

PENETRATION OF CONTROLLED DROP SPRAY OF BACILLUS THURINGIENSIS INTO CHRYSANTHEMUM BEDS COMPARED WITH HIGH VOLUME SPRAY AND THERMAL FOG

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Summary Mature all year round chrysanthemum plants in dense beds were treated with a thermal fog or sprays containing the microbial insecticide, Bacillus thuringiensis (B.t.), and the fluorescent dye, saturn yellow. Six arbitrary categories of dye coverage were made after each treatment. The bacteria at each category were estimated by viable spore counts on agar plates. Application of these values to visual scans of whole sample plants gave a quantified distribution of coverage. The method is suitable for use with other crops. Less B.t. was wasted by CDA (40%) and thermal fog (45%) than by high volume spray (HV) (67%), but this economy was nullified by uneven distribution of the deposit. Compared with peripheral foliage, CDA gave x 80 less deposit on the upper surface of leaves on the lower parts of plants in inner rows of beds, while the deposit on the under surfaces in this position was negligible. Fogs gave a similar distribution of deposits on upper surfaces and virtually no under-leaf deposit anywhere. HV gave even cover throughout the bed with about twice as much deposit on upper surfaces as on lower surfaces. HV would control caterpillars of all ages. CDA and fog would protect only peripheral foliage and buds, against only large caterpillars.

#### INTRODUCTION

Because of the high labour input required for high volume (HV) sprays, growers are increasingly using labour saving ultra low volume and low volume methods for applying pesticides in glasshouses. The value of these methods for glasshouse crops is at present poorly understood. All year round chrysanthemums present severe difficulties, even with HV, because they are grown close together in beds and much of the foliage is near ground level. The use of the microbial insecticide, Bacillus thuringiensis (B.t.) against lepidopterous caterpillars, such as Phlogophora meticulosa presents further difficulty since the bacteria must be eaten to take effect: there is no contact action. B.t. survives passage through pulse-jet type fogging machines and good coverage was obtained over the upper surfaces of leaves of tomato crops (Burges and Jarrett, 1977). CDA and thermal fog appeared likely to penetrate the dense foliage of chrysanthemum beds.

In the present work, the effectiveness of CDA and thermal fog is compared with HV on chrysanthemums.

## METHODS AND MATERIALS

### Year round chrysanthemum crop

Plants were grown at 10-cm spacings in non-raised beds, of 10 rows of 35 plants. Terminal buds were removed to produce many blooms per plants (spray blooms). The experiments were conducted when the plants were fully grown (60 cm high) with buds beginning to show colour.

### Controlled drop application

The spray (Table 1) was applied with a hand-held spinning cup, at a flow rate of 0.39 ml/sec, which produced drops of 40  $\mu$ m number median diameter (nmd), of which 16% were 29  $\mu$ m or less (nd16) and 84% were 55  $\mu$ m or less (nd84). The corresponding volume median diameter (vmd) was 57  $\mu$ m (vd16, 42  $\mu$ m; vd84, 80  $\mu$ m). These drop sizes were obtained by allowing the drops to fall on to slides coated with aluminium oxide and measuring the crater diameters under a low power microscope (May, 1950). In an attempt to obtain maximum coverage and penetration of the dense foliage, the spray was applied evenly from all four sides of the bed at a distance of 40 cm, continually rotating the machine through an arc of 120°, and then along the length of the bed from above, directing the spray towards the plants at an angle of 45° to the long axis of the bed. While spraying, the mixture was continually agitated to prevent settling of the bacteria in the cannister.

Table 1  
Composition of sprays and fog

Method of application	Rate of application	Concn w/v		
		Bacteria <sup>1</sup>	Saturn yellow	Additives to water base
CDA	40l/ha	10%	2.5%	1.0% Triton X-100 12.5% Ulvapon oil
HV	4000l/ha	0.1%	0.1%	0.1% Triton X-100
Thermal fog	40l/ha	10%	1.0%	methanol, 2-ethoxyethanol <sup>2</sup>

1. The wettable powders saturn yellow MF-7 (H. Haeffrom and Co. Ltd. Chepstow), containing particles of 1-2  $\mu$ m, and bacteria (Dipel; Abbott Laboratories, North Chicago, Illinois, U.S.A.) in suspension were homogenised in a Waring blender to avoid blocking the nozzles of the machines.

