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# Spray Application Factors and Plant Growth Regulator Performance: I. Bioassays and Biological Response†

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**Abstract:** Bioassays were adapted to investigate effects of droplet size and carrier volume on performance of daminozide, gibberellic acid ($GA_3$) and 2,4-D using *Phaseolus vulgaris* L. as a model system. Response to plant growth regulators was indexed by inhibition (daminozide), promotion of internode elongation ($GA_3$) or ethylene production (2,4-D). Elongation of first plus second internodes above primary leaves was evaluated 14 days after treatment of primary leaves, while ethylene production was determined from head-space samples of incubated leaves 24 h after treatment. Daminozide inhibition of internode elongation was related to decreased cell size and number in pith and epidermis (range 49–70% of the untreated control). $GA_3$ increased cell size and number in both tissues 2·3- to 4·8-fold. Responsiveness to daminozide and 2,4-D markedly decreased as seedling age increased from 8 to 12 days, but responsiveness to $GA_3$ increased. Decreasing droplet size (10–0·5 µl) and increasing carrier volume (10–200 µl per leaf) at constant dose of daminozide (100 µg per leaf) and 2,4-D (100 µg per leaf) significantly increased performance, but had little effect on performance of $GA_3$ (2 µg per leaf). Effects of application factors on performance were related to their effects on the interface area between droplets and leaf surface. Significant positive linear relationships were obtained between plant response and the logarithm of the droplet/leaf interface area for all growth regulators. © 1998 Society of Chemical Industry

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## 1 INTRODUCTION

The effects of spray application variables on pesticide performance have been a matter of debate, since the introduction of chemical crop protection. Numerous studies have investigated the effects of droplet size and carrier volume on the performance of fungicides, insecticides, herbicides and plant growth regulators. However, the results were often inconsistent. Spray application variables were shown to increase, have no effect or decrease performance.[1–4] At first sight, the inconsistency of the data suggested that the effects of application factors on performance were specific for the application equipment, compound or species studied.[5] However, other factors should be considered.

First, performance of systemic agrochemicals following spray application is the integrated result of a series of sequential transfer processes of the AI from the spray nozzle to binding to the target site at the response level. The transfer stages involved include: (1) droplet formation at the spray nozzle, (2) droplet flight to the target, (3) droplet impaction, retention and deposit formation on the plant surface, (4) foliar uptake and transport to the responding tissue, (5) AI binding to a receptor site and (6) the induction of the biological response.[6–8] Any one of the transfer steps may be affected by changing droplet size and carrier volume.[7,9] Indeed, when individual transfer stages were considered, common trends among effects of application factors could be identified.[4]

Second, when using commercial spray applicators, effects of application factors are difficult to control independently, and for hydraulic spray nozzles the broad drop-size distribution is a serious limitation in data interpretation. Hence, in many experiments application factors were confounded and the conclusions were limited to the particular operating parameters investigated.[4]

The consequences of this situation are threefold.

(1) In order to assure acceptable field performance, manufacturers' recommended use rates for agrochemicals are generally based on a worst-case scenario that provides acceptable performance. Thus, in all but the worst case, dose rates are higher than needed. The increasing number of recent publications on the effects of reduced dose rates on pesticide performance supports this contention.[10,11]

(2) While in the course of crop production overdosing may not be a serious problem with fungicides, insecticides or herbicides—provided that selectivity is not altered and that there is no phytotoxicity—it may be critical for plant growth regulators (PGR). Optimum performance of PGRs is limited to a narrow dose range between no effect and overdosing (e.g. fruit thinning).[12,13] Altering efficiency of application by varying spray application factors may alter performance in an unpredictable manner. In fact, spray application technology is considered to be a limiting factor in performance of many plant growth regulators, and information on effects of application factors on performance is urgently needed to improve consistency.[12,13]

(3) Successful integrated pest management practices rely on maintaining an active population of predators and other natural control agents. These are at risk when agrochemicals are applied inefficiently.

To develop a better understanding of the complex interactions in the spray application process, we adopted several bioassays permitting evaluation of spray application variables under defined conditions. In this paper, we report on (1) the development of simple and sensitive bioassays for evaluating plant response to selected foliar-applied growth regulators and (2) the effect of droplet size and carrier volume on performance. Daminozide, gibberellic acid ($GA_3$) and 2,4-D were selected because foliar absorption is a prerequisite for action, they consistently induce measurable plant responses, they differ in polarity and are representatives of important PGRs in crop production.

