaf blade, avoiding major veins above which the cuticle has been shown to differ in polarity, and totally excluding the shoot as a target. Also, there was no droplet coalescence or

Table 4. Comparison of selected characteristics of spray deposits applied either by hand using a microsyringe in our system[7] (syringe) or by microsprayer using a monosize droplet applicator in the system of Stevens and Bukovac[8] (sprayer)

Deposit characteristic	*GA_3*		*2,4-D*		*Daminozide*	
	Syringe	*Sprayer*	*Syringe*	*Sprayer*	*Syringe*	*Sprayer*
Dose rate (g ha^{-1})	4	n.a[a]	200	4–421	200	5–215
Carrier volume (litre ha^{-1})	20–400	n.a	20–200	21–188	20–400	11–91
Droplet diameter (μm)	1241–2673	n.a	985–2673	56–771	1241–2673	51–771
Droplet number per unit area (cm^{-2})	0.02–4	n.a	0.02–4	2–6018	0.02–4	2–3681
Coverage (% leaf area)	0.3–10.6	n.a	0.5–11.3	2–58	0.3–10.6	3–37
Amount of AI deposited per unit wetted area (g m^{-2})	0.004–0.15	n.a	0.18–4.3	0.0002–0.94	0.19–7.6	0.0003–0.45

[a] Not applied in experiments utilizing the microsprayer.[8]

overstrike, which often occurs during spray application. Further, to avoid confounding between effects of application factors and effects of formulation on uptake and translocation, which may be specific for the factors being investigated (ie plant species,[11] AI[12]), we selected in our study PGRs that are usually applied in aqueous solutions without spray additives. These differences must be kept in mind when attempting to extrapolate our findings to spray application of other AIs. Published information on effects of droplet size and carrier volume on herbicide performance has been compiled and reviewed recently and those interested in field performance are referred to those reports.[5,13]

In the present discussion, we will focus on (1) theoretical aspects of application factors in an ideal system and (2) how our data relate to findings derived from other studies under comparably controlled conditions. For the latter comparison we selected a study by Stevens and Bukovac[8] who used a similar system, but extended the drop-size range far below the one investigated in this and our previous contribution,[7] thereby more closely simulating spray application in the field (Table 4). Further, in contrast to the data presented in this and our previous paper, Stevens and Bukovac[8] did not detect an effect of droplet size or carrier volume on uptake and translocation of 2,4-D or daminozide by field bean. Later studies on carrier volume effects on 2,4-D performance in field bean confirmed these findings.[14]

According to Hartley and Graham-Bryce,[15] penetration from a small donor reservoir of volume V_{don} through an interfacing membrane into a large receiver reservoir of volume V_{rec} is given by eqn (1):

$$\frac{-P * A * t}{V_{don}} = \ln\left(\frac{C_{don}}{C_0}\right) \qquad (1)$$

V_{don}, the interfacial area (A) between V_{don} and the membrane and the permeance (P), a measure of membrane conductance, were all assumed to be constant and independent of time. The receiver concentration was assumed to be negligibly low. Further, the hypothetical membrane was assumed to be of zero thickness. C_{don}/C_0 represented the fraction of AI remaining in the donor at time t. Accordingly, $1\text{-}C_{don}/C_0$ corresponded to the AI fraction that penetrated the membrane. Plotting $1\text{-}C_{don}/C_0$ versus t for various A values yielded the hypothetical penetration time-courses depicted in Fig 5. Provided that the initial donor concentration (C_0) at $t = 0$ did not exceed the AI solubility, the ratio of C_{don}/C_0 decayed exponentially with time, while the fraction penetrated ($1\text{-}C_{don}/C_0$) increased at a decreasing rate. Plotting the logarithm of C_{don}/C_0 versus t yielded a linear relationship, where the slope corresponded to the first-order rate constant (k) give by eqn (2) (Fig 5, inset):

$$k = \frac{-P * A}{V_{don}} \qquad (2)$$

At constant V_{don} and constant P the rate of penetration is proportional to A. Thus, increasing interfacial area at constant dose is expected to increase the rate