2. "VK2 carrier".

### High volume spray

The plants were sprayed to run off at 6.9 bars, using a number three Allman hollow cone nozzle with a cone angle of 60°, delivering 1.6 l/min, endeavouring to obtain an even coverage of both upper- and lower-leaf surfaces. This involved spraying the plants from the sides of the bed, ensuring that the spray was directed from ground level up towards the undersides of the leaves, and also from above. The spray (Table 1) was allowed to dry for 4h before sample plants were taken.

### Thermal fog

The fog (Table 1) was applied with a Pulsfog K10 machine, using jet size number

12. The sizes of drops falling on slides were nmd, 17  $\mu\text{m}$ ; nd16, 9  $\mu\text{m}$ ; nd84, 30  $\mu\text{m}$ ; vmd, 42  $\mu\text{m}$ ; vd16, 27  $\mu\text{m}$ ; vd84, 65  $\mu\text{m}$ . The fog also contained an undetermined number of drops < 10  $\mu\text{m}$  in diameter, which do not make craters on the slides, but which would have made very little difference to the vmd. While fogging, the machine was held horizontally, 0.8m off the ground, starting at one end of the house and moving across its width to facilitate even dispersal of the fog.

The fog was applied on a still day to minimise escape of fog through spaces between glass panes and around ventilators etc. The fog was allowed to settle for 2.5h before sampling.

#### Sampling techniques

After each treatment, a minimum of 20 intact sample plants were removed from the bed. An initial visual examination of the foliage was made in a darkroom, under u.v. light, to establish subjectively six distinct arbitrary categories of coverage as shown by the deposit of fluorescent tracer dye. The leaves from each of the sample plants were then systematically removed and the area of each measured using a photo-electric area meter (Evans Electro-selenium Ltd., Halstead). The percentage of the area of each leaf in the different coverage categories was estimated visually under u.v. light.

The bacterial content at each category of coverage was estimated from six 1.4-cm diam. leaf discs cut from the examined leaves. The spores were removed by placing each disc in 10 ml of sterile phosphate buffer, pH 7.2, in a screw cap vial, containing glass balls, and shaking on a vibratory shaker for 10 min. The washings were suitably diluted and the number of viable spores estimated by pour plating (Burgess and Thompson, 1971) 1-ml samples into Lab Lemco agar (Oxoid Ltd., London). Bacterial colonies were counted after 24 and 48 h incubation at 30°C.

### RESULTS

#### Selection of CDA machine

An initial examination of coverage achieved with a number of sprayers, using only the dye in the spray, indicated poor deposition except on upper surfaces of leaves with drops less than 20  $\mu\text{m}$  in a low speed airstream (20 m/s at nozzle decreasing to 1 m/s within 1 m) achieved with the Microgen HCS 1-2 sprayer. Too strong an air velocity (22 m/s at nozzle decreasing to 14 m/s at 1 m) achieved with a fan (28 cm diam., 6000 rev/min) driven by a 2 stroke engine was liable to damage plants. This compares with the Turbair Tot (20 m/s at nozzle 8 m/s at 1 m). As a compromise, further tests were made with a hand-carried spinning cup, producing drops of 40  $\mu\text{m}$  (nmd) and fitted with a fan providing 10 m/s at the nozzle decreasing to 2 m/s at 1 m distance. Coverage of individual leaves was often patchy due to the shading effects of leaves. In a static test to assess the degree of penetration across a bed, coverage of leaves was assessed using an arbitrary scale of 0-10. Most leaves of the outer plants were scored 4-10 on either upper or lower surfaces, with some leaves having satisfactory cover on both surfaces. Coverage on the next row was usually in the range 1-6 but plants in the centre were scored 0-1. It was concluded that this machine was the best for comparing CDA with other application methods. A system for directing the spray into the bed from many directions was developed (see "Methods").

#### Comparison of CDA, thermal fog and HV

B.t. and saturn yellow were added to the sprays (Table 1). The percentage of each leaf in each subjective dye category, obtained by scanning, were converted to quantitative values of B.t. deposit by applying the estimated numbers of B.t. spores

in each category, obtained from the viable spore counts. This gave a quantitative three-dimensional picture of the distribution of deposit in each bed of plants.