## 2 EXPERIMENTAL METHODS

### 2.1 Plant material

Beans (*Phaseolus vulgaris* L.) of three cultivars (cv. Green Ruler (GR) or Nerina (NE), both determinate types and cv. Black Seeded Blue Lake (BSBL) indeterminate type) were used. Development of daminozide and $GA_3$ assays was carried out using the cultivars BSBL and GR, respectively. Since a consistent supply of BSBL and GR became difficult, subsequent experiments were conducted using NE. Seeds (three or four per pot) were planted in peat (9 cm diam.) or plastic (0·5 litre vol.) pots containing a commercial greenhouse growing medium. Following emergence, seedlings were selected for uniformity and freedom from defects and thinned to one plant per pot. Plants were watered daily. Fertilizer was provided either initially in the growing medium (8 g litre$^{-1}$ Osmocote 14-14-14; Scotts-Sierra Horticultural Prod. Co., Marysville, OH 43041) or at regular intervals by watering with a water-soluble fertilizer (Peters 20-20-20; Scotts-Sierra Horticultural Prod. Co.) or a diluted Steiner nutrient solution. Growing conditions for BSBL and GR bean (assay development) were: growth cabinet at 25/20($\pm$2)°C day/night temperature and 45/62 ($\pm$5)% RH, 200 μmol m$^{-2}$ s$^{-1}$ photosynthetic photon flux density (PPFD) at the primary leaf level supplemented by 1% red light (tungsten filament bulbs) during a 16-h photoperiod. Plants were maintained under these conditions for the entire experimental period. Unless otherwise specified, plants were treated 8 days after seeding. For studies on effects of droplet size and carrier volume on daminozide and $GA_3$ performance (both NE) growing conditions were: growth cabinet at 24/19 ($\pm$2)°C day/night temperature and 75/

75 (±5)% RH, 400 μmol $m^{-2}$ $s^{-1}$ PPFD during a 14-h photoperiod at the primary leaf level. One day prior to treatment, plants were transferred to a greenhouse and grown until harvested. Growing conditions in the greenhouse were: 23/20 (±5)°C day/night temperature and 70/90 (±10)% RH. Photoperiod was extended for 0·5 h after sunset using tungsten filament bulbs. Pots were placed in trays and trays were repositioned every other day. NE beans used in the 2,4-D assays were raised under the same conditions, but remained in the growth chamber for the entire experiment.

## 2.2 Chemicals

Spray solutions were prepared with deionized water using daminozide >99% pure, technical (Uniroyal Chemical Crop., Middlebury, CT 06749) or analytical grade (Sigma Chemical Co., St. Louis, MO 63178)), gibberellic acid ($GA_3$ content > 90%, Sigma Chemical Co.) or 2,4-D (99%, Aldrich Chemie Benelux, 1030 Brussel, Belgium). No surfactant, buffer or solvent, other than water, was added. 2,4-D was converted to the triethanolamine salt by adding an equimolar quantity of triethanolamine (98%, Sigma Chemical Company).[14] 2,4-D concentrations refer to the acid equivalent, $GA_3$ concentrations assume a $GA_3$ content of 100%.

## 2.3 Experiments

### *2.3.1 Development of bioassays*

Earlier studies established a consistent daminozide and $GA_3$ elongation response in bean[15,16] and the physiological basis was investigated by determining cell numbers and sizes of epidermal and pith tissue of the first internode above primary leaves of BSBL bean. Plants were sprayed to run-off with solutions containing daminozide (4.8 g $litre^{-1}$) or $GA_3$ (100 mg $litre^{-1}$). Untreated plants served as a control. Internode elongation was followed over a 10-day period or until internode elongation ceased. Cell sizes and numbers were determined in semi-thin sections of the pith under a binocular microscope. For epidermal tissue, cell numbers were calculated by dividing internode length by mean cell sizes determined in tissue excised from the median portion of the internode. Preliminary investigations have shown that cell sizes in the median portion of the internode are representative for the entire internode (Bukovac, M. J., unpublished). Internode length was determined with 10, cell numbers and sizes with three or four replications.

Auxin-induced ethylene production has been used earlier as a model system for studying penetration.[17–20] This system was adopted for our studies: 2,4-D was applied to the adaxial surface of one of the primary leaves. The treated leaf was excised 24 h after droplet application and transferred to a glass test tube (vol. 55·5 ml) with petioles immersed in 0·5 ml of deionized water. Tubes were sealed with a rubber septum, incubated for 2 h in the dark at 30°C, and a 1-ml headspace sample was taken (1-ml disposable syringe, Terumo Europe N.V., 3030 Leuven, Belgium) for ethylene measurement by gas chromatography (GC; Packard Model 439, Packard Instrument Co. Inc., Downers Grove, IL 60515). The GC was equipped with a Porapack Q column (prepacked, 8′ × 1/8″, 80–180 mesh; Packard Instrument Company Inc.) and a flame-ionization detector. Operating conditions were: 60, 80 and 125°C injector, column and detector temperatures, respectively; $N_2$ carrier gas at 25 ml $min^{-1}$. GC calibration was checked at regular intervals using a custom-prepared ethylene standard. Preliminary experiments established that (1) prolonging the incubation period up to 8 h significantly increased the amount of ethylene for 2,4-D treated, but not for untreated leaves and (2) rates of ethylene production were independent of incubation time for 2,4-D treated leaves ($P = 0{\cdot}05$; Knoche, M., unpublished). For convenience, a 2-h incubation period was selected for subsequent experiments.

Time courses of internode elongation (0 to 10 days after treatment with daminozide or $GA_3$) and ethylene production (0 to 48 h after treatment with 2,4-D) were established following spray application until run-off (daminozide or $GA_3$) or droplet application (10 × 1- μl droplets for 2,4-D). Concentrations of simulated spray solutions were 4·8, 0·1 and 10 g $litre^{-1}$ for daminozide, $GA_3$ and 2,4-D, respectively. Length of first plus second internodes (daminozide and $GA_3$) above primary leaves and ethylene production (2,4-D) were measured periodically. Number of replications was 10.