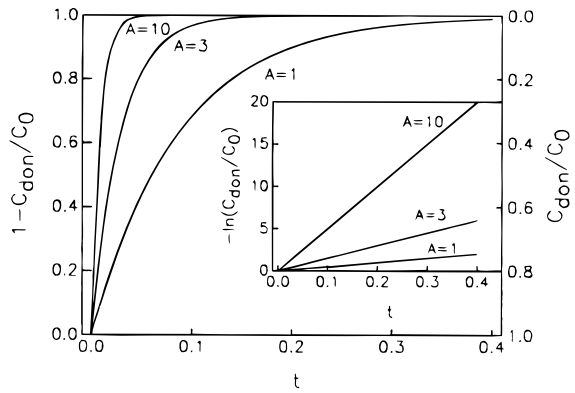

Figure 5. Time-course of fractional penetration ($1\text{-}C_{don}/C_0$) through an ideal membrane at different contact areas A. Inset: Plot of logarithm of the AI fraction remaining in the donor versus time at different contact areas. For details see text.

of penetration and decrease the time to reach penetration equilibrium. The effect of increasing A on penetration rates will decrease as penetration approaches an equilibrium. Finally, at equilibrium the amount penetrated is expected to be independent of A.

In our study the apparently dry deposits served as donors, since most penetration occurred after the droplets appeared dry. This is supported by other studies which demonstrated that deposits from dried-down droplets serve as effective donors for subsequent penetration.[16–18] Since dose was held constant in our study, V_{don} (following droplet drying) was constant even when the initial carrier volume was varied. The assumption of a non-saturated deposit in the above model, however, is somewhat unrealistic. First, deposits appeared macroscopically dry. Second, deposits of 2,4-D at low interfacial areas appeared white and powdery, suggesting that some crystallization of the AI occurred, while those of GA_3 were translucent. If AI concentration in the donor deposit exceeded solubility during the drying process, precipitation and crystallization of the AI would occur. Assuming that the precipitated AI was solubilized, replacing any AI that penetrated, C_{don} would remain constant until the AI precipitate became exhausted. At this point C_{don} and, hence, penetration rates would decrease exponentially until the entire amount of AI available for uptake had penetrated the membrane. Under these circumstances, the initial phase of penetration would be characterized by a constant penetration rate, levelling off only after the precipitated AI in the deposit was exhausted. Thus, increasing A would be expected to (1) increase initial rates of penetration, (2) decrease the time to reach penetration equilibrium and (3) not alter the total amount penetrating, provided that solubilization of the precipitated AI in the deposit equalled (ie replaced) the amount that penetrated. Therefore, the expected effects of application factors on uptake are qualitatively identical, irrespective of whether deposits with or without precipitated AI serve as the donor.

Our uptake data and those of Stevens and Bukovac[8] are in general agreement with this qualitative effect predicted by the above models, provided that uptake at 24 h in the study of Stevens and Bukovac,[8] but not in our study, had reached equilibrium. Further, based on the above considerations and the smaller effect that interface area had on GA_3 compared with 2,4-D uptake (Fig 3), we would also expect GA_3 penetration to be closer to equilibrium. While direct evidence is limited, there is some indirect evidence supporting this hypothesis. First, for compounds that do not affect their own transport one would expect AI translocation to stem and roots to be a constant fraction of the amount taken up, ie translocation efficiency to be constant, if the system was at equilibrium.[19] This was the case for the study by Stevens and Bukovac,[8] while, in our study, translocation to shoot and roots (calculated as percentage of uptake) depended on the amount taken up. Further, for GA_3 the x-axis intercept of a plot of uptake (% of applied) versus translocation (% of applied) was at 4.0% uptake, but for 2,4-D at 10.4% (Fig 2A and B). If the system had reached equilibrium, both regression lines should intercept the origin. Also, rates of penetration in our time-course study averaged 0.3 (8–24 h) and 0.8% h^{-1} (12–24 h) for GA_3 and 2,4-D, respectively (Fig 1A and B). These data indeed suggest that GA_3 was closer to reaching a penetration equilibrium than was 2,4-D. Second, coverage and, hence, interfacial area was larger in the study by Stevens and Bukovac[8] and, thus, penetration should indeed reach equilibrium earlier.