#### Spatial distribution

Comparing the upper-leaf cover obtained with the three application methods (Tables 2 and 3), CDA gave good coverage on outer plants and the upper parts of inner plants. The bottom quarters of outer plants received only half the coverage of higher regions. The lower half of inner plants was severely under treated with a x 80 difference between top and bottom quarters. Results with thermal fog were similar. HV gave even cover throughout the bed.

Under-leaf cover (Tables 2 and 3) was less than that on the upper surface and spatial differences were more extreme. With CDA, under-leaf cover was a third of upper-leaf cover in the best position high on the plants and it worsened lower down the plants, being negligible low down on the inner rows. Thermal fog gave negligible under-leaf cover virtually everywhere, noticeable cover being present only on 0.2% of the foliage inverted during plant culture operations. With HV, under-leaf cover was usually about half that on the upper surface and nearly as even. Extreme mean under-leaf values varied by about x 2, with no consistent difference between outer and inner rows, and a slight trend to poorer cover down the plants.

#### Distribution as proportions over total foliage area

Of the bacteria retained on the plants, 92% were on the uppersides of leaves with CDA, virtually all with the thermal fog and 68% with HV.

Table 2

Effect of application method on the distribution of spray deposits across the width of a year round chrysanthemum bed. The deposits are expressed as the average number of viable spores ( $\times 10^3$ ) per  $\text{cm}^2$  of leaf

Application method	Leaf surface	Row					Mean	Mean of upper and lower
		Outer	2nd	3rd	4th	Centre		
CDA	Upper	588	239	231	250	334	328	177
	Under	68	46	10	2	5	26	
HV	Upper	151	126	136	140	155	141	104
	Under	75	80	44	75	63	67	
Thermal fog	Upper	318	230	196	250	273	253	126
	Under	negligible underleaf cover						

The proportion of the foliage covered sufficiently to control caterpillars of all sizes ( $>$  about  $200 \times 10^3$  spores/ $\text{cm}^2$ ) can be seen from the proportion of the foliage in each coverage category (Table 4). CDA and fog adequately covered about half the upper leaf surface and HV 91%. Of the under-leaf surface, however, CDA adequately covered only about 4%, fog virtually none and HV about 47%.

#### Spray wastage

With CDA about 60% of the spray was deposited on the crop, with fog about 55% and with HV only 33% (Table 5). The mean spore deposits over upper- and under-leaf

surfaces were 177, 126 and  $104 \times 10^3$  spores/cm<sup>2</sup> respectively (Table 2), the fog deposit being nearer that of HV than CDA. This relative difference arises partly because the whole glasshouse was fogged, thus taking account of the area of pathways - the amount of B.t. per unit area of crop was 36% less than with the spray treatments. The estimated CDA deposit of 60% is probably a slight underestimate because some of the 40% apparently lost was probably deposited on adjacent beds.

Table 3

Effect of application method on the vertical distribution of bacteria on outer and inner plants of a year round chrysanthemum bed. The deposits are expressed as the average number of viable spores ( $\times 10^3$ ) per cm<sup>2</sup> of leaf

Application method	Position	Outer row		Innermost rows	
		Upper-leaf surface	Under-leaf surface	Upper-leaf surface	Under-leaf surface
CDA	Top quarter	634	204	629	46
	2nd "	789	170	415	4
	3rd "	649	36	111	0.7
	Bottom "	378	1	8	0.1
HV	Top quarter	156	105	161	93
	2nd "	164	87	155	87
	3rd "	155	85	154	73
	Bottom "	134	50	145	71
Thermal fog	Top quarter	581	-	592	-
	2nd "	435	-	368	-
	3rd "	273	-	66	-
	Bottom "	176	-	22	-

Table 4

Effect of application method on the proportion of leaf area in each coverage category