The effect of seedling age on response to daminozide (1000 μg per leaf), $GA_3$ (20 μg per leaf) and 2,4-D (100 μg per leaf) was investigated in 8- to 12-day-old plants. For daminozide and $GA_3$, spray solutions were applied as 20 × 5-μl droplets to both primary leaves, for 2,4-D as 10 × 1-μl droplets to one of the primary leaves. Final internode elongation (daminozide and $GA_3$) was measured. Ethylene production was determined 24 h after 2,4-D application as described above. Number of replications was five (daminozide and $GA_3$) or 10 (2,4-D).

To investigate the relationship between initial internode length at the time of daminozide application and final length of the first internode, seven- to nine-day-old BSBL bean were selected for initial internode length and grouped in the three length classes averaging <0·3, 1 and 2 cm. Daminozide solutions (4·8 g $litre^{-1}$) were applied as described above. Cell sizes and numbers were determined in pith and epidermal tissues. Measurements were carried out with five replications.

Dose-response relationships were established by dipping primary leaves of BSBL and GR bean in daminozide or $GA_3$ solutions. Since primary bean leaf surfaces were easy to wet (contact angle <90°), dipping

**TABLE 1**
Effect of Saturating Doses of Daminozide and $GA_3$ on Development of the First Internode above the Primary Leaves of *Phaseolus vulgaris* (cv. Black Seeded Blue Lake)[a]

| Treatment | Internode length (cm)[b] | Cell size (μm)[b] | | Cell number[b] | |
|---|---|---|---|---|---|
| | | Pith | Epidermis | Pith | Epidermis |
| Control | 2·3 (100)a | 86 (100)a | 33 (100)a | 289 (100)a | 716 (100)a |
| Daminozide | 0·8 (35)a | 45 (53)b | 23 (70)b | 169 (59)b | 352 (49)b |
| $GA_3$ | 25·3 (1097)b | 316 (367)c | 76 (230)c | 847 (293)c | 3422 (478)c |

[a] Mean initial internode length was <0·3 cm.
[b] Means within columns followed by the same letter are not significantly different according to the Tukey test at $P = 0·05$. Percentage of control given in parenthesis.

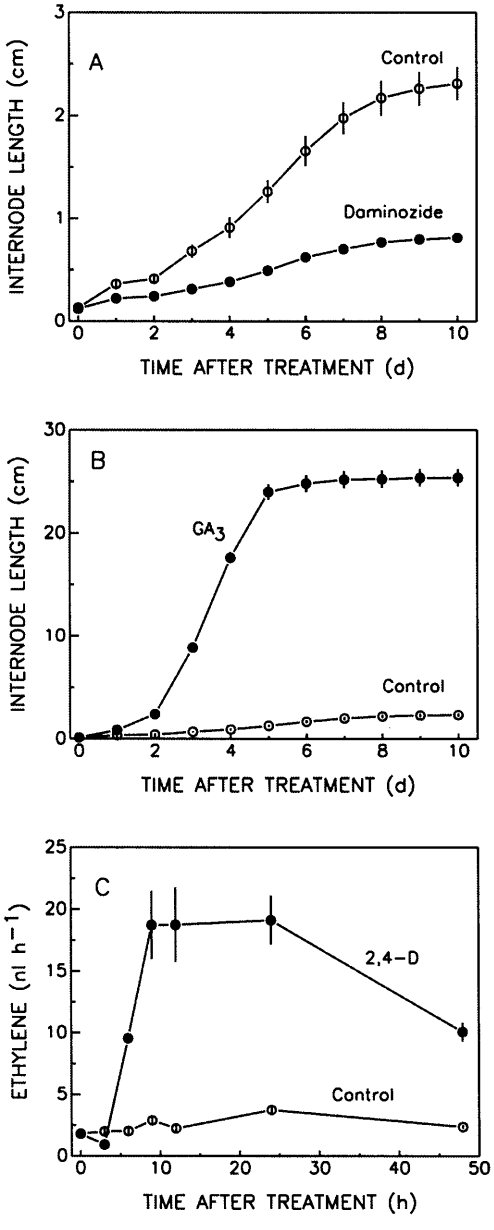


**Fig. 1.** Time course of (A) daminozide inhibition of internode elongation, (B) $GA_3$ promotion of internode elongation and (C) 2,4-D stimulation of ethylene production in *Phaseolus vulgaris*.

resulted in uniform wetting. Concentrations of simulated spray solutions ranged from 0 to 10 g litre$^{-1}$ for daminozide and 0 to 40 mg litre$^{-1}$ for $GA_3$. The dose retained per leaf was calculated from spray retention measured on leaves of the same age and cultivar. Retention was determined gravimetrically after leaves were dipped and allowed to drain for 30 s, while held vertically. Number of replications was eight. The dose-response relationship for the 2,4-D assay was established by applying 2,4-D solutions (concn 0 to 30 g litre$^{-1}$) as 10 × 1-μl droplets to one of the primary leaves of NE bean. Number of replications was 10.