Although the models presented above were consistent with our and the published data on effects of application factors on uptake and translocation, several questions remain to be answered:

First, what is the basis for the optimum-type relationship between coverage and GA_3 uptake (Table 1, Fig 3A)? Since (1) high coverage was achieved at large carrier volumes (equivalent to low concentrations), (2) GA_3 solutions were not buffered and hence, pH increased from 3.4 at 10 µl per leaf to 4.7 at 100 µl per leaf and (3) GA_3 uptake decreased as pH increased,[9] the apparent decrease of uptake at high carrier volumes may have been a reflection of the change in pH.

Second, penetration equilibria significantly below 100% are difficult to explain based on the above models, but have been reported in many studies[9,20] on foliar uptake, including that of Stevens and Bukovac[8] and the present one. Possible explanations include partition characteristics between deposit and cuticle that favour the AI remaining in the deposit[17,21] or AI crystallization upon droplet drying.

Third, at present it is not clear whether penetration at equilibrium is affected by variation of application factors. If V_{don} was independent of application factors (eg the deposit represented the donor and dose was held constant) and the entire amount of AI in the deposit was available for uptake, a change in A would be unlikely to affect the penetration equilibrium.

Fourth, assuming a constant P implied that (1) the deposit/cuticle and the cuticle/water partition coefficients ($K_{dep/cut}$ and $K_{cut/water}$, respectively) and (2) the diffusion coefficient (D) were independent of concentration. Indeed, many sorption and diffusion studies have shown that $K_{cut/water}$ and D are independent of concentration (for review see references[18,22,23]). However, most of these studies have been limited to dilute solutions, while deposits represent highly concentrated mixtures/solutions of an AI in a deposit.[24] Also, many formulations of agrochemicals contain surfactants, some of which alter the D of an AI in a concentration-dependent

manner.[25] Since a decrease in A at constant dose increased the amount of AI deposited per unit interface area, the amount of surfactant in the cuticle and, hence, the D of an AI may be inversely related to A. Consequently, decreasing A under these circumstances may increase penetration.

Last, deposits may be far more complex than the simple homogenous deposit solutions assumed in the discussion presented above. For example, droplets upon drying frequently form annular deposits on the leaf surface, where the amount of AI per unit interface area within a deposit differs markedly.[26–29] The contact area covered by such deposits is significantly smaller than the original droplet footprint at equilibrium.[29] Further, in such deposits, A may change with time as the deposit center becomes depleted as penetration proceeds. Additional complications may arise from phase separation of AI and spray additives during droplet drying which has been demonstrated by Falk and Schulke[30] and Bukovac *et al.*[31] Little is known about the donor characteristics of such deposits.

5 CONCLUSION

Clearly, further studies are necessary to provide a mechanistic understanding of effects of application factors on uptake and performance of systemic agrochemicals. The data presented herein demonstrate that application factors may alter performance by affecting foliar uptake. There is some evidence that droplet geometry is critical and, hence, using spray droplet sizes that are common to agricultural sprays is highly desirable. However, at present there is no satisfactory application technique commercially available that permits precise placement of small, individual, mono-size droplets in defined patterns within a small defined area such that the dose applied is maintained constant. These criteria are essential for critical investigation of the effect of application factors on uptake of radiolabeled pesticides. Further research efforts should also focus on the role of deposits in foliar penetration. There is increasing evidence that the deposit may represent a significant and, for some AI, perhaps the primary donor for penetration. Critical properties of deposits with respect to penetration are (1) the interface area A of the deposit with the leaf surface, (2) the partition coefficient between deposit components and cuticle, (3) the volume and composition of the deposit and (4) the AI concentration and distribution within the deposit. Thus far, deposits of spray droplets have received relatively little attention and, consequently, limited information is available on their physical/chemical characteristics relevant to penetration. A detailed understanding of these characteristics and how they are affected by application factors is necessary to provide a basis for increasing efficacy of spray application by optimizing the factors of droplet size and carrier volume.

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