Application method		% of total leaf area in each category					
		0	1	2	3	4	5
CDA	Spores/cm <sup>2</sup> $\times 10^3$	0	4.4	11	55	537	999
	Upper-leaf	4.0	11	14	22	33	16
	Under-leaf	62	17	11	6	3	0.9
HV	Spores/cm <sup>2</sup> $\times 10^3$	0	1.3	11	75	260	1100
	Upper-leaf	0.8	1.3	1.4	6	85	6
	Under-leaf	3.2	8	17	25	46	0.4
Thermal fog	Spores/cm <sup>2</sup> $\times 10^3$	0	10	79	226	638	-
	Upper-leaf	10	22	18	20	31	-
	Under-leaf	ca99	-	-	-	-	-

The greatest loss was with HV sprays, due mostly to spray running off leaves on to the soil, although with high pressures small drops could drift on to adjacent beds.

Table 5  
Effect of method of application on the proportion of bacteria deposited on foliage

Application method	% of total bacteria on:-		
	Upper leaf surface	Lower leaf surface	Upper + lower leaf surface
CDA	55	5	60
Fog	55	ca0	55
HV	22	11	33

#### DISCUSSION

The use of fluorescent dye (Courshee and Ireson, 1961) combined with B.t. spore counts proved to be a valuable technique for quantifying the distribution of bacterial spray deposits within densely planted beds of year round chrysanthemums. The method would also be suitable for use on other crops. In quantitative studies of chemical pesticide distribution, B.t. could be incorporated in sprays as well as dye to improve the quantitative value of deposit estimates.

To compare the efficiency of the three machines when each was used to its best effect, the operations were thorough, probably more so than in average commercial applications. Even though the thoroughness of application can influence the results, a number of definite conclusions can be made. CDA failed to penetrate adequately to the upper-leaf surfaces of the inner plants, because the dense foliage rapidly reduced the air speed, while under-leaf cover was poor on most plants and very poor on the lower parts of inner plants, because there was little upward component to the air movement. HV gave much more even cover, with half as many spores on under-leaf surfaces as on upper surfaces. This was because the spray carried better than CDA and was directed into the beds from more directions, including upwards and horizontally from positions near the soil surface. Fog gave a similar cover of upper-leaf surfaces to that of CDA, but no under-leaf cover, because once the fog had left the immediate vicinity of the fogger, the air speed was low with little impingement due to the small size of drops, which settled mostly downwards on to the leaves. The results with fog were similar to results with fog on tomato plants (Burges and Jarrett, 1977), in which only 0.5% of the bacteria were deposited on the undersides of horizontal leaves and leaves slanting at 45°, and only 12% on the undersides of vertical leaves. The comparatively small wastage with CDA on chrysanthemums is similar to the results of Morgan (1976) with apple trees, on which CDA wasted less spray than HV.

In commercial practice, slightly better coverage of chrysanthemums might be obtained by using several CDA machines pointing in different directions on a boom, which could improve air movement within the crop. Further work is needed to assess this possibility. To obtain even coverage with commercially applied fogs, the machine must be moved along the glasshouse during fogging. The commercial application of HV would probably be less thorough than that in the present work. Even so, the lesser wastage with CDA (40%) and fog (45%) compared with HV (67%) would be nullified by uneven distribution and poor under-leaf cover. Only HV sprays of B.t. on chrysanthemums would be adequate to control caterpillars of all ages in all

regions of the bed. This is because good under-leaf coverage is required, since the caterpillars feed underneath the leaves for 7-14 days after hatching, leaving the upper epidermis intact, and since B.t. is a particulate, insoluble material, with no translamina effect, active only when ingested. After this period, the caterpillars eat completely through the leaves, ingesting upper-surface deposits. Young caterpillars are more susceptible than old ones (Burgess and Jarrett, unpublished) so under-leaf cover half as concentrated as that on the upper surface, as obtained with HV, is effective. Sometimes it is required to protect only marketable upper foliage and buds shortly before harvest against large caterpillars surviving previous control treatments. At these times, CDA and fog would be effective. Fog has the advantage of extremely rapid application. CDA has an advantage over fog of providing limited under-leaf coverage. It is quicker than HV, taking 1.5 min per bed compared with 2 min for HV.