*2.3.2 Effects of droplet size and carrier volume*

Effects of droplet size and application volume on daminozide inhibition and $GA_3$ promotion of internode elongation and 2,4-D stimulation of ethylene production were investigated. Droplets of 0·5 (2,4-D only), 1, 2, 5 and 10 μl volume were applied using a microliter syringe fitted with a mechanical dispenser (Hamilton Bonaduz AG, 7402 Bonaduz, Switzerland) for total application volumes of 10, 20, 50, 100 and 200 (daminozide and $GA_3$ only) μl per leaf. Application volume was established by varying droplet number. Since dose was held constant (100, 2 and 100 μg per leaf for daminozide, $GA_3$ and 2,4-D, respectively), concentrations of simulated spray solutions differed among volumes. Daminozide and $GA_3$ concentrations were 10, 5, 2, 1 and 0·5 g litre$^{-1}$ and 200, 100, 40, 20 and 10 mg litre$^{-1}$ for the 10, 20, 50, 100 and 200 μl per leaf volumes, respectively. 2,4-D concentrations were 10, 5, 2 and 1 g litre$^{-1}$ for the 10, 20, 50 and 100 μl per leaf volumes, respectively. Assuming a leaf area index of 1 and an average primary bean leaf size of 50 cm$^2$, the daminozide, $GA_3$ and 2,4-D doses applied corresponded to 200, 4 and 200 g ha$^{-1}$, respectively. Accordingly, carrier volume ranged from 20 to 400 litre ha$^{-1}$ (equivalent to 10–200 μl per leaf) for daminozide and $GA_3$ and 20–200 litre ha$^{-1}$ (equivalent to 10 to 100 μl per leaf) for 2,4-D, respectively. Growth regulators were applied to both (daminozide and $GA_3$) or one of the primary leaves (2,4-D) of 10-day-old NE bean. Elon-

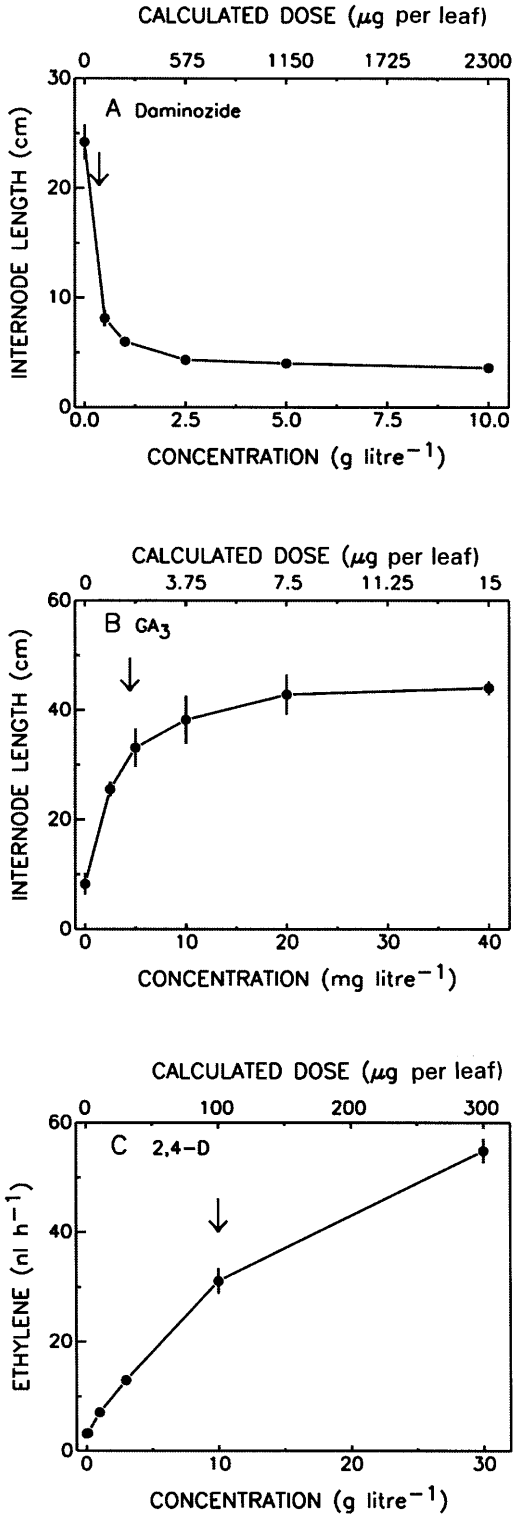


**Fig. 2.** Dose-response of (A) daminozide inhibition of internode elongation, (B) $GA_3$ promotion of internode elongation and (C) 2,4-D stimulation of ethylene production in *Phaseolus vulgaris*. Arrows indicate the dose selected for the study of effects of application factors on performance.

gation of first plus second internodes above primary leaves was measured 14 days after treatment (daminozide and $GA_3$), while ethylene production was determined after 24 h. Experiments were carried out

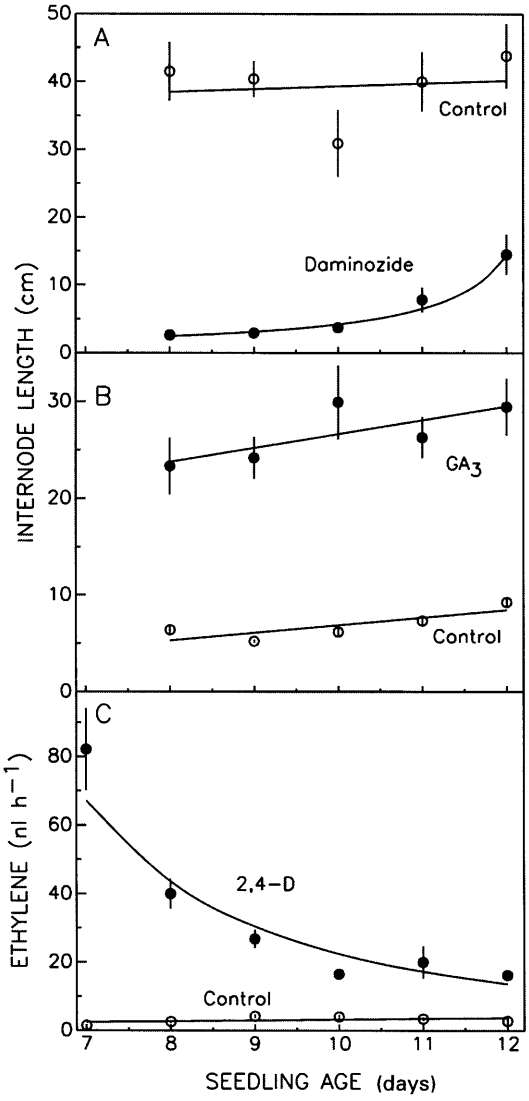


**Fig. 3.** Effect of seedling age on (A) daminozide inhibition of internode elongation, (B) $GA_3$ promotion of internode elongation and (C) 2,4-D stimulation of ethylene production in *Phaseolus vulgaris*.

with 10 (daminozide and $GA_3$) replications. Data for 2,4-D-induced ethylene production represent the average of three experiments with three or four replications each.

## 2.4 Statistics

Experiments were completely randomized. Where appropriate, data were analyzed by analysis of variance as complete factorials. Standard errors were included in all graphs. Where not shown, error bars were smaller than data symbols. Significance of regression equations at the 5, 1 and 0·1% probability level is indicated by *, ** and ***, respectively. Unless otherwise specified, regression analysis was carried out using treatment means.

**TABLE 2**
Effect of Initial Internode Length at the Time of Daminozide application on Development of the First Internode above the Primary Leaves of *Phaseolus vulgaris* (cv. Black Seeded Blue Lake)

| *Initial internode length (cm)* | *Final internode length (cm)*[a] | *Cell size (μm)*[a] | | *Cell number*[a] | |
|---|---|---|---|---|---|
| | | *Pith* | *Epidermis* | *Pith* | *Epidermis* |
| Control | 4·9 (100)a | 103 (100)a | 41 (100)a | 515 (100)a | 1194 (100) a |
| <0·3 | 1·3 (26)d | 57 (55)b | 22 (54)c | 219 (43)c | 577 (48) b |
| 1·0 | 2·3 (47)c | 70 (68)c | 24 (59)bc | 328 (64)b | 974 (82) a |
| 2·0 | 3·1 (63)b | 75 (73)d | 26 (63)b | 437 (85)a | 1184 (99) a |

[a] Means within columns followed by the same letter are not significantly different according to Duncan's multiple range test at $P = 0{\cdot}05$. Percentage of control given in parenthesis.

## 3 RESULTS

### 3.1 Development of bioassay

Daminozide inhibited elongation of the first internode of BSBL bean by 65%, but $GA_3$ promoted internode elongation more than 10-fold. Daminozide inhibited both cell division and elongation in pith and epidermal tissues, but $GA_3$ promoted division and elongation in both tissues (Table 1).

Time-course studies of internode elongation following daminozide and $GA_3$ application revealed that internode elongation was complete within 10 days after treatment (Fig. 1A,B). Upon 2,4-D application, ethylene production increased, reached a maximum between 10 and 24 h after application and decreased thereafter (Fig. 1C). Based on these experiments, 14 days after treatment was selected for assessing effects of spray application parameters for daminozide and $GA_3$ and 24 h for 2,4-D.

Daminozide inhibition, $GA_3$ promotion of internode elongation and 2,4-D stimulation of ethylene production were positively related to the dose applied (Fig. 2). All PGR responses increased at a decreasing rate with increasing dose. Based on these dose-response relationships, doses of 100, 2 and 100 μg per leaf were selected for the study of the effect of application variables on daminozide, $GA_3$ and 2,4-D performance, respectively (see arrows in Fig. 2A–C).