With the use of chemical pesticides against other chrysanthemum pests and diseases, CDA and fogs would be effective against leaf miners because adults lay eggs only on the upper-leaf surface of upper foliage. Only HV would be effective against most other pests and diseases because they mainly occur throughout the beds and many are found only on lower-leaf surfaces. Translamina pesticides applied by CDA or fog would be effective except in the lower part of the centre of beds.

These experiments are part of a continuing programme of spray application research at the Glasshouse Crops Research Institute.

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NOTES



BEHAVIOUR OF SINGLE DROPS AND SPRAYS IN DIFFERENT  
CLIMATIC CONDITIONS IN A CLIMATE TOWER

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Summary The construction of the climate tower used for the experiments is described. The tests covered the evaporation behaviour of individual water drops and clouds of water drops without and with an additive to inhibit evaporation. In the case of the single drops, the change in diameter after falling was measured, starting from differing drop sizes. The behaviour of a cloud of drops due to the changes in climate and height of fall was determined. The adding of the additive agent resulted, in the case of both medium-dry and dry climate, in a volume median diameter (VMD) which was about 10  $\mu\text{m}$  larger than that of the water spectrum, after a fall of 6 metres.

INTRODUCTION

The study of the evaporation behaviour of drops is aimed at achieving more effective plant protection measures causing as little contamination of the environment as possible through wind drift. Because drops partially evaporate in flight, plants are not treated with the assumed drop spectrum. As a result of evaporation a portion of the smaller drops can remain suspended in space. Besides the choice of an appropriate time of day for application, the adding of preparations which inhibit evaporation can help to reduce the evaporation of the drops.

Theoretical studies on the evaporation of water drops were carried out by Ranz and Marshall (1952), Williamson and Threadgill (1974), Law and Bowen (1975) and Heidt (1976), to name only a few. Seymour (1960) measured the life span of water drops in a wind tunnel with air of differing relative humidity. Arsdon (1962) observed a decrease in the evaporation of the solvent when a preparation for inhibiting evaporation was added.

With the aid of a climatic tower, we have been investigating the evaporation behaviour of single water drops as well as clouds of water drops. Above all, the climate tower is meant to serve as a testing ground for the various anti-evaporation additives. Because these preparations differ from one another and especially from water in terms of their physical properties, it is not possible to calculate the evaporation rate simply on the basis of a theoretical model.

## CLIMATE TOWER

The climate tower built for the experiments has a square cross section measuring 200 by 200 cm and a height of 600 cm. Its walls are thermally insulated. The temperature of the air can be varied from 10° to 40° C, and the relative humidity from 15% to 95%. The desired climate can be produced within 10 to 70 minutes of air circulation depending on the climate change required. The experiment is conducted, however, only after the air circulation has ceased and the air has become calm. The height of the drop generator can be varied between 50 and 600 cm (Fig. 1).

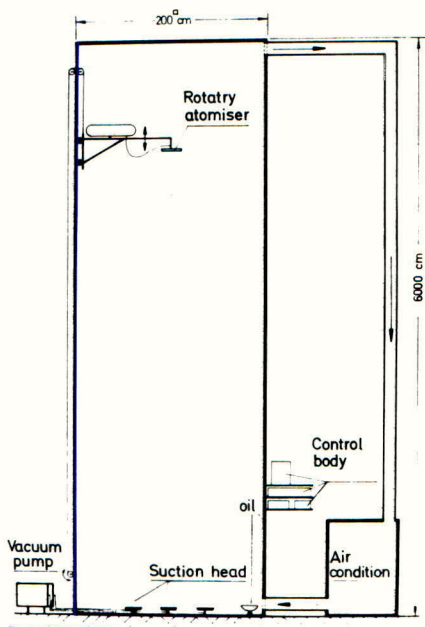


Fig. 1 Schematic diagram of climate tower

EXPERIMENTAL STUDIES OF THE EVAPORATION BEHAVIOUR OF INDIVIDUAL  
WATER DROPS

In order to investigate the evaporation behaviour of single water drops, a drop generator as developed by Bouse et al (1974) was constructed. This generator functions according to the Rayleigh disintegration principle, with the periodic disturbance being brought about by a Piezo-electric crystal. The experiments were conducted at a temperature of 40°C and a relative humidity of 15%. A 0.1% solution of BSP (Brilliant-sulfo-flavin) was used as the spray liquid.