Seedling age significantly affected daminozide inhibition and $GA_3$ promotion of internode elongation and 2,4-D stimulation of ethylene production (Fig. 3). There was little difference in response to daminozide for 8- to 10-day-old seedlings. Responsiveness of the tissue dramatically decreased with age after 10 days (Fig. 3A). $GA_3$ promotion of internode elongation was positively

**TABLE 3**
Effect of Droplet Size and Application Volume at Constant Daminozide Dose (100 μg per leaf) on Length of First plus Second Internodes above the Primary Leaves of *Phaseolus vulgaris* (cv. Nerina)

| *Droplet size (μl)* | *Internode length (cm)*[a] | | | | | |
|---|---|---|---|---|---|---|
| | *Volume (μl per leaf)* | | | | | |
| | *10* | *20* | *50* | *100* | *200* | *Mean*[b] |
| 1 | 7·4 | 8·5 | 7·3 | 8·5 | 7·6 | 7·8c |
| 2 | 12·0 | 10·6 | 9·3 | 8·9 | 7·8 | 9·7b |
| 5 | 14·1 | 11·6 | 11·5 | 10·3 | 8·2 | 11·1a |
| 10 | 13·0 | 11·1 | 12·8 | 10·5 | 9·0 | 11·3a |
| Mean[b] | 11·6a | 10·5b | 10·2b | 9·5b | 8·1c | |

[a] Length of first plus second internodes of untreated plants was 16·7 (±1·0) cm.
[b] Means followed by the same letter are not significantly different according to Duncan's multiple range test at $P = 0{\cdot}05$. Interaction not significant.

and linearly related to seedling age (Fig. 3B). Compared to daminozide, response to $GA_3$ was less dependent on seedling age. 2,4-D-induced ethylene production decreased with increasing seedling age (Fig. 3C). Therefore, 10-day-old plants were chosen for studies on effects of spray application factors.

Daminozide inhibition of elongation of the first internode was markedly dependent on initial internode length (Table 2). Application at increasing initial internode length from $<0{\cdot}3$ to 2 cm resulted in a decreased response from 1·3 to 3·1 cm (control 4·9 cm). The decreased responsiveness was primarily related to a decreasing daminozide effect on cell division in pith and epidermis (Table 2).

### 3.2 Effect of droplet size and carrier volume

Response to daminozide, $GA_3$ and 2,4-D was related to droplet size and/or carrier volume (Tables 3, 4 and 5) with no interaction between droplet size and carrier volume. For daminozide, increasing application volume and/or decreasing droplet size significantly decreased internode elongation and hence, increased performance (Table 3). Some localized phytotoxicity at the treatment site was observed in the low volume/large droplet size treatments, but due to the high variability no relationship was apparent between phytotoxicity and internode elongation. $GA_3$ promotion of internode elongation was positively related to application volume, but droplet

**TABLE 4**
Effect of Droplet Size and Application Volume at Constant $GA_3$ Dose (2 μg per leaf) on Length of First plus Second Internodes above the Primary Leaves of *Phaseolus vulgaris* (cv. Nerina)

| | *Internode length (cm)*[a] | | | | | |
|---|---|---|---|---|---|---|
| *Droplet size* | *Volume (μl per leaf)* | | | | | |
| *(μl)* | *10* | *20* | *50* | *100* | *200* | *Mean*[b] |
| 1 | 29·9 | 30·6 | 32·3 | 33·8 | 33·7 | 32·0a |
| 2 | 29·9 | 32·9 | 33·9 | 32·5 | 36·0 | 33·0a |
| 5 | 30·2 | 31·4 | 32·8 | 33·9 | 35·8 | 32·8a |
| 10 | 30·6 | 27·7 | 32·3 | 31·8 | 33·7 | 31·2a |
| Mean[b] | 30·2c | 30·6bc | 32·8ab | 33·0ab | 34·8a | |

[a] Length of first plus second internodes of untreated plants was 15·5 (±0·8) cm.
[b] Means followed by the same letter are not significantly different according to Duncan's multiple range test at $P = 0{\cdot}05$. Interaction not significant.

**TABLE 5**
Effect of Droplet Size and Application Volume at Constant 2,4-D Dose (100 μg per leaf) on Ethylene Production of *Phaseolus vulgaris* (cv. Nerina)

| | *Ethylene (nl $h^{-1}$)*[a] | | | | |
|---|---|---|---|---|---|
| *Droplet size* | *Volume (μl per leaf)* | | | | |
| *(μl)* | *10* | *20* | *50* | *100* | *Mean*[b] |
| 0·5 | 32·7 | 43·4 | 53·7 | 67·8 | 49·4a |
| 1 | 27·2 | 38·2 | 58·9 | 75·4 | 49·9a |
| 2 | 18·7 | 29·1 | 51·9 | 64·0 | 40·9b |
| 5 | 12·6 | 40·4 | 41·2 | 59·6 | 38·5b |
| 10 | 12·9 | 21·1 | 30·4 | 45·1 | 27·4c |
| Mean[b] | 20·8d | 34·4c | 47·2b | 62·4a | |

[a] Ethylene production of untreated leaves was 4·9 (±0·4) nl $h^{-1}$.
[b] Means followed by the same letter are not significantly different according to Duncan's multiple range test at $P = 0{\cdot}05$. Interaction not significant.

size had no significant effect on performance (Table 4). Increasing carrier volume and decreasing droplet size significantly increased ethylene production by 2,4-D (Table 5). Further, 2,4-D increased the fresh weight of the internode tissue between cotyledons and primary leaves and internode fresh weight was related to carrier volume (Knoche, M., unpublished). Phytotoxic symptoms were restricted to leaf tissue treated with high concentrations (low volumes) of 2,4-D. Further, larger droplets tended to induce more phytotoxicity than small droplets.

## 4 DISCUSSION

The performance of foliar-applied PGRs is the integrated result of all transfer processes of the AI from the spray nozzle to binding to the target site.[6,8] Our assays included the transfer stages from foliar uptake to biological response based on a constant dose. The application technique used ensured that there was no confounding between droplet size and carrier volume. Unfortunately, droplets applied using this technique were restricted to droplet volumes $\geqslant 0{\cdot}5$ µl (equivalent to 985 µm diameter). For comparison, Arnold[21] reported volume median diameters (VMD) of 230 to 257 µm for a commonly used 110-degree-flat fan nozzle (equiv. to 0·006 to 0·009 µl, respectively; range for transverse VMDs measured at 5 cm distance and 300 kPa) and for CP nozzles used in aerial applications, VMDs between 133 and 406 µm (equiv. to 0·001 to 0·035 µl) depending on orifice size and operating conditions have been measured.[22] However, at present there is no effective technique commercially available providing a comparable degree of control and capable of generating small droplets.

The assays developed were effective for evaluating effects of application factors on PGR performance, since (1) the changes in response were sufficiently meaningful that they could be measured in our system, and (2) comparisons among treatments were made at constant dose (Tables 3, 4 and 5).

Performance of foliar-applied agrochemicals has been related to the degree of leaf coverage, such that increasing coverage at constant dose increased performance.[5,23,24] In our experiments, coverage changed as droplet size and carrier volume was varied. If coverage is a critical factor, then effects of droplet size and carrier volume on performance should be dependent on their effects on coverage. Therefore, we examined the relationship between response and interface area between droplets and adaxial surfaces of primary leaves of NE bean. Interface areas of individual droplets of the respective solutions were calculated from droplet diameter measurements (determined by vernier caliper, average of 14 to 18 determinations, e.g. 2,4-D: range 1·9 to 5·4 mm diam. for 0·5- to 10-µl droplets, respectively) and multiplied by the number of droplets applied per plant. Decreasing droplet size from 10 to 1 µl at constant carrier volume and increasing carrier volume from 10 to 200 µl per leaf at constant drop size increased interface area two- and 20-fold, respectively. Total interface area ranged from 13 to 528 $mm^2$ per leaf (for daminozide and $GA_3$) and from 23 to 566 $mm^2$ per leaf (for 2,4-D) corresponding to an estimated leaf coverage ranging from approximately 0·3 to 11·3% for an average 50 $cm^2$ bean primary leaf. Plotting response, i.e. internode elongation (daminozide and $GA_3$) and ethylene production (2,4-D) versus the logarithm of the interface area between spray solution and leaf surface yielded significant linear relationships (Fig. 4, Table 6). These relationships were qualitatively the same for com-

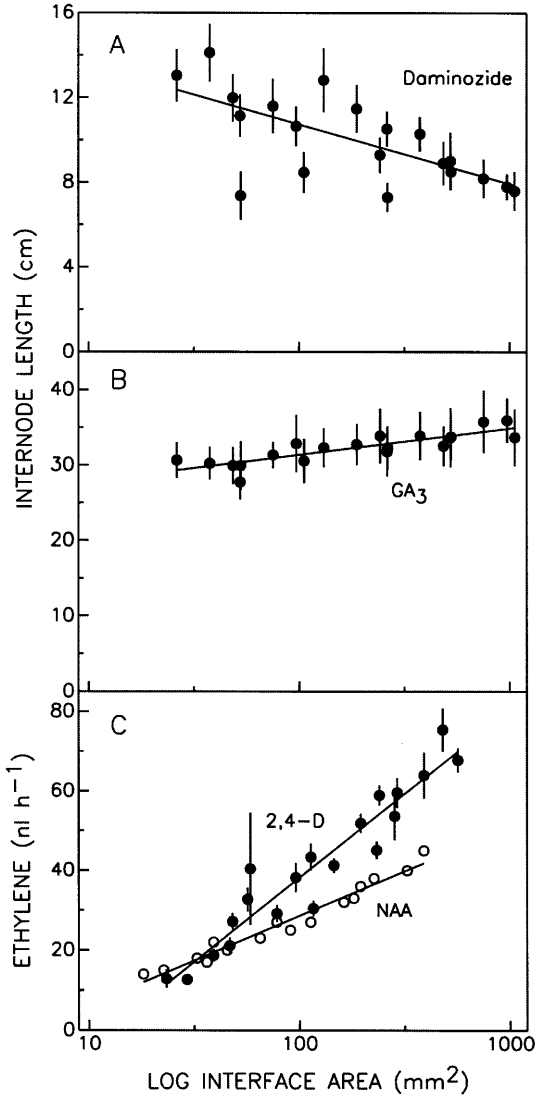


**Fig. 4.** Relationship between the droplet/leaf interface area as affected by spray application factors and performance of selected growth regulators in *Phaseolus vulgaris* (daminozide, $GA_3$, 2,4-D) or *Vigna unguiculata* (NAA). (A) Daminozide inhibition of internode elongation. (B) $GA_3$ promotion of internode elongation. (C) 2,4-D and NAA stimulation of ethylene production. Data for NAA redrawn from Tables 2 and 5 in Crabtree and Bukovac.[19]