Drops with diameters of 130, 165 and 258  $\mu\text{m}$  were produced. After having been collected from different heights in silicon oil they were photographed with the aid of an ultra violet flash.

The results of the experiment reveal that the drops with a diameter of 258  $\mu\text{m}$  lose 10% of their diameter through evaporation after having fallen 3 metres in air of the above mentioned temperature and humidity. Under the same conditions, the drops with 165  $\mu\text{m}$  diameter lose 20% of their diameter. In the case of the smaller diameters, the evaporation rate increases rapidly. Thus, the drops with 130  $\mu\text{m}$  diameter were reduced after a fall of 2 metres to half of their original size. (Fig. 2).

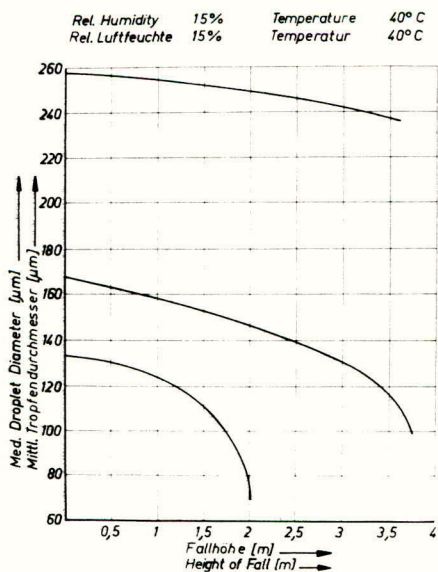


Fig. 2 Evaporation of water drop

EXPERIMENTAL STUDIES OF THE EVAPORATION BEHAVIOUR OF A SPECTRUM  
OF WATER DROPS

On account of the differences in drop size, the catching of drops in oil does not permit a convincing judgement of the extent of evaporation of a drop spectrum. Especially when a climate is dry and only a portion of the drops reaches the oil tray. A large number of drops are suspended in the air and do not settle, whereas a further portion crystallizes. In order to record the spectrum of drop size a modified cascade impactor was used. Diaphragms served as object slides. By means of a light suction, very small drops suspended above the bottom can also be included. Here, as well, the drops are photographed with the aid of an ultra violet flash. Moreover, it is possible in normal light to determine the number of crystallized drops on the object slides. This number can be taken as a relative measure for estimating the evaporation behaviour of a drop spectrum. A rotary atomizer was used to carry out the experiments. The drop spectrum of this atomizer had a VMD of  $140 \mu\text{m}$  with a relative humidity of 95% and a temperature of  $15^\circ\text{C}$ . The atomizer was 2 metres above ground level.

Measurement of the drop spectrum of the same atomizer in a climate of 45% relative humidity and  $25^\circ\text{C}$  revealed a VMD of  $80 \mu\text{m}$ . With 15% relative humidity and  $40^\circ\text{C}$ , this value was reduced to  $40 \mu\text{m}$ ; in this case, because many small drops had evaporated, the number of drops measured was considerably lower than in the previous experiments (Fig. 3).

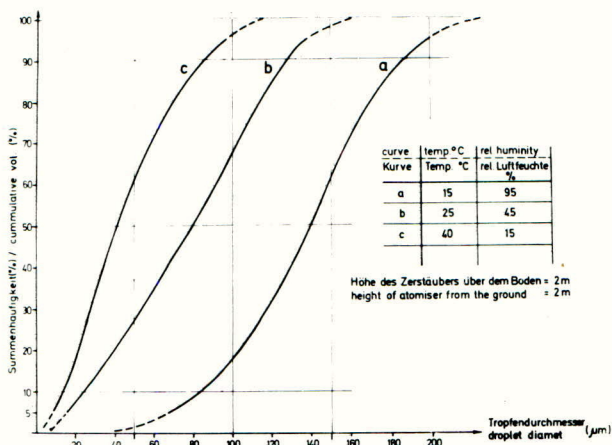


Fig. 3 Effects of different climatic conditions on the drop spectrum

In further trials the change in the drop spectrum of the same atomizer as a result of evaporation was studied under the same climatic conditions but with different heights of fall. In these trials under climatic conditions of 45% relative humidity and 25°C and with the atomizer height raised from 2 to 4 metres, a decrease from a VMD of 90  $\mu\text{m}$  to a VMD of 30  $\mu\text{m}$  was recorded. When the height of the atomizer was increased to 6 metres, a drop spectrum with a VMD of 23  $\mu\text{m}$  was measured (Fig. 4).

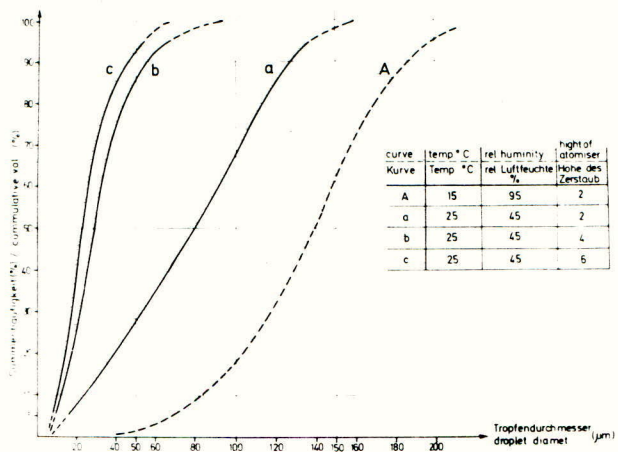


Fig. 4 Drop spectrum at different heights

The results of the experiments with a 15% relative humidity and a temperature of 40°C revealed that increasing the height of the atomizer from 4 to 6 metres leads to only a slight change in the drop spectrum. The number of crystals was about the same in both these experiments (Fig. 5).

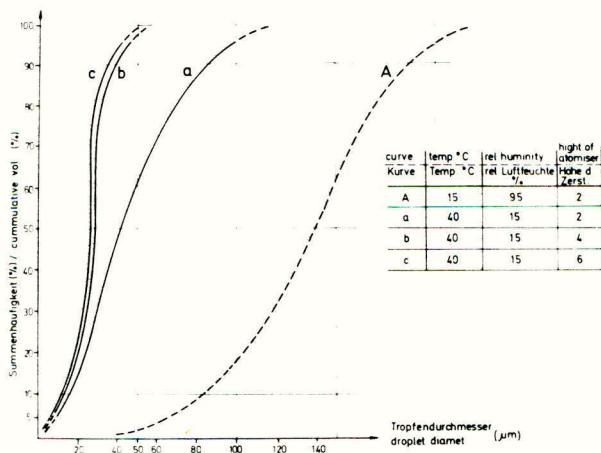


Fig. 5 Drop spectrum at different heights

EXPERIMENTAL STUDIES OF THE EVAPORATION BEHAVIOUR  
OF A CLOUD OF DROPS WITH AN ADDITIVE

As an example of an additive for improving the deposit on plants a 5% solution of Synergid was chosen. The addition of this preparation to water had negligible effect upon the drop spectrum. This holds true for centrifugal as well as hydraulic atomization. For the trials in the climate tower, the same rotary atomizer as in the previous experiments was used. The height of the atomizer was 6 metres. Trials were conducted at 25°C and 25% relative humidity as well as with a drier climate of 40°C and 15% relative humidity.

In comparison with the spectrum of water drops, the VMD was roughly 10  $\mu\text{m}$  larger using the same atomizer and similar climatic conditions (Fig. 6).

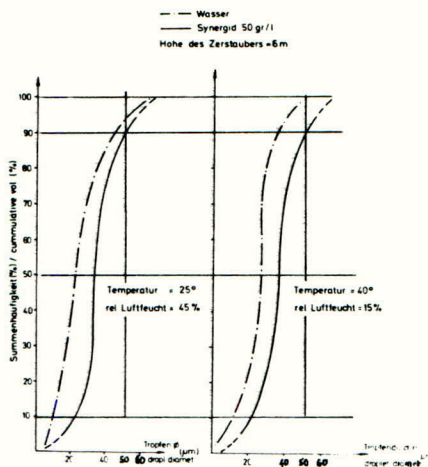


Fig. 6 Effects of additive (Synergid) on the volatility of drops

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