**TABLE 6**
Regression Equations for the Relationship between the Logarithm of the Droplet/Leaf Interface Area and Performance of Plant Growth Regulators

| *Compound* | *Performance index (units)* | *Regression coefficient (± standard error)* | | *Coefficient of determination* ($r^2$) |
|---|---|---|---|---|
| | | *a* | *b* | |
| Daminozide | Internode length (cm) | −2·8 (±0·7) | 16·4 (±1·7) | 0·457** |
| $GA_3$ | Internode length (cm) | 3·5 (±0·5) | 24·3 (±1·2) | 0·722*** |
| 2,4-D | Ethylene (nl $h^{-1}$ per leaf) | 42·2 (±3·0) | −46·2 (±6·4) | 0·956*** |
| 2,4-D | Internode fresh weight (mg) | 24·2 (±5·1) | 152·1 (±10·7) | 0·747*** |
| NAA[a] | Ethylene (nl $h^{-1}$ per leaf) | 22·4 (±1·2) | −16·1 (±2·3) | 0·963*** |

[a] Data for NAA recalculated from Tables 2 and 5 in Crabtree and Bukovac.[19] Regression equations were of the form $Y = a \times \log(\text{interface area in mm}^2) + b$.

pounds of different modes of action (i.e. systemic response to daminozide and $GA_3$ versus localized response for 2,4-D) and widely differing physicochemical characteristics. Further, the same qualitative relationship was obtained with published data[19] on NAA stimulation of ethylene production in cowpea (*Vigna unguiculata* L.; Fig. 4C). These data agreed with findings reported by Merritt[25] who observed an increase of difenzoquat performance in *Avena fatua* L. when coverage increased by decreasing droplet size or increasing carrier volume at constant dose. Therefore, the following conclusions were derived.

First, the effects of changing droplet size or carrier volume can be accounted for by changes in interface area between spray solution and leaf surface whether achieved by altering droplet size or carrier volume. This is of practical importance because decreasing droplet size at constant volume may also increase spray drift and hence off-target deposition. In contrast, increasing coverage by increasing carrier volume will improve performance without compromising the efficacy of droplet transfer to the target, provided that the volume applied does not exceed the retention capacity of the leaves (target). Mechanistically, this observation further suggested that differences in drying time between the various size droplets had no significant effect on performance. This conclusion is in general agreement with reports in the literature indicating that penetration during the liquid phase of a spray droplet is frequently negligible compared to penetration following droplet drying.[20,26] $GA_3$ promotion of internode elongation and 2,4-D-induced increase of internode fresh weight were only affected by carrier volume (Table 4 and Knoche, M., unpublished) and may be attributed to the greater effect of carrier volume on coverage compared to droplet size.

Second, the linear relationship between performance of growth regulators and the logarithm of the interface area indicated that increasing interface area increased performance at a decreasing rate. Qualitatively similar relationships have been reported[3] and were observed in spray application trials with daminozide (Knoche, M., Lownds, N. K. & Bukovac, M. J., unpublished). Thus, performance of foliar-applied PGRs may become less dependent on coverage as coverage increases.

Although changes in performance as a function of coverage were qualitatively identical for the compounds studied, they were quantitatively different (see slope of regression lines in Table 6). Three processes may account for these differences, namely, (1) foliar uptake, (2) translocation to the target site (daminozide and $GA_3$) and (3) the responsiveness of the target site to a given amount of AI at that site (response per unit dose at site). Unfortunately, data are not available on effects of application factors on foliar uptake and subsequent translocation for our or a comparable system. However, we assume that at least the rate of uptake is positively related to interface area, since (1) transcuticular penetration is a diffusive process,[27] (2) the driving force for penetration is independent of initial concentration, if dried droplet deposits represent the donor[26] and (3) the initial flow rate is proportional to the interface area based on Fick's laws of diffusion.[28] This hypothesis does not account for dose dependence of cuticle permeance, which has been demonstrated for some AI.[29] Unfortunately, direct evidence for the above hypothesis is currently lacking.

The third process potentially accounting for differential effects of application factors in our system is the induction of the biological response. The amount of response per unit AI translocated to the responding site ('response efficiency') may have differed between assays. An approximate 'response efficiency' in the assays may be estimated from the slope of the dose-response curves (see Fig. 2). Although a quantitative comparison of the different dose-response curves is difficult, qualitative conclusions may be drawn. The steeper the slope of the dose-response curve, the greater will be the change in

performance when dose or efficacy of dose transfer is varied. Hence, dependence of performance on spray application factors is expected to decrease with increasing dose, provided that the response to a given dose is qualitatively similar to the one given in Fig. 2. This conclusion is in agreement with findings demonstrating that the effect of drop size on herbicide performance decreased with increasing herbicide dose.[25]

## 5 CONCLUSIONS

Our data established that application factors altered performance of plant growth regulators such that decreasing droplet size and increasing carrier volume improved performance. This effect was related to a corresponding change in coverage. There was no qualitative difference between the three systemic compounds, i.e. daminozide, $GA_3$ and 2,4-D (internode development), or the localized effect of 2,4-D and NAA on inducing ethylene production.[19] We expect these relationships to be equally applicable to pesticides of similar chemistry requiring foliar uptake for biological response. The quantitative differences in effects of interface area on performance among PGRs were most likely related to (1) foliar uptake, (2) translocation to the target site and/or (3) the amount of response per unit AI. Further studies in this series will address effects of application factors on these processes.